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Immunomodulators and Human Health

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Foreword

Modulation of the immune system has a vital role in the management of health and disease progressions in humans. The genesis of the immune system modulation is from the necessity to eradicate and modulate pathogenic as well as nonpathogenic microorganisms that could hamper the body's capability to uphold homeostasis. There are a variety of immunomodulators that originated from nature and of synthetic origin. Scientists across the globe have endeavored to explore several strategies for the treatment/cure of the diseases. Thus, it becomes quite essential to have the adequate immunity level to combat challenging and devastating pathogens so as to ensure a state of well-being of mankind. The present book entitled "**Immunomodulators and Human Health**" is an attempt to pool the relevant information contributed by authors around the world and update the readers about fundamentals and recent advancements in the domain of immunomodulators. It is noteworthy to state that some of the chapters have been exclusively devoted to COVID-19 and its management strategies.

I am quite sure that readers would find it a fascinating piece of scholarly compilation.

Best wishes.

National Institute of Immunology
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Amulya K. Panda

Preface

This book, **"Immunomodulators and Human Health"** depicts a full picture of the state-of-the-art research and development of actionable knowledge in medical science. As is evident from the latest discussions at various conferences and seminars held across the globe based on diverse medical, biotechnology strategies in the current scenario of the Covid-19 pandemic, people are more concerned about the immune system and immune boosters. A major reason for the above situation, we believe, is the gap between academic research and real-time clinical applications and needs. The present book includes four parts (I, II, III, and IV) and among that Part I, Neutraceuticals and Plant Metabolite, contains four chapters, Part II, Nanotechnology and Cancer, includes three chapters, and Parts III (Infectious and Autoimmune Diseases) and IV (Enzyme, Hormone, and Biomolecules) include five and four chapters, respectively.

Neutraceuticals and Plant Metabolites

Chapter 1 entitled "Classification, Mode of Action and Uses of Various Immunomodulators," written by Kumar and colleagues, states that immunology is the most fundamental area of pharmaceutical research, and it has fantastic guarantees concerning the anticipation and treatment of a broad scope of disarranges, for example, the provocative maladies of the skin, gut, respiratory tract, joints, and focal organs. Immunomodulators are turning into a feasible assistant to build up modalities that offer a novel methodology for treating irresistible ailments in the coming many years of the twenty-first century.

Chapter 2 entitled "Potential Role of Herbs and Spices on the Immune System" was written by Anandharamakrishnan and his coauthors that the natural body's defense system plays a critical role to keep away the person from infections and minimize the risk of falling sick regularly. The smart way to improve immunity is by changing the lifestyle by consuming food that has immunomodulatory activity. Spices/herbs have been used as preservatives as well as traditional medicines since ancient times due to their disease prevention capability. Numerous results from preclinical and clinical trials over few spans have shown the beneficial role of spices/herbs and their active components in the control and prevention of several

complications such as arthritis, respiratory diseases, cancer, cardiovascular disease, glucose impairment, and brain disorders.

Chapter 3 entitled "Immune Booster Activity of Prebiotics" is described by Oyedepo and her associates that the key to good health is a functioning and strong immune system. A lot of studies in dietary and food biotechnology are ongoing about new alternatives for disease prevention. Extensive studies conducted on the link between the gut microbiome and immunity have led to increased interest in functional foods. The need to improve health and quality of life has brought forth the concept of functional foods and nutraceuticals, which have nutritional value and certain biological activities. This chapter highlights the interaction between the immune system and functional foods/nutraceuticals in terms of modulation of immune functions by a variety of mechanisms.

Chapter 4 entitled "Antioxidants and Immunomodulation" by Dubey and his associates described that the immune system, one of the most sophisticated defense systems of the body, is capable to recognize and eliminate the unlimited varieties of foreign and undesirable agents. A strong defense mechanism is needed for a balanced and disease-free body. But nowadays lifestyle and stress cause variations in endogenous systems and physicochemical conditions triggering wreckage and alteration in the immunity leading to the generation of free radicals, which subsequently causes several diseases like cancer, aging, and neurological and cardiological diseases. In this chapter, the action of antioxidants on free radicals, their mechanism of immunomodulation, sources, and occurrence along with classification and potential health effects have been discussed.

Nanotechnology and Cancer

Chapter 5 entitled "Nanotechnology and Immunomodulators in Cancer" by Agop and coauthors describes that since the last ten years, immunotherapy represents a promising strategy for treatment in cancer without massive damaging normal cells, by reprogramming and activating antitumor immunity. However, the adverse events of immunotherapy related to the low specificity in tumor cell targeting represent limits of immunotherapy efficacy. The potential of nanotechnologies is represented by the possibilities of carrying immunotherapeutic agents by nanoparticles with various material types, with different shapes, sizes, coated ligands, loading method, hydrophilicity, elasticity, and biocompatibility. This chapter summarizes different types of cancer immunotherapy already approved for cancer treatment or currently studied in clinical trials which can be possibly correlated with nanotechnologies.

Chapter 6 entitled "Advancements in the Field of Oral, Intravenous and Inhaled Immunomodulators Using Nanotechnology" by Parijat Pandey and coworkers describes that, despite great progress in the field of conventional delivery of immunomodulators, the development of newer techniques and drugs is greatly required due to intrinsic instability of immunomodulators in vivo, related toxicity, and the required multiple administrations. The focus of this chapter is on summarizing the recent condition and developing a way in such nanotechnology-

based oral, intravenous, and inhaled immunotherapies as well as the function of nano-size particles as a carrier of immune-modulators, and depots for sustained immunostimulation along with associated advantages and limitations.

Chapter 7 entitled "Phytochemicals as the Source of Natural Immunomodulator and Their Role in Cancer Chemoprevention" by Gupta et al. In the present chapter, authors have depicted that a well-functioning immune system of the host body plays a pivotal role in the maintenance of ordinary physiological and immunological functions as well as internal environment. Balanced immunity enhances defense mechanism against infection, diseases, and unwanted pathogens to avoid hypersensitivity reactions and immune-related diseases.

Infectious and Autoimmune Diseases

Chapter 8 entitled "Immunomodulators and Autoimmune Liver Diseases," written by Prameela Kandra et al., describes that autoimmune liver disease (AiLD) is a series of progressive and chronic inflammation to the bile duct and liver cells arising due to impaired coordination between the components of one's immune systems ultimately leading to the destruction of the liver. This disease primarily constitutes autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC), and primary sclerosing cholangitis (PSC) under its wing. Immunomodulatory therapy established itself as a robust approach by providing a platform to treat such diseases. This chapter unfolds with a brief perspective on the epidemiological, pathogenetic, and clinical studies of AiLDs and dives deep into understanding the intricate dynamics of the immune response during the pathogenesis of AIH. This study also highlights the numerous immunomodulators emphasizing their therapeutic potential for treating AiLDs.

Chapter 9 entitled "Immuno-modulators Role in the Treatment and Management of Tuberculosis," written by Rao et al., discusses that tuberculosis is caused due to Mycobacterium tuberculosis (M-tb), which leads to major therapeutic challenges causing several immune dysfunctions by affecting various immune checkpoints. Over the past decades, many research efforts have been made to control infections. However, the etiology of tuberculosis reveals that M-tb has coevolved with human immune response and hijacks various defense mechanisms of natural and synthetic antimicrobial agents contributing to the development of multidrug resistance. Henceforth, the strategy of immunomodulation, such as host-directed therapy (HDTs), emerges as an important therapeutic modality in treating infectious diseases like tuberculosis. Thus, the present chapter discusses the efficacy of various immunomodulation against the etiology of M-tb infections and challenges in the development of different classes of immunomodulatory agents.

Chapter 10 entitled "Role of Immunomodulators in Autoimmune Diseases" by Das et al. narrates that the immune system comprising an intricate network of various specialized cells and associated molecules is crucial to the extermination of pathogens from the host's body, and thus vital to human survival. Along with the generation of an immune response, the immune system is also responsible for the maintenance of tissue homeostasis in a continuously fluctuating environment.

Autoimmune disorders have many manifestations and can either be localized such as rheumatoid arthritis or be systemic such as systemic lupus erythematous to name two. Current therapies for most autoimmune disorders include immune suppression in general, aiding in the reduction of exaggerated immune response and inflammation. The current chapter is focused on the current strategies of immunomodulation along with their advantages and disadvantages.

Chapter 11 entitled "Psychology, Epigenetics, Immunomodulation, and Immune Dysfunction: Understanding the Connection," written by Goswami and coworkers. This chapter explains in brief the essential concepts relating to health and diseases that are important for having a complete understanding of immunomodulatory processes and immune dysfunction. This chapter aims in addressing the knowledge gap by pointing out the importance of considering the existence of natural immunomodulators and their relevance in the regulation of immune system functioning while designing and administering artificial immunomodulators.

Chapter 12 entitled "Immunomodulators Based Ayurvedic Plants: Against Virulent Infectious COVID-19," written by Rinki Kumari and colleagues, highlights the role of traditional medicines in the management of COVID-19. The chapter commences with a description of principles forming the basis of the relationship of life with nature and spans through a variety of plant sources observed to be useful to combat viral infections.

Enzyme, Hormone, and Biomolecules

Chapter 13 entitled "Role of Cytokines as Immunomodulators," written by Kaur and Ghorai, describes that the resilience of our immune system is remarkable. It is always on guard against various pathogens that we encounter whether we eat, work, or sleep. The chapter here deals with cytokines and their action as immunomodulators at different levels of the immune system of our body and their role in immunotherapies.

Chapter 14 "Immunomodulatory Properties of Proteins and Peptides: Food Derivates Approach," written by Gloria A. Martínez and associates, describes that food represents a millennial source of multiple molecules with potential as health enhancers, not only from a nutritional point of view, and proteins are described as one of them. Proteins and their derived peptides could interact in a wide range of biological levels but claim attention as immunomodulating agents. This chapter aims to analyze the food-derived protein and peptide's role in the immune response with an emphasis on their employment as health promoters, the involved mechanisms, and their potential incorporation in products.

Chapter 15 entitled "Fatty Acids and Immunomodulation," authored by Shahrul and Tasyriq, states that cells require energy as a source for survival. The chapter describes the underlying molecular mechanisms concerning health and disease pathogenesis. It addresses the importance of dietary intervention as a mode of therapeutic lifestyle modification for chronic diseases such as metabolic syndrome, cardiovascular disease, and several other diseases. Additionally, clinical strategies of

targeted therapy to modifying fatty acids to overcome the increasing burden of chronic diseases as well as emerging diseases are also presented.

Chapter 16 entitled "Immunomodulatory Effects of Endocrine Disrupting Chemicals" by Kaur and Ghorai entails about endocrine-disrupting chemicals (EDCs) and their role in immunomodulation states that EDCs have become an integral part of the human environment, be it in cosmetics, food, plastic packaging materials, toys, pesticides, and numerous other amenities. The nexus between environmental EDCs and epigenetic regulation of genes has also been underlined. The article also stresses the need for enhanced research on existing and emerging EDCs along with advocating the involvement of individual and scientific society and its stakeholders in communicating and implementing changes in public policy and awareness.

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Contents

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Part I

Nutraceuticals and Plant Metabolites

Classification, Mode of Action and Uses of Various Immunomodulators

1

Prashant Kumar, Sweta Rai, Sunil Kumar Verma, P. Shakti Prakash, and Dheeraj Chitara

Abstract

Immunomodulators are substances found in nature that aid in immune system regulation. They are concoction operators that take part in insusceptible frameworks of the immune system. Commonly present immunomodulators were less significant compared to therapeutic immunomodulators. Immunomodulatory medicines, like 6-mercaptopurine and mycophenolate mofetil, conceal the safe framework and minimise irritation in the stomach tract in persons with inflammatory bowel disease and ulcerative colitis Crohn's disease. Their advantages come from their capacity to invigorate conventional and versatile safeguard systems, a kind of cytokines that empower the whole body. Immunosuppressants and immunostimulants are two categories of immunomodulators. Immunosuppressants are involved in smothering the invulnerable framework and handle neurotic safe reactions in the immune system like sickness and unite dismissal. Immunostimulants are agents that enhance the body's resistance in case of infections. It also improves the immune response, and people with the depletion of response are immunotherapeutic operators. For

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example, various scatters, such as immunodeficiency state, immune system sickness, malignant growth, and viral contamination, can be treated with immunostimulants. In the subsequent decades of the twenty-first century, immunomodulators will become a viable assist in generating modalities that will provide a unique approach for treating irresistible diseases.

Keywords

Antigen · Antibodies · Transplantation · Immunomodulators · Immunosuppressant

1.1 Introduction

Immunology is characterised as natural defence mechanism against many diseases or disorders. The invulnerable framework is the body's most complex biological mechanism. The term insusceptibility characterises the natural defence system against the many diseases. The human immune system is very sophisticated and highly advanced among vertebrates; this immune framework is capable of generating boundless variety of cells and caught enormous spectrum of infections and foreign particles. The substances capable of inducing, amplifying or restraining any component or phase of the invulnerable framework are referred to as immunostimulators. Immunostimulators and immunosuppressants are two types of immunomodulators are known for use. Immunopharmacology is a more current branch of pharmacology concerned with immunomodulators (Patil et al. 2012). Administration of immunostimulators as in the case of AIDS and the utilisation of immunosuppressor in the cases of an exaggerated response of a safe framework are appreciating to reconstitute the normal resistant framework and increase the longevity of life. Immunomodulator intake, along with antigen, is meant to support the insusceptible framework. The modulator is the resistant framework's key role in distinguishing self from non-self. Immunisation can take place in two ways: actively or passively. In inactive immunisation, an antigen is stimulated to aid the body's development of immunological defences against potential exposure. Passive immunisation entails administering antibodies that have been preformed to the person who has already been exposed or is soon to be exposed to antigen. The activity of immunostimulant has been reported in several plants, and these plants utilised traditionally for rejuvenation of the immune system and treatment of chronic diseases in India, China and European countries. Currently, some stimulation of antigen-specific or non-specific invulnerability is evidenced by an increase in haemagglutinating antibody (Ha) titre and plaque-forming cells treated with half ethanolic extract of these plants in mice. The studies prove that these plant products play a vital role in rejuvenation therapy and chronic ailments of Indian traditional medicine (Patil et al. 2012). Around 122 chemicals inferred from plants have been

identified as therapeutic substances that are also used in commercial drugs. For example, the bark of the willow tree is rich in salicylic acid, which is also an active metabolite of aspirin, and this bark has been identified as a therapeutic substance (Goodman 1996). Some of the medications which are as often as possible utilised by the physicians are also determined from plant sources, for example, aspirin, digoxin, quinine, opium, etc. (Goodman 1996). They have been used as an herbal tranquilliser for a long time. There is a growing interest in using these medicinal plants as modulators of the complex safety system. Many chemical forms of alkaloids, flavonoids, terpenoids and polysaccharides have been discovered through many sorts of research conducted in the area (Puri et al. 2013); lactones and glycosides are the main factors for modification in the immunomodulatory properties.

1.2 Subtypes of Immunomodulators

Immunomodulators are divided into the three subtypes:

(a) Immuno-adjuvants improve vaccine efficacy and can be employed as particular immunostimulants. Immuno-adjuvants are the actual modulators of the fast response, acting as selectors between immune-protective and immune-destructive T1 (Th1) and T2 (Th2) cells (Dias et al. 2012).
(b) Immunostimulants are inalienably non-specific resistant to infection. These immunostimulants work in both innate and adaptive invulnerable responses. These immunostimulants serve as prophylactic and promoter compounds such as immunopotentiators—the immunostimulants with invulnerable response, which act as immunotherapeutic compounds (Wadood et al. 2013).
(c) Structurally and functionally, immunosuppressants are a diverse class of drugs frequently used in combination regimens to treat various autoimmune and allergy diseases (Billiau and Matthys 2001). Immunosuppressant decrease in resistance to infections may occur as a result of chemotherapeutic factors (Billiau and Matthys 2001).

Immunosuppressant clinical applications are as follows:

- To prevent transplanted organs and tissues from being rejected
- To treat graft-versus-host disease in bone marrow transplants
- To treat myasthenia gravis, systemic lupus erythematosus, rheumatoid arthritis, psoriasis and ulcerative colitis, which are not entirely understood autoimmune components in their pathogenesis
- Avoiding Rh haemolytic disease in newborns with selective immunosuppression

1.2.1 Immunostimulant

The immunostimulation comprises a therapeutic concept which stimulates the non-specific immune system; this means that non-antigen-dependent stimulation is used to boost the efficacy of granulocytes, macrophages and natural killer cells.

Immunomodulator Drug Side Effects

Pulmonary toxicity, myelosuppression, alopecia and an increased risk of infection have been reported as side effects of these drugs (Kremer et al. 1994).

1.2.1.1 Pharmacognostic Approaches

Pharmacognostic approaches are briefly discussed in Table 1.1.

1.2.1.2 Chemistry of Phytoconstituents Used as Immunostimulants

1.2.1.2.1 Glycosides

These are organic compounds derived from plant and animal sources that, when hydrolysed by enzymes or acids, give one or more sugar moieties known as glycone parts. When water is lost, they create acetals and ether forms that connect with the hydroxyl groups of sugar and non-sugar moieties. Many glycosides are available for the ideal immunomodulatory action, such as:

- *Picrorhiza scrophulariiflora*, anthraquinone glycosides.
- Three novel sesquiterpene glycosides have been isolated from the stems of *Dendrobium nobile*: *Andrographis paniculata*, Dendroside and Dendronobilosides.

1.2.1.2.2 Flavonoids

Flavonoids are 15-carbon (6-3-6) skeletons with two phenyl rings that bind three-carbon.

Various forms of flavonoids, including apigenin, exhibit immunomodulatory activities.

- Oligomeric proanthocyanidins.
- Isoflavonoids, flavones and anthocyanins such as flavonoids are found in *Terminalia arjuna*.

1.2.1.2.3 Coumarins

Glycosides derived from benzo-a-pyrone are known as coumarins. Furanocoumarins are made by fusing furan ring to a coumarin at the 6 and 7 positions or the 7 and 8 positions (Leung et al. 2005), and these molecules show immunomodulatory activities. 6,7-Dihydroxycoumarin (esculetin) is a coumarin derivative extracted from a range of plants, including *Artemisia capillaris*, *Citrus limonia* and *Euphorbia lathyris*. It shows pleiotropic biological activities, such as inhibition of lipoxygenase, suppression of the oxidative reaction that damages DNA, inhibition of tyrosinase

Table. 1.1 Summary of immunomodulators produced from plants

Category	Botanical (family)	Ayurvedic name	Part used	Chemical constituents	Other biological activities
Sesquiterpenes	*Ocimum sanctum Linn. (Labiatae)*	Tulsi	Whole plant	Eugenol, carvacrol, derivatives of ursolic acid, apigenin, anthocyanins, flavonoids	Carminative, stomachic, antispasmodic, antiasthmatic, hepatoprotective (Nadkami 2005; Khare 2008; Singh et al. 2007; Vaghasiya et al. 2010)
Flavonoid	*Morus alba Linn. (Moraceae)*	Brahmdaru	Leaves, Bark, Fruits	Flavonoids, anthocyanins	Expectorant, hypocholesterolaemic, diuretic (Bharani et al. 2010)
Glycoside	*Panax ginseng Wall. (Araliaceae)*	Ninjin	Fruits, Root	Saponins such as ginsenosides, panaxdiol, panaxtriole, oleanolic acid	Adaptogenic properties, antiarrhythmic (Khare 2008)
Flavonoid	*Achillea millefolium C. Koch (Compositae)*	Yarrow	Leaves	Flavonoids, alkaloids, polyacetylenes, coumarins, triterpenes	Anti-inflammatory, antispasmodic, antipyretic, diuretic (Sharififar et al. 2009)
Anthraquinone glycosides	*Aloe vera Tourn. Ex Linn. (Liliaceae)*	Kumari, Ghritkumari	Leaves	Anthraquinone glycosides	Purgative, Emollient anti-inflammatory (Cooper and Turcasso 1999; Khare 2008; Hamman 2008; Sikarwar et al. 2010)
Diterpene	*Andrographis paniculata Nees (Acanthaceae)*	Kaalmegha	Leaves	Diterpenes	Hepatoprotective, antispasmodic, blood purifier, febrifuge (Khare 2008; Varma et al. 2011)
Saponin glycoside	*Asparagus racemosus wild. (Liliaceae)*	Shatavari	Roots	Saponins, sitosterols	Ulcer healing agent, nervine tonic, anti-gout (Nadkami 2005; Bopana and Saxena 2007)
Terpenoid	*Murraya koenigii (L) Spreng. (Rutaceae)*	Surabhini-Nimba	Leaves	Coumarin, Carbazole alkaloids, glycoside	Antifungal, insecticides (Khare 2008; Shah et al. 2008)

(continued)

Table. 1.1 (continued)

Category	Botanical (family)	Ayurvedic name	Part used	Chemical constituents	Other biological activities
Flavonoids	*Couroupita guianensis Aubl. (Lecythidaceae)*	Nagalinga	Fruits, Flower	Steroids, flavonoids, phenolics	Antifungal (Pradhan et al. 2009)
Alkaloid	*Tinospora cordifolia Miers (Menispermaceae)*	Amrita, Guduchi	Entire herb	Alkaloids such as berberine, tinosporic acid	Hypoglycaemic agent, antipyretic (Kirti et al. 2004; Nadkami 2005)
Flavonoid	*Lagenaria siceraria Mol. (Cucurbitaceae)*	Katu-tumbi	Leaves, fruit	Cucurbitacin, beta-glucosidase	Purgative, emetic (Deshpande et al. 2008)
Triterpenoid and flavonoid	*Terminalia arjuna Roxb. (Combretaceae)*	Arjuna	Leaves, Bark	Flavonoids, oligomeric proanthocyanidins, tannins	Cardiotonic, diuretic, hypertensive (Halder et al. 2009)
Flavonoid	*Bauhinia variegate Linn. (Caesalpiniaceae)*	Kanchana	Roots, Bark, Buds	Flavonoids, Beta-sitosterol, lupeol	Antifungal, astringent (Ghaisas et al. 2009)
Flavonoid	*Urena lobata Linn. (Malvaceae)*	Nagabala	Roots, Flower	Flavonoids	Diuretic, emollient, antispasmodic (Rinku et al. 2009)
Saponin glycoside	*Gymnema sylvestre R. Br. (Asclepiadaceae)*	Gurmar	Leaves	Sapogenins	Antidiabetic, diuretic, antibilious (Malik et al. 2009)
	Cordia superba Cham and C. rufescens A. DC. (Boraginaceae)	Shleshmaataka	Leaves, Fruit, Bark	Alpha-amyrin	Anti-inflammatory, antimicrobial (Costa et al. 2008)
Glycoside	*Picrorhiza scrophulariiflora Benth. (Scrophulariaceae)*	Kutki	Roots	Iridoid glycosides, amphicoside	Antioxidant (Smit 2000)
Flavonoid	*Heracleum persicum Desf. (Apiaceae)*	Golpar	Shrub	Flavonoids, furanocoumarins	Antimicrobial (Sharififar et al. 2009)
Alkaloids	*Cissampelos pareira Linn. (Menispermaceae)*	Paatha	Roots	Hayatine alkaloids	Antipyretic, analgesic, antilithic (Bafna and Mishra 2010)

Flavonoids	*Abutilon indicum Linn. (Malvaceae)*	Atibalaa	Whole plant	Flavonoids	Diuretic, antibacterial (Dashputre and Naikwade 2010)
Saponins	*Chlorophytum borivilianum Sant. F (Liliaceae)*	Safed musli	Roots	Saponins	Antifungal (Thakur et al. 2007)
Flavonoids	*Altherananthera tenella Coibola (Amaranthaceae)*	Snowball	Herb	Flavonoids, triterpenes	Antitumour, anti-inflammatory (de Agostino Biella et al. 2008)
Carbohydrate	*Cistanche deserticola (Orobanchaceae)*	Cistanche	Herb	Polysaccharide	Immunomodulator, mitogenic, comitogenic (Habijanic et al. 2001)
Coumarin	*Cliona celata (Clionaidae)*	Boring sponge	Sponge	Clionamide, dehydrocoumarin	Antibacterial (Kannan et al. 2007)
	Cordyceps militaris L. (Clavicipitaceae)	Militaris	Fungus	Cordycepin, cordyceps acid	Anti-inflammatory (Lu et al. 2007)
Alkaloids	*Crinum latifotium Andr. (Amaryllidaceae)*	Milk and wine lily	Herb	Alkaloids	Immunomodulator (Ismail and Asad 2009)
Terpenoids	*Dracocephalum kotschyi (Lamiaceae)*	Dragon'shead	Herb	Essential oil	Immunomodulator (Mikhaeil et al. 2003)
Carbohydrates	*Echinacea angustifolia (Asteraceae)*	Cone flower	Flowers	Polysaccharide	Treatment for common cold, immunomodulator (Gaur et al. 2009)
Glycoside	*Eclipta alba L. (Compositae)*	Bringraja	Leaves	Triterpenoid glycoside	Anticancer, antileprotic, analgesic, antioxidant, antimyotoxic (Jain et al. 2005)
Tennin	*Euphorbia hirta Linn. (Euphorbiaceae)*	Asthma weed	Herb	Quercitol, myricitrin, gallic acid	Anti-inflammatory, sedative, anxiolytic activity (Satpute et al. 2009)
Alkaloid	*Evolvulus alsinoides Linn. (Convolvulaceae)*	Shankhpushpi	Herb	Alkaloids	Brain tonic (Jafarian et al. 2010)

(continued)

Table. 1.1 (continued)

Category	Botanical (family)	Ayurvedic name	Part used	Chemical constituents	Other biological activities
Phenols	*Haussknechtia elymatica (Apioideae)*	Haussknechtia	Herb	Phenolics	Immunomodulator (Tanaka et al. 1999)
Carbohydrates	*Inonotus obliquus Pers. (Hymenochaetaceae)*	Chaga mushroom	Mushroom	Polysaccharide	Antitumour (Jeong et al. 2006)
Lignin	*Larrea divaricata DC. (Zygophyllaceae)*	Creosote bush	Herb	Lignans	Anti-inflammatory (Hung et al. 2007)
Carbohydrates	*Lycium barbarum Linn. (Solanaceae)*		Fruit	Polysaccharide-protein complexes	Antioxidant (Zheng et al. 2006)
Proteins	*Matricaria chamomilla (Rhabdoviridae)*	Chamomile	Flowers	Protein	Immunomodulator (Cherng et al. 2008)
Glycoside	*Mollugo verticillata L. (Molluginaceae)*	Carpetweed	Herb	Quercetin, triterpenoid glycosides	Immunomodulator (Kobayashi and De Mejía 2005)
Saponin	*Moringa oleifera L. (Moringaceae)*	Sahijan	Leaves	Vitamin A, B, C, carotenoids, saponins	Antioxidant (Wang et al. 2010)
Terpenoid	*Pestalotiopsis leucothes (Amphisphaeriaceae)*		Fungus	Terpenes	Immunomodulator (Noori et al. 2004)
Alkaloid	*Piper longum L. (Piperaceae)*	Pipali	Fruits	Alkaloids	Antioxidants (Steinbach and Stevens 2003)
Phenol	*Rhodiola imbricate Gray. (Crassulaceae)*	Roseroot	Rhizomes	Phenolics	Immunostimulating properties (Ballarin 2008)
Flavonoids	*Silybum marianum L. (Asteraceae)*	Milk thistle	Flowers	Flavonoids	Antioxidant (Chang et al. 2007)
Carbohydrate	*Salicornia herbaceae (Chenopodiaceae)*	Glasswort	Herb	Polysaccharides	Immunomodulator (Mungantiwar et al. 1999)
Carbohydrate	*Viscum album L. (Loranthaceae)*	Mistletoe	Leaves, young twigs, berries	Viscotoxins, polyphenols, polysaccharides	Antitumoural (Sredni et al. 1990)

Carbohydrate	*Thuja accidentalis (Arborvitae)*	White cedar	Leaves	Polysaccharides	Immunomodulator (Bonacorsi et al. 2009)
Flavonoids	*Ganoderma lucidum (Fr.) P. karst. (Polyporaceae)*	Reishi mushroom	Whole plants	Flavonoids, triterpenes	Antioxidant (Chang et al. 2007)
	Nyctanthes arbor-tristis L. (Oleaceae)	Paarijaata	Leaf, Seeds	Iridoid glucosides	Anti-inflammatory, antispasmodic (Jiménez-Medina et al. 2006)
Alkaloid	*Actinidia macrosperma C. F. Liang (Actinidiaceae)*	Actinidia	Fruits	Alkaloids, saponins	Antileprotic (Bhatt et al. 2010)
Carbohydrate	*Acacia catechu Wild. (Leguminosae)*	Khadira	Leaf	Flavonoids, quercetin	Hypoglycaemic, astringent (Killestein et al. 2003)
Carbohydrate	*Boswellia spp. (Burseraceae)*	Shallaki	Gum resin	Triterpenes, Ursanes	Hypoglycaemic (Ordway et al. 2003)
Terpenoid	*Hibiscus rosa sinensis Linn. (Malvaceae)*	Japaa	Flowers	Cyclopropanoids	Antidiarrhoeal, anti-inflammatory (Mali and Hatapakki 2008)
	Cleome gynandra Linn. (Capperdiceae)	Tilaparni	Leaf, seeds	Hexacosanol, kaempferol	Anti-inflammatory (Ebringerova et al. 2002)
Terpenoids	*Hyptis suaveolens (L) Poit. (Lamaceae)*	Tumbaaka	Leaf, Flowers	Lupeol, Beta-sitosterol	Carminative, antispasmodic (Sugumaran and Robinson 2010)
Saponin	*Randia dumetorum Lamk. (Rubiaceae)*	Madana	Fruits	Saponins, triterpenes	Chlorosis, antiarthritic (Hsu et al. 2008)
Flavonoids	*Allium hirtifolium Boiss. (Alliaceae)*	Persian shallot	Herb	Thiosulphinates, flavonoids	Antirheumatic, anti-inflammatory (Zvetkova et al. 2001)
Flavonoids	*Citrus natsudaidai Hayata (Rutaceae)*	Japanese summer grapefruit	Fruits	Auraptene, flavonoids	Antioxidant (Amirghofran et al. 2000)
Carbohydrate	*Acanthopanax sessiliflorus (Rupr. & Maxim.) (Araliaceae)*	Prickly spine	Shoots, roots	Biopolymers	Lympho-proliferative activity (Senchina et al. 2005)

(continued)

Table. 1.1 (continued)

Category	Botanical (family)	Ayurvedic name	Part used	Chemical constituents	Other biological activities
Lipid	*Agelas mauritianus (Porifera)*	Agelas	Sponge	Glycolipid	Phagocytic activity (Jayathirtha and Mishra 2004)
Carbohydrate	*Aphanothece halophytica (Chroococcales)*		Cyanobacterium	Exopolysaccharide	Inhibits influenza virus (Patel et al. 2009)
Flavonoids	*Apium graveolens Linn. (Apiaceae)*	Celery seeds	Leaves, Seeds	Flavonoids, coumarins	Anti-inflammatory (Ganju et al. 2003)
Protein	Genus *Ardisia (Myrsinaceae)*	Marlberry	Shrub, Branches	Peptides, saponins, isocoumarins	Antimetastatic drug, anti-HIV property (Amir et al. 2007)
Tennin	Genus *Aristolochia (Aristolochiaceae)*	Pipevine	Leaves	Aristolochic acid	Antiangiogenic, employed in prostate cancer (Chen 2007)
Alkaloid	*Artemisia annua Linn. (Compositea)*	Wormwood	Herb	Artemisinin	Immunosupressive (Davicino et al. 2007)
Hydrocarbon	*Genus aspergillus (Trichonomaceae)*	Aspergillus	Fungus	Polyene, triazole	Antifungals (Gan et al. 2003)
Lipid	*Botryllus schlosseri*	Botryllus	Tunicates	Cytokines	Antioxidant, antiviral, antimicrobial, antitumoural (De Souza Reis et al. 2008)
	Bidens pilosa L. (Asteraceae)	Beggar-ticks	Flowers, Leaves	Polyacetylenes	Anti-inflammatory, immunosuppressive, Antimalarial, antibacterial (Ferreira et al. 2003)
Alkaloids	*Boerhaavia diffusa (Nyctaginaceae)*	Punarnava	Herb	Alkaloids	Immunostimulatory (Gupta et al. 2010)
	Bugula neritina L. (Bugulidae)	Brown bryozoans	Marine invertebrates	Macrocyclic lactones	Immunomodulator (Kumar et al. 2005)
Flavonoids	*Byrsonima crassa Nied. (Malpighiaceae)*	Byrsonima	Leaves	Flavonoids, terpenes, tannins	Antimicrobial, antioxidant (Sunila and Kuttan 2004)

Carbohydrates	*Calendula officinalis L. (Asteraceae)*	Garden marigold	Flowers	Polysaccharides, proteins, fatty acids, flavonoids, carotenoids, triterpenoids	Antitumour, antiviral, anti-HIV propertis (Mishra et al. 2008)
Tennin	*Camellia sinensis L. (Theaceae)*	Tea	Leaves	Epigallocatechin gallate, quercetin, gallic acid	Anticancer, Lipid lowering, Anticataract activity, Hepatoprotective, antioxidant (Meeran et al. 2006)
Alkaloid	*Cannabis sativa (Cannabaceae)*	Common hemp	Leaves	Cannabinoids	Immunomodulator (Im et al. 2006)
Alkaloid	*Carpobrotus edulis L. (Aizoaceae)*	Fig marigold	Flowers, fruit	Alkaloids	Immunomodulator (Elluru et al. 2007)
Glycoside	*Centella asiatica Linn. (Umbelliferae)*	Brahmi	Herb	Triterpenoid saponins	Immunomodulator (Gohla et al. 1992)

activity and antitumour activities (Thanh et al. 2004). The root of *Angelica dahurica* is used to assess cytotoxic coumarins (Jafarian et al. 2010).

1.2.1.2.4 Sapogenins

Sapogenins such as triterpenoid saponins and diterpenes modulate a broad spectrum of immunomodulatory activities. Some examples are *Gymnema sylvestre*, *Chlorophytum borivilianum*, *Boswellia* spp. and *Randia dumetorum*.

1.2.1.2.5 Alkaloids

These essential compounds, found in natural and synthetic forms, contain one or more nitrogen atoms. These alkaloids are found in heterocyclic form and have particular physiological effects on humans or animals. Some well-known alkaloids are *Achillea millefolium*, *Murraya koenigii*, *Cissampelos pareira* and *Actinidia macrosperma*.

1.2.1.2.6 Thiosulphinates

These compounds, such as *Allium hirtifolium*, have potent immunomodulatory as well as adaptogenic effects (Alamgir and Uddin 2010).

1.2.1.2.7 Volatile Oils and Terpenoids

Terpene is a hydrocarbon (C5H8) chain, while terpenoids are hydrocarbons with oxygenated derivatives. Terpenes and terpenoids are plant and animal origin volatile oils. Many plants show immunomodulatory activity with terpene moiety display, e.g. eugenol derived from *Ocimum sanctum*.

1.2.1.2.8 Polysaccharides

The regulation of innate susceptibility and, more particularly, the macrophage characteristic of polysaccharides has several therapeutic advantages. Both microbial and botanical polysaccharides bind to the surface receptors in macrophages that induce immunomodulatory responses. Both forms of organisms share these evolutionarily conserved polysaccharide structural features. The evaluation of botanical polysaccharides reveals beneficial immunomodulatory properties, providing a rare opportunity to discover novel therapeutic compounds—polysaccharides activate monocytic cells and induce monocytic cell differentiation into macrophages (Allison 2000; Ríos 2010).

1.2.2 Immunostimulant Synthetic Drugs

Immunostimulants are attractive substances that activate the invulnerable framework of animals to improve the natural resistance to many bacterial and viral infections. These biologically active substances are derived from natural sources or synthesised using various chemical properties and action mechanisms. In general, immunostimulants induce the amalgamation of specific antibodies and cytokines for the treatment of infectious diseases. These immunostimulants are divided into

two groups: first, specific immunostimulants that function as antigens for the stimulation of immune responses (e.g. vaccines) and, second, non-specific immunostimulants that have no antigenic properties yet enhance healthy responses to other antigens (e.g. adjuvants and non-specific immunostimulants).

The origin and mode of action of these immunostimulants are classified (Labh and Shakya 2014).

1.2.2.1 Functions of Immunostimulants

Immunostimulants activate various components of the insusceptible framework in animals and humans. They develop the non-specific immunotherapy and immunoprevention by stimulating the significant factors of the resistant framework including phagocytosis, properdin and complement frameworks protective secretory Iga antibodies, α- and γ-interferon release, T- and B-lymphocytes, combination of specific antibodies and cytokines, and blend of pulmonary surfactant (Petrunov et al. 2007). There are several reasons to use immunostimulants to treat various infectious diseases, including bacteria's antibiotic resistance, allergic reactions to antibiotics, immunosuppressive effects of antibiotics and poor effects of antibiotics in viral infections (Petrunov et al. 2007).

1.2.2.2 Types of Immunostimulants

Immunostimulants were divided into seven groups for better understanding: bacterial products, complex carbohydrates, vaccines (antigens and adjuvants), cytokines, immunoenhancing products, plant extracts and animal extracts. Few immunostimulatory drugs (endogenous immunostimulants or synthetic immunostimulants) have been developed to induce humoral or cellular fast responses against bacterial or viral infections, immune deficiency diseases and cancer. They were classified as follows:

1.2.2.2.1 Levamisole (Ergamisol)

Levamisole is a synthetic immunostimulant that stimulates B- and T-lymphocytes, as well as monocytes and macrophages. It was used in adjuvant therapy with 5-fluorouracil after surgical resection in patients with colon cancer. Allergy, nausea, influenza and muscle pain are some of the common disadvantages. Levamisole has been successfully used in combination with polymers to treat dermatological disorders. For example, it was combined with cimetidine to treat recalcitrant warts and with prednisolone to treat aphthous ulcers of the mouth (Patil et al. 2012; Biswajit et al. 2014).

1.2.2.2.2 Thalidomide

Thalidomide or immunoprin (C13H10N2O1) is an immunomodulatory drug. In patients with erythema nodosum leprosum, thalidomide can reduce circulating TNF-α. In HIV-positive patients, however, it increased TNF-α. Furthermore, it was able to overcome its therapeutic effects in severe rheumatoid arthritis and angiogenesis. Isoprinosine (Inosiplex/Imunovir): Isoprinosine (C52H78N10O17) is a combination of inosine, acetamidobenzoic acid and dimethylaminoisopropanol.

Isoprinosine could enhance the levels of cytokines, including IL-1, IL-2 and IFN-γ. In response to mitogenic or antigenic stimuli, it boosted lymphocyte proliferation. Isoprinosine also augmented active T-cells and induced T-cell surface markers on prothymocytes. It was utilised to treat Herpes simplex infections, Epstein-Barr and measles infections. Its disadvantages include a milder CNS depressant, temporary nausea and higher serum and urine uric acid levels (Patil et al. 2012).

1.2.2.3 Immunocynin

Immunocynin is a stable form of hemocyanin, a copper-containing protein, which is found in molluses and arthropods. It was utilised to treat urinary bladder cancer with reduced side effects, such as rare yellow fever (Patil et al. 2012).

1.2.2.3.1 Bestatin

Bestatin, a dipeptide [(2S, 3R)-3-amino-2-hydroxy-1-phenylbutanoyl]-L-leucine, is a low-toxicity immunostimulant that binds to lymphocytes and macrophages and enhances both humoral and cellular safe responses. It is an inhibitor of leucine aminopeptidase and aminopeptidase-B—bestatin possesses antitumour activity and also increases the antitumour activity of bleomycin and adriamycin. Bestatin was effective in preventing the metastasis of P388 leukaemia when the antibiotic was regularly injected after tumour inoculation (Tsuruo et al. 1981); the dipeptide was immunorestorator in the elderly and cancer patients and HIV-infected subjects. In vitro enhanced granulocytopoiesis and thrombocytopoiesis, which might restore them in myelohypoplastic men (Mathe 1991).

1.2.2.3.2 Bacterial Products

The effect of immunostimulatory are due to the release of cytokines from bacteria. Its immunostimulatory mechanism is caused by bacteria that (a) induce a granulomatous reaction at the site of administration and (b) prevent and treat carcinoma forms. This mechanism causes phagocytosis and resistance to infection through B- and T-cell-mediated responses. Some disadvantages are excessive touchiness, fever, shock and complex insusceptible disease (Patil et al. 2012).

1.2.2.3.3 Recombinant Cytokines

Many interferons and interleukins stimulate immune reactions. After stimulation with mitogens, interferons could be obtained from trout leucocytes. It could cause in vitro resistance to pancreatic necrosis infection in trout cells. Low doses of interferon could induce stable positive outcomes in mammals without causing side effects. On the other side, vaccination of animals with the recombinant IL-2 increased the protective effects against specific infections. In large dosages, IL-2, on the other hand, was a highly hazardous compound, causing symptoms, such as fever and diarrhoea. The cleaned cytokines produced unsatisfactory results in clinical trials because the resistant responses were produced by a blend of cytokines generated by the safe cells rather than against a single cytokine. In this way, non-specific cytokine amalgamation enhancers will develop safe responses and solve the problem (Galeotti 1998). Thus, recombinant cytokines are produced

recently in various expression frameworks (e.g. plants) and utilised in clinical trials, such as interferons, TNF-α and IL-2 (Sirko et al. 2011).

1.2.2.4 Complex Carbohydrates

Several types of complex carbohydrates were described as follows:

1.2.2.4.1 Glucans

The β-(1→3)-linked chain of glucose units is an essential class of immunostimulants.

There are β-(1→6)-branched glucose units in the main chain. The β-glucans were derived from unusually well-preserved structural components of cell walls in organisms, algae and yeast and have a wide range of molecular weights ranging from 5 to 200 kDa. Depending on the source, the length and frequency of these branches vary. B-glucan has been used to stimulate antitumour mechanisms (e.g. increased macrophage activity) and to improve host resistance to a variety of microbial pathogens in mammals. Glucan may also be beneficial in preventing aflatoxin's carcinogenic effects. The β-glucan was thought to be a stimulator of cell invulnerability. In fact, in the presence of glucans, mammalian macrophages or monocytes have specific receptors for glucans and their precursors, such as cytokines (e.g. IL-1, IL-9, TNF-α) and prostaglandins (Sahoo and Mukherjee 2001; Madrigal-Bujaidar et al. 2015). In Japan, β-glucans such as lentinan, derived from shiitake mushroom, and Polysaccharide-K, derived from *Coriolus versicolor*, were licensed as anticancer drugs. Lentinan may induce protective Th1 insusceptible responses to control the proliferation of malaria parasites in red blood cells by stimulating the maturation of Dcs; increasing the expression of MHCII, CD80/CD86 and Toll-like receptors (TLR2/TLR1) and the level of IL-12; and forestalling the adverse effects of Tregs. The primary roles of glucans have been discovered in the treatment of cancer, infection resistance, stress reduction and the restoration of damaged bone marrow. Zymosan, a combination of polysaccharides isolated from the cell walls of *Saccharomyces cerevisiae*, could potently stimulate macrophages and induce neutrophil cytokine release. In reality, β-glucan in zymosan was recognised as its active component for non-specific immunomodulation. Also, β-glucan may also be able to reverse myelosuppression generated by chemotherapeutic medicines by targeting the C3 fragment of complement and circulating antibodies. Recent studies have shown that daily therapy with soluble or insoluble β-glucan reduced tumour size by 70–95%. To be sure, after the coupling of antibodies on the surface of cancer cells, C3 fragments of complement could coat the cancer cells at that point, β-glucan-prepared cells, such as neutrophils, macrophages and NK cells, correctly recognised these complement-antibody complexes and executed the tumour cells. The cooperation of β-glucan with antitumour antibodies is a practical approach in combination treatment (Vetvicka 2011).

1.2.2.4.2 Trehalose

Trehalose dimycolate (TDM), muramyl dipeptide (MDP) and lipopolysaccharides (LPS) as bacterial products promote the production of antibodies, stimulate lymphocyte activation and elicit specific susceptibility to bacterial infections. Trehalose dimycolate, a glycolipid found in *Mycobacterium*'s cell wall, is a potent immunostimulant that inhibits tumour growth and improves resistance to bacterial, parasitic and viral infections. Because of their amphipathic properties, they can interact with membranes. TDM primes murine macrophages to produce nitric oxide (NO) and develop the antitumour activity. TDM, as an adjuvant, enhances both cellular and humoral invulnerability while eliciting a more robust cellular response. TDM could induce potent safe responses against malaria antigens in comparison to groups infected with malarial antigens and Freund's adjuvant. The results showed that in macrophage-drained mice injected with silica particles, the protective effect of TDM is reduced, indicating the role of macrophages.

T-lymphocytes were not required for TDM to activate peritoneal macrophages. Trehalose diesters could activate IL-12p10 and IFN-γ mRNA (Parant et al. 1978; Oswald et al. 1997).

1.2.2.4.3 Prebiotics

Prebiotics are inedible filaments that increase beneficial gut commensal bacteria, improving the health of the host. Prebiotics, such as fructooligosaccharide, mannan oligosaccharide, inulin or β-glucan, are known as monosaccharides. They significantly boost innate insusceptible reactions, such as phagocytic activation, neutrophil activation, alternative complement framework activation and increased lysozyme activity. Immunosaccharides interact with pattern recognition receptors (PRR) conveyed on innate invulnerable cells to directly activate the innate safe framework. In order to activate innate safe cells, they can also be linked to microbe-associated molecular patterns (MMPs). Probiotics activate the innate safe framework in two ways: (a) by directly stimulating the innate invulnerable framework and (b) by boosting the growth of commensal microbiota (Song et al. 2014).

1.2.2.5 Immunostimulants Used in Vaccines

Vaccines include a vast variety of immunostimulants; for example, an adjuvant heat-labile enterotoxin from *Escherichia coli* (LT), administered in the form of immunostimulant (LT-IS) patch on the skin, may improve insusceptible responses to influenza vaccination in the elderly. The invulnerable activation induced by LT-IS enhanced the potency of generating Alzheimer's disease (AD)-specific vaccination reactions as an adjuvant in the clinical trial (Davtyan et al. 2014). Co-administration of a potent adjuvant in IS patches containing heat-labile enterotoxin from *E. coli*. The anti-influenza antibody insusceptible response was significantly increased when *E. coli* was applied to the skin at the location of DNA vaccination (Mkrtichyan et al. 2008); adjuvants enhance and modulate resistant responses to antigens. This is important when the sanitised antigens do not elicit effective innate or adaptive resistant frameworks. Adjuvants are diverse in the sorts and levels of invulnerable responses. Expected advantages of adjuvants contain more robust resistant

preparing, effective invulnerable responses in low-response populations (e.g. the older or immunocompromised patients), the utilisation of smaller amounts of the antigen and safety profile (Garcon et al. 2011). New adjuvants have already applied to more efficient influenza vaccines, as well as vaccines targeting hepatitis B (HBV) and human papillomavirus (HPV) (Frech et al. 2005). On the other hand, CpG oligonucleotides and imiquimod drugs (an antiviral compound) could activate dendritic cells, induce in situ maturation and migration of Dcs and augment both humoral and cellular insusceptible responses (Frech et al. 2005). The unmethylated CpG motif in bacterial DNA was recognised as a B-cell stimulating adjuvant, and synthetic oligodeoxynucleotides (ODNs) containing the CpG motifs were shown to induce potent therapeutic activities in various infections and tumour animal models. Imiquimod was topically utilised for patients with anogenital warts as well as basal-cell carcinoma. The investigations indicated that CpG ODNs and imiquimod (resiquimod) drugs act as synthetic ligands for TLR9 and TLR7, respectively, and both stimulate Dc maturation efficiently (Frech et al. 2005).

1.2.3 Immunosuppressant

1.2.3.1 Synthetic Drugs: Manufactured Medications

Medications to smother human response against resistant have been used for couples of the decade. Such compounds were used for patient treatment undergoing organ transplantation or suffering from autoimmune diseases. The major stumble back of primitive immunosuppressive compounds was due to absence of specificity. Wide suppression of safe cell replication and cell function sometimes leads to extreme toxicities and related adverse symptoms. As the knowledge of invulnerable framework response for molecular and cellular level evolved, more current with specific compounds were developed which target particular component with its safe response. These modern immunosuppressive compounds do not have potential adverse effects with their efficacy and safety had significantly risen above to there predecessor compounds (Allison and Eugui 2005).

1.2.3.2 Immunosuppression for Organ Transplantation

Medications that suppress the human resistive response are widely used to prevent the rejection of transplanted organs (alloimmunity) and to treat autoimmune disease (autoimmunity). Solid-organ transplantation mostly involve the heart, liver, kidney and lungs (Libby and Pober 2001). The main objectives of transplant immunosuppression are:

- To forestall rejection of transplanted organs
- To limit sedate toxicity along with side effect
- To limit hazard of infection

In an ideal condition, these three goals could be fulfilled utilising most minor medications and the minimum possible dosage that could be effective in patients

along with graft survival. Transplantational tissue rejection occurs in three phases: hyper-acute, acute and chronic. Hyper-acute rejection includes a spontaneous (in practically no time) response from the recipient's an resistant framework against transplanted tissue and, which is expected due to measures taken by antibodies counter to donor H.L.A (human leukocyte antigen or A.B.O antigen-6 Eradication of transplanted tissues could be quick and broad. Precise matching between donor and recipient tissues can forestall this rejection type. Acute rejection type is most likely to incur inside initially 1–3 months post-transplant. Acute rejection is caused primarily due to host T-cells. When triggered by foreign antigens on the donor tissue, cytotoxic T-cells enter the organ and begin disintegration by releasing cytotoxic catalysts and proteins (e.g. perforins). Treatments that decrease T-cell activity work effectively for this type of acute rejection. In acute rejection, humoral-mediated rejection is crucial because host B-cells sharpen to donor tissue by producing antibodies against it. Antibodies directed against endothelial cells of the heart tissue can cause vasculature damage in acute rejection. This method of treating acute rejection is not particularly well known.

1.2.4 Inhibitor of Lymphocyte Gene Expression

Immunosuppression is used in transplant patients for various reasons, including preventing acute rejection in the days following the transplant. In order to do this, induction therapy is started during the transplantation surgery and lasts typically for 7–10 days. The infusion of a robust immunosuppressive antibody that blocks T-cell activation is typically used in induction treatment. Daclizumab and basiliximab, two of these drugs, are antibodies to the T-cell D25 (D 5 cluster of differentiation) receptor. Interleukin-2 has a high affinity for activating this T-cell receptor (IL-2). Even though activated T-cells can only transmit D25, these agents are very selective for T-cells already activated by MH. Daclizumab is a "humanised" antibody with 90% human components and is expected to be less antigenic than basiliximab, which has 75% human components. For both induction and acute rejection therapy, two polyclonal anti-thymocyte globulins are available. The first antibody, tgam, is generated from horses, while rabbits determine the second (Thymoglobulin). Both are linked to lymphocyte D receptors in a variety of ways. Once bound, antithymocyte globulins promote complement-mediated lysis of T-cells, resulting in their depletion. Both compounds are robust immunosuppressants, and transplanted patients might be exposed to a variety of infections because of their broad mechanism. Restriction of the globulins can cascade a chain of release for cytokines from T-cells, leading to "cytokine release syndrome", which causes headaches, fever, cold and vomiting in patients. The murine-inferred monoclonal muromonab (OKT3) is the third type of immunosuppressive antibody. This globulin binds to the CD3 cell surface receptor on T-cells, crucial in T-cell activation. Patients may produce donor organ trustworthiness and function since OKT3 is a murine protein. Cellular and humoral processes appear to be involved. Chronic inflammation of the donor tissue is a central feature of chronic rejection. T-cells release

cytokines when they are activated, which attract and activate macrophages. The donor tissue is then infiltrated by macrophages, which attack it with cytolytic compounds. Continuous antibody production by activated B-cells and the resulting activation of complement proteins may lead to chronic rejection. The retransplanting approach is the only option because there is no effective pharmacologic therapy for avoiding chronic rejection.

1.2.5 Antibodies Against Specific Immune Cell Molecules

Antibodies are Y-shaped proteins produced by the immune system in response to infection. For example, they help remove disease-causing bacteria from the body by crushing them or preventing them from contaminating cells. Antibodies function profoundly by perceiving and adhering to particular proteins, such as those present on the surfaces of pathogens and microorganisms, when the body encounters an organism simply because insusceptible cells develop antibodies that directly perceive proteins relevant to that specific microorganism. In the wake of recouping from a disease or getting an immunisation, few of these counteracting agents creating resistant cells, for the most part, stay in the body as memory cells, furnishing insusceptibility to future contaminations with a similar bug. Since memory cells and antibodies are now present, the body experiences a similar organism; the invulnerable reaction is quicker and can prevent the disease from grabbing hold. Antibodies that perceive the body's proteins rather than proteins from irresistible organisms can cause hurt. In immune system ailments, such as lupus, numerous sclerosis and rheumatoid joint pain, individuals produce antibodies that adhere to their body's proteins and assault solid cells.

Hypersensitivities include an exceptional class of antibodies called immunoglobulin E (IgE). When these antibodies recognise allergens, they cause invulnerable cells to release histamine and other irritating particles, resulting in severe side effects from unfavourably susceptible responses. Antibodies are mostly used in biomedical research because of their unique ability to recognise and cling to specific proteins, such as determining whether a given protein is present in a sample or where a specific protein is located within a cell.

1.2.5.1 Polyclonal Antibodies Antithymocyte Globulin (ATG)

Antithymocyte globulin is a pure type of gamma-globulin derived from rabbit serum immunised against human thymocytes (Sharma and Sharma 2007).

1.2.5.1.1 Mechanism of Action

Antithymocyte globulins have cytotoxic antibodies that will bind to CD2, CD3, CD4, CD8, CD11a, CD18, CD25, CD44 and CD45, as well as HLA class I and II in the surface of human T-lymphocyte cells. The antibody drains circulating lymphocyte by direct cytotoxicity of complement and cell-mediated, which in turn block lymphocyte function by binding to the cell surface molecule engaged in cell activity regulation (Katzung 2012).

1.2.5.1.2 Therapeutic Uses

One of the major uses is in severe renal transplantational rejection.

1.2.5.1.3 Adverse Effects

Rigors, hypotension, serum sickness, glomerulonephritis, leucopenia with thrombocytopenia and increased risk of infection with malignancy are some of the main effects when several immunosuppressive compounds are used together (Golan et al. 2011).

1.2.5.2 Monoclonal Antibodies: Muromunab (Anti-CD3 Antibodies, OKT3)

An antibody targeting CD3, a trimeric structural molecule adjacent to the T-cell receptor in the surface of human T-lymphocytes, has been used in human transplantation with remarkable success since the early 1980s (Sharma and Sharma 2007).

1.2.5.2.1 Mechanism of Action

Muromonab-CD3 binds to the CD3 chain, a monomorphic component of the T-cell receptor complex involved in antigen recognition, cell signalling and preference.

The use of antibodies induces a rapid internalisation of T-cell receptors, which prevents antigen recognition. The antibody is administered by depleting and extracting full T-cells from peripheral lymph organs (Carlos and Harlan 1994). The lack of traceable T-cells from individual lymphoid organs leads to secondary T-cell death, characterised by implementation activation, activation-induced T-cell passing and the marginalisation of T-cells in the vascular endothelial wall as well as redistribution of T-cells to the non-lymphoid organs. Muromonab-CD3 reduces T-cell function, as explained by a lack of interleukin-2 production coupled with massively reduced production of several cytokines, except interleukin-4 and interleukin-10 (Katzung 2012).

1.2.5.2.2 Therapeutic Uses

Cases of severe organ transplant rejection.

1.2.5.2.3 Adverse Effects

High fever, cold with headache, tremor, nausea, diarrhoea, abdominal pain, malaise, myalgias and arthralgias along with generalized weakness; minor effects such as skin allergy, cardiorespiratory issues and central nervous system disorders including aseptic meningitis; and potential fatal severe pulmonary oedema and acute respiratory distress syndrome (Tortora Gerard and Derrickson Bryan 2008).

1.2.6 Inhibitors of Immune Cell Adhesion

Cell-cell and cell-lattice attachments are known to assume critical jobs in the enlistment and enactment of insusceptible T-cells. Noticeable among the subatomic attachment parts are integrins (β1 and β2) that intercede the collaborations of an

assortment of insusceptible cells to extracellular frameworks and other resistant cells, separately (Adutler-Lieber et al. 2014).

Adhesion molecules can be classified into four major groups: integrins, selectins, cadherins and immunoglobulin superfamily (IgSF), including nectins and mucins (Samanta and Almo 2015). Along with the conventional adhesion molecules, the specific enzyme vascular adhesion protein 1 (VAP-1) plays an important role in cell adhesion (Jalkanen et al. 2007). Compounds that block leucocyte adhesion, transmigration with expression of related CAMs present therapeutic model as immunosuppressive and anti-inflammatory drugs (Hynes 1992).

For the most part, a cell adhesion inhibitor are classified as target site for cell-cell adhesion with expression of cell adhesion molecules (Jia et al. 2015), though certain small molecules such as flavonoids (Kobuchi et al. 1999) and others (Mun et al. 2011). When the affecting experience of a cell adhesion molecule is known, specific inhibitors for cell-cell contact is limited (Jin et al. 2010).

1.2.6.1 Efalizumab

Efalizumab (lymphocyte function-associated antigen-1 inhibitor) is a humanised gG1 mAb that targets the CD11, a chain of the LFA-1 (lymphocyte function-associated antigen).

1.2.6.1.1 Mechanism of Action

Efalizumab attached to lymphocyte function-associated antigen-1 and blocked the lymphocyte function-associated antigen-1-ICAM (intercellular adhesion molecule) interaction to avoid T-cell adhesion, trafficking and onset.

1.2.6.1.2 Pharmacokinetics

Efalizumab offers a certain saturation with 80 per cent modulation of CD11 within a time frame of 24 hours of administration, according to pharmacokinetic and pharmacodynamic studies.

1.2.6.1.3 Therapeutic Uses

Survival of murine skin, heart allografts and psoriasis along with renal transplantation (Sharma and Sharma 2007).

1.2.7 Tolerogens or Inhibitors of Immune Cells

A tolerogen is a foreign antigen that suppresses the immune response or induces immunological tolerance, unlike an immunogen that stimulates an immune response. Instead of inducing the immune system to be active, the tolerogen binds to the lymphocytes' antigen receptor to suppress it.

1.2.8 Inhibitors of Lymphocyte Gene Expression to Reduce Inflammatory Response

1.2.8.1 Mechanism of Action

Cell-cell and cell-cross-section connections are known to expect principal employment to select and establish immune T-cells. Observable among the subnuclear connection parts are integrins (β1 and β2) that intervene in the joint efforts of various invulnerable cells to extracellular systems and other safe cells independently (Adutler-Lieber et al. 2014).

Along with the conventional adhesion molecule, the enzyme ex-vascular adhesion protein 1 (VAP-1) plays a vital role in cell adhesion (Jalkanen et al. 2007). The compound that inhibits leucocyte adhesion, transmigration and expression of associated CAM presents in a therapeutic model for immunosuppressive and anti-inflammatory drugs (Hynes 1992).

Cell adhesion inhibitor will be classified for target for cell-cell adhesion along with expression of cell adhesion molecules (Jia et al. 2015). With impact on expression of cell adhesion molecules is known, specific inhibitors for cell-cell contact are very little (Jin et al. 2010).

1.2.8.2 Therapeutic Uses

Transplant rejection, graft-versus-host disease in bone marrow transplantation, rheumatoid arthritis, SLE and various conditions of skin, asthma, allergic disorders, inflammatory bowel and ophthalmic diseases (Sharma and Sharma 2007).

1.2.8.3 Adverse Effects

Some major effects are growth retardation in minor, avascular bone necrosis, osteopenia, cataract, hyperglycaemia and hypertension.

1.2.9 Inhibitors of Lymphocyte Signalling to Prevent Immune Cell Activation and Proliferation: Calcineurin Inhibitors

1.2.9.1 Cyclosporine

Cyclosporine (cyclosporin A) is a cyclic polypeptide chain with a total of 11 AA produced by the fungal species *Beauvera nivea*. Cyclosporine overpowers T-cell subordinate immune system pathways as transplant rejection and pathway for autoimmunity. It also prevents T-lymphocytes from receiving antigen-triggered signals, effectively reducing the expression of numerous lymphokines, interleukin-2 and anti-apoptotic proteins. Cyclosporine binds to make a complex with cyclophilin, a specific type of cytoplasmic receptor present in target T-cells. This complex binds to calcineurin, inhibiting Ca2+-stimulated dephosphorylation of the cytoplasmic component of the nuclear factor of activated T-cells (NFAT). When NFAT is dephosphorylated and translated, it attaches to the nuclear component required for complete T-cell activation, including the activation of the L-2 and other lymphokine genes. After physical interaction with the cyclosporine/

cyclophilin complex, calcineurin phosphatase activity is stopped. This inhibits NFAT dephosphorylation, resulting in NFAT not entering the nucleus transcription activated and the T-lymphocyte failing to respond to antigenic stimulation.

1.2.9.1.1 Pharmacokinetics

Cyclosporine administered orally or IV. Oral bioavailability is less around 30%. Food stops its absorption. It is metabolised by CYP3A, resulting in drug-to-drug interaction. Inactive metabolite is ejected primarily through the bile and faeces but minimally in urine (Chaudhuri 1997).

1.2.9.1.2 Therapeutic Uses

Organ transplantation, rheumatoid arthritis, psoriasis, early engraftment, extending kidney graft survival and cardiac and liver transplantation (Sengupta 2009).

1.2.9.1.3 Adverse Effects

Renal dysfunction, tremor, hirsutism, hypertension, hyperlipidaemia, gum hyperplasia, hyperuricaemia, hypercholesterolaemia, nephrotoxicity, diabetogenic and increase in LDL cholesterol.

1.2.9.2 Tacrolimus

Tacrolimus (PROGRAF, FK506) is a macrolide antibiotic synthesised by *Streptomyces tsukubaensis*.

1.2.9.2.1 Mechanism of Action

T-cell activation is inhibited via blocking calcineurin. Tacrolimus binds to an intracellular protein FK506-binding protein-12 (FKBP-12), an immunophilin, which is structurally linked to cyclophilin. A complex of tacrolimus-FKBP-12, Ca2+, calmodulin, calcineurin forms and calcineurin phosphatase activity is stopped. As described for cyclosporine, the inhibition of phosphatase activity inhibits dephosphorylation and nuclear translocation of NFAT and stops T-cell activation.

1.2.9.2.2 Pharmacokinetics

Tacrolimus can be administered orally or IV; the liver metabolises 99% by CYP3A and has plasma half-life of 7–8 h (Singhal 2007).

1.2.9.2.3 Therapeutic Uses

Prophylaxis of solid-organ allograft rejection, kidney transplantation and paediatric liver transplantation.

1.2.9.2.4 Adverse Effects

Nephrotoxicity, GI complaints, diabetes, neurotoxicity, hypertension, hyperkalaemia and hyperglycaemia.

1.2.10 Mammalian Target of Rapamycin (mTOR) Inhibitors: Sirolimus

1.2.10.1 Mechanism of Action

Sirolimus blocks T-lymphocyte activation and proliferation downstream of the interleukin-2 and other T-cell growth factor receptors. Sirolimus formation of a complex with immunophilin FKBP-12, but the sirolimus-FKBP-12 complex does not affect calcineurin activity. It binds and blocks protein-kinase targeted mammalian target of rapamycin (mTOR), which is a key protein in cell-cycle progression. Regulation of mTOR stops cell-cycle progression at the G1- to S-phase transition (Goodman 1996).

1.2.10.2 Pharmacokinetics

Oral bioavailability 15% Protein binding 40–45% is against albumin, where it is metabolised by the liver with the help of CYP3A4. Sirolimus excreted 91% through faeces and 2.5% through urine with a plasma half-life of 62 h.

1.2.10.3 Therapeutic Uses

Organ transplant inhibitor is incorporated into stents to inhibit local cell proliferation and blood vessel occlusion.

1.2.10.4 Adverse Effects

Increased level in serum cholesterol, triglycerides, impaired renal function, prolong postponed unite function, lymphocele and anaemia with leucopenia.

1.2.11 Cytotoxic Agents to Reduce Lymphocyte Proliferations

1.2.11.1 Antimetabolites: Azathioprine

Azathioprine (Imuran) is a purine antimetabolite and imidazolyl derivative of 6-mercaptopurine.

1.2.11.1.1 Mechanism of Action

With the exposure to nucleophiles is cleaved to 6-mercaptopurine, which result in conversion to extra metabolites which prohibit de novo purine synthesis. 6-Thio-IMP is changed into 6-thio-GTP, which is incorporated into DNA. Cellular proliferation leads to dysfunction a type of lymphocyte function.

1.2.11.1.2 Therapeutic Uses

Allergenic kidney transplantation and organ transplant rejection.

1.2.11.1.3 Adverse Effects

Bone marrow suppression, leucopenia, thrombocytopenia (not common) and/or anaemia (not common) along with increased susceptibility to infections, hepatotoxicity, alopecia, nausea, vomiting, abdominal pain, mucositis and pancreatitis.

1.2.11.2 Mycophenolate Mofetil

Mycophenolate mofetil (CellCept) is a 2-morpholinoethyl ester of mycophenolic acid (MPA).

1.2.11.2.1 Mechanism of Action

Mycophenolate mofetil is pro-medicate which is hydrolysed into active tranquilise, i.e. mycophenolic acid (MPA) that is a selective, non-competitive and reversible inhibitor of inosine monophosphate dehydrogenase (IMPDH), which is an integral part in the de novo pathway of guanine nucleotide synthesis. B- and T-lymphocytes are dependent on this pathway, while other cell types use salvage pathway for cell proliferation; MPA therefore selectively inhibits lymphocyte proliferation along with some vital function, i.e. antibody formation and cellular adhesion along with migration (Rang 2007).

1.2.11.2.2 Pharmacokinetics

Mycophenolate mofetil undergoes rapid complete metabolism to MPA after oral/intravenous administration of MPA, which results in metabolising to an inactive phenolic glucuronide MPAG. Out of which 87% is excreted through urine as MPA.

1.2.11.2.3 Therapeutic Uses

Prophylaxis of transplant rejection and renal transplant.

1.2.11.2.4 Adverse Effects

Various adverse effects can be seen, such as leucopenia, diarrhoea, vomiting and sepsis associated with cytomegalovirus (Finkel et al. 2009).

1.2.12 Alkylating Agents

1.2.12.1 Cyclophosphamide

Cyclophosphamide is a unique immunosuppressant, and its function is to enhance T-cell responses despite suppressing B-lymphocyte proliferation.

1.2.12.1.1 Mechanism of Action

Alkylating agents add alkyl groups by forming covalent bonds with nucleophilic moieties ex-phosphate, sulfhydryl, hydroxyl, carboxyl, amino and imidazole groups occuring in DNA/RNA. By forming cross-links between the strands of DNA, they prevent the method of cell division as well as protein synthesis. These drugs are lethal to rapidly reproducing tissues and inducing cell death until they are exposed to the division method. The abovementioned drug's cytotoxicity deals with a degree of DNA alkylation (Goodman 1996).

1.2.12.1.2 Therapeutic Uses

Autoimmune disorder in patients having acquired factor-XIII antibody, bleeding syndromes, antibody-induced unadulterated red cell aplasia and Wegener's granulomatosis.

1.2.12.1.3 Adverse Effects

Pancytopenia, haemorrhagic cystitis, unite-versus-host disease syndrome, nausea, vomiting, cardiac toxicity and electrolyte disturbances (Mythili et al. 2004).

1.2.13 Cytokine Inhibitors (Anticytokine Antibodies)

Tumour necrosis factor-α and IL-1 are pro-inflammatory cytokines found in the pathogenesis of rheumatoid arthritis and Crohn's disease. Activated T-lymphocytes link to the IL-2, swhich promotes its proliferation (Hilmer and Ford 2009).

1.2.13.1 TNF-α Inhibitors

Activated cytotoxic TH1 cells secrete tumour necrosis factor-α that to tumour necrosis factor receptors (TNFR1 or TNFR2) are present in fibroblasts, neutrophils and vascular endothelial cells except for these; there are soluble forms of tumour necrosis factor-α receptor present in serum and synovial fluid. Release of cystine L-1, L-6 and adhesion molecules caused by tumour necrosis factor activation, which promotes leucocyte activation and trafficking (Golan et al. 2011).

1.2.13.2 Etanercept

It's a genetically modified fusion protein made up of two soluble tumour necrosis factor p75 receptors connected to the Fc portion of human-IgG1. The medication works on external administered soluble tumour necrosis factor-α receptor, which provides artificial binding sites to tumour necrosis factor-α, leading to inhibition of tumour necrosis factor-α from attaching to the film-bound TNFR-1 and TNFR-2. The medication is mainly used for treatment of rheumatoid arthritis along with psoriatic arthritis (Saif 2005).

1.2.13.3 Infliximab

It's chimeric monoclonal antibody produced of exposure from mice to human tumour necrosis factor-α. The resultant antibody is fused to constant region IgG-1, which lowers the drug's antigenicity. The medication cross links with film bounded tumour necrosis factor-α receptor on cell surface to block T-cell and macrophage function forcing to stop the release of other pro-inflammatory cytokines. It has longer half-life and does not bind tumour necrosis factor-β. Infliximab is used to treat Crohn's disease and rheumatoid arthritis (Rang 2007).

1.2.13.4 Adalimumab

It is a human recombinant monoclonal antibody to tumour necrosis factor-α, which is significantly less antigenic than infliximab since it lacks the foreign component. Its serum half-life is 2 weeks.

1.2.14 Miscellaneous: Immunostimulants

Indifferent to immunosuppressive agent that blocks the rejection of immune response and autoimmunity, different immunostimulatory drugs are designed with different functionality to infection, immunodeficiency and cancer. They work on both cellular and humoral immune system.

1.2.14.1 Bacillus Calmette-Guerin (BCG)

Live bacillus Calmette-Guerin (BCG: TICE BCG, TheraCys) is made from live culture from the bacillus of Calmette and Guerin strain of *Mycobacterium bovis.*

1.2.14.1.1 Mechanism of Action

At the site of granulomatous reaction.

1.2.14.1.2 Therapeutic Uses

Prophylaxis along with treatment for urinary bladder carcinoma and T1 papillary after transurethral resection.

1.2.14.1.3 Adverse Effects

Shock, hypersensitivity, chills and fever (Goodman 1996).

1.2.14.2 Levamisole

Levamisole (Ergamisol) is manufactured as an anthelmintic yet promises to repair weak immune response.

1.2.14.2.1 Therapeutic Uses

Adjuvant treatment with the help of 5-fluorouracil after surgical resection in patients suffering from Duke's stage C colon cancer and agranulocytosis.

1.2.14.2.2 Adverse Effects

Symptoms related to influenza, nausea, allergic reactions and body ache.

1.2.14.3 Thalidomide

1.2.14.3.1 Mechanism of Action

Thalidomide is proven to lower circulation of tumour necrosis factor-α of patients dealing with erythema nodosum leprosum yet to elevate it in subjects who are HIV seropositive. Indifference to it had been suggested that the medications result in angiogenesis.

1.2.14.3.2 Therapeutic Uses

In rheumatoid arthritis (Heidari 2011).

1.2.14.3.3 Adverse Effects

One of the effects is teratogenicity.

1.2.15 Recombinant Cytokines

Therapeutic uses of recombinant cytokines and their effects (Parnham and Nijkamp 2005; Sharma and Sharma 2007; Katzung 2012):

- Interferons: e.g. alpha, beta and gamma interferons work by induction of various enzymes along with inhibition of cell proliferation, increased phagocytosis by macrophages and augmentation of specific cytotoxicity. They are used in hairy cell leukaemia, malignant melanoma, follicular lymphoma, Kaposi's sarcoma and chronic hepatitis B. They have certain adverse effects such as hypotension, arrhythmias, myocardial infarction, gastrointestinal distress, loss of apetite and weight loss.
- Interleukins: e.g. aldesleukin and des-alanyl-1, serine-125 human IL-2. Cellular immunity is profoundly activated via lymphocytosis, eosinophilia and thrombocytopenia with the release of several cytokines. It has many adverse effects such as capillary leak syndrome, hypotension, reduced organ perfusion and death.

Colony stimulating factors: e.g. filgrastim works by increasing the number and differentiation of myeloid progenitors. It is used in leucopenia and ganciclovir-induced neutropenia. It has many adverse effects such as myocardial infarction and anorexia.

1.2.15.1 Isoprinosine

Isoprinosine aka inosiplex is the complex of the pacetamido-benzoate salt of N, N-dimethylamino-2-propanol and inosine in a molar ratio of 3:1.

1.2.15.1.1 Mechanism of Action

Isoprinosine promises to enlarge production of cytokines as IL-1, IL-2 and IFN-γ. It also increases proliferation of lymphocytes in response to mitogenic or antigenic stimuli, increases active T-cell rosettes and induces T-cell surface markers on prothymocytes.

1.2.15.1.2 Therapeutic Uses

Herpes infections, subacute sclerosing panencephalitis, Epstein-Barr and measles viruses.

1.2.15.1.3 Adverse Effects

Minor CNS depressant and transient nausea along with rise of uric acid in serum and urine (Parnham 2005).

1.2.15.2 Immunocynin

It is a balanced form of haemocynin, which is a non-heme oxygen carrying along copper-containing protein present in arthropods and molluses.

1.2.15.3 Therapeutic Uses

Used to treat some form of urinary bladder cancer.

1.2.15.4 Adverse Effects

Uncommon mild fever. The main three classes of drugs currently utilised for maintenance therapy are antimetabolites, lymphocyte signalling inhibitors and corticosteroids, which are all examples of antimetabolites. Older compounds such as azathioprine and methotrexate as well as more recent compounds such as mycophenolate mofetil and leflunomide are all examples of antimetabolite immunosuppressants. Tamper with critical metabolic pathways in a variety of safe cells, which can stifle their proliferation and induce apoptosis. Azathioprine was the first compound of its kind to be used for immunosuppression in relation to organ transplants. It is a mercaptopurine prodrug and a tranquiliser that interferes with purine nucleic acid metabolism and, as a result, lymphoid cell replication. One major disadvantage of using older drugs such as azathioprine is their lack of specificity and potential for suppressing replication in other highly proliferative tissues, including the bone marrow and stomach. When azathioprine is used in conjunction with allopurinol, increase in drug blood levels is observed (Brooks et al. 1982).

These are the immunosuppressive drugs used for solid organ transplant (Rifle et al. 2005):

- Glucocorticoids inhibit inflammatory gene transcription and induce lipocortins. They are used in maintenance therapy and treatment of acute rejection. They cause hyperglycaemia, osteoporosis, hypercortisolism, growth impairment and impaired wound healing.
- Cyclosporine inhibits IL-2 expression and lymphocyte activation. It is used in maintenance therapy. It causes nephrotoxicity, neurotoxicity, hypertension, hirsutism and gingivital hyperplasia.
- Tacrolimus inhibits IL-2 expression and lymphocyte activation. It is used in maintenance therapy. It causes nephrotoxicity, hypertension, hyperglycaemia, gastrointestinal disturbances and myelosuppression.
- Sirolimus suppresses IL-2 signalling as well as lymphocyte activation. It is used in maintenance therapy and treatment of acute rejection. It causes hypertension, peripheral oedema, hyperlipidaemia and myelosuppression.
- Mycophenolate mofetil inhibits lymphocyte guanosine synthesis. It is used in maintenance therapy and causes hypertension, gastrointestinal disturbances and myelosuppression.

- Azathioprine inhibits purine nucleic acid metabolism. It is used in maintenance therapy. It causes gastrointestinal disturbances and myelosuppression.
- Monoclonal antibodies (e.g. muronomab) inhibit purine nucleic acid metabolism. They are used in maintenance therapy. They cause cytokine release syndrome, pulmonary oedema and hypersensitivity.

These are the immunosuppressive drugs used to treat autoimmune diseases (Libby and Pober 2001):

- Methotrexate inhibits lymphocyte and folate metabolism. It is used in inflammatory bowel disease. It causes nausea, diarrhoea and alopecia.
- Leflunomide is an inhibitor of lymphocyte and pyrimidine synthesis. It is used in rheumatoid arthritis. It causes hepatotoxicity, renal impairment, teratogenic and gastrointestinal disturbances.
- Etanercept, infliximab and adalimumab are TNF-a inhibitors. They are used in rheumatoid arthritis, psoriasis and inflammatory bowel disease. They cause infection and myelosuppression.
- Glucocorticoids inhibit inflammatory gene transcription and induce lipocortins. They are used in rheumatoid arthritis and inflammatory bowel disease. They cause hyperglycaemia, osteoporosis, hypercortisolism, growth impairment and impaired wound healing.

1.3 Conclusion

Immunology, it was reasoned, is presumably the most rapidly developing sector of clinical biotechnology. It's a great way to prevent and cure a variety of problems, including inflammatory skin, gut, respiratory system, joints and specific organ disorders. Immunomodulators would be a major part of medicine in the twenty-first century. Helping the system help itself by enhancing the insusceptible framework is of focal significance in the general public so pushed, horribly supported and presented to poisons that a large portion of us are probably going to have undermined invulnerable frameworks. Immunomodulation, on the other hand may, is a normalising method that corrects feeble invulnerable frameworks and temper insusceptible frameworks that are overactive, yet it does not help the safe framework. Immunomodulators are becoming a viable addition to established modalities, providing a unique methodology for treating incurable diseases in the next decades of the twenty-first century.

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Potential Role of Herbs and Spices on the Immune System

2

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Abstract

The natural body's defence system plays a critical role to keep away the person from infections and minimize the risk of falling sick regularly. The smart way to improve immunity is changing the lifestyle by consuming food that has immunomodulatory activity. Spices/herbs have been used as preservatives as well as traditional medicines since ancient times due to their disease prevention capability. Numerous preclinical and clinical trials over few spans revealed the benefits of spices/herbs and their bioactive compounds in the control and prevention of several complications such as arthritis, respiratory diseases, cancer, cardiovascular disease, glucose impairment, and brain disorders. This chapter deals with the active compounds of spices and herbs and their potential health benefits in the management of the immune system. Overall, extensive research is required to support claims on the immune-boosting effects of foods.

Keywords

Immunomodulator · Phytochemicals · Biomolecules · Immunoregulator · Autoimmune diseases · Detoxification

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2.1 Introduction

In recent days, plants have been studied for their immunomodulating action against various infectious diseases. The term "herbal medicine" or "phytotherapy" refers to the therapeutical application of the plants or plant-derived compounds for treating infectious diseases. These agents include either the whole plants or parts of the plants including fruits, stem, leaves, bark, seeds, etc. (Khanal et al. 2020a; Prajapati and Kumar 2020). The World Health Organization (WHO) stated that about three-quarters of the globe relies on herbal medicine for their health care (Kumar et al. 2012). The natural body defence system plays a critical role to keep away the person from infections and minimize the risk of falling sick regularly. The simple method that can be adopted to improve immunity is by consuming a balanced diet. Ayurvedic (India), Greco-Arab, or Unani-Tibb (South Asia), Egyptian, Chinese, and Kampo (Japan) are the natural or traditional medicine that has been commonly practiced for a long time to improve the health status in different regions. Spices/herbs are typically characterized as aromatic plant parts, including seeds, roots, pods, leaves, and bark, which not only provide versatility in the human diet but also contribute to hedonic response (Shantilal et al. 2018).

Spices and herbs contain a considerable amount of phytochemicals such as phenylpropanoids, isothiocyanates, terpenes, sulfur, and diarylheptanoids compounds, which might be helpful to protect the human body against various diseases including parasitic diseases, viral infections, dermatological disorders, inflammation, etc. (Dhama et al. 2016; Hannen 2018; Anywar et al. 2020). Based on the phytochemicals' existence, extensive research has been conducted to explore the immunomodulatory response of species/herbs against numerous cancer cell lines including pancreas, colon, breast, and lung. Namrata Singh and colleagues have claimed that Indian medicinal plant extracts with proper dosage stimulate the immune response against bacterial, viral, and other diseases. The major Indian medicinal spices and herbs with immunostimulant properties include *Withania somnifera*, *Morus alba Linn*, *Sophora subprosrate*, *Acacia catechu*, *Jatropha curcas L.*, *Achillea wilhelmsii*, *Picrorhiza scrophulariiflora*, *Plantago asiatica L.*, *Panax ginseng*, *Allium sativum*, *Cynodon dactylon*, *Schisandra arisanensis*, *Rhus toxicodendron*, *Pteridium aquilinum*, *Actinidia erantha Benth*, *Boerhaavia diffusa*, *Dioscorea japonica*, *Andrographis paniculata*, *Curcuma longa*, and *Tinospora cordifolia* (Singh et al. 2015, Singh et al. 2016). This chapter reviews the immunomodulatory effects of some traditional spices and herbs mostly used in India.

2.2 Immunity and the Immune System

The immune system in humans is an extremely complex linkage of specialized cells, which through modulating, moderating, and engulfing malignant and foreign cells prevent infections and diseases. Besides, the bone marrow and lymph nodes correspondingly contribute to the immune system by storing and generating different antigens that shape an immune cell. Depending on the function, the immune system

was divided into two different groups, such as the adaptive immune system (specific or acquired immune system) and the innate immune system (nonspecific immune system) (Vesely et al. 2011). T cells and B cells are the major types of immune cells of which B cells can transform into plasmocytes and are responsible for producing antibodies (Abs), whereas T cells destroy the antigens through cell immunity. In the adaptive immune system, T cells play a critical role and thus develop and induce tolerance against antigens and cause an appropriate immune response. Phagocytes (macrophage, granulocytes, and natural killer cells) release interferon bodies, which ultimately brought out immunoregulatory function during infection. Cytokines such as monokines and lymphokines are chemical mediators that attract the neutrophils through chemotaxis and thereby regulate immune reactions (Jantan et al. 2015). Some natural or synthetic compounds modulate the immune system positively or negatively and are categorized as "suppressors" or "stimulants" or "adjuvants" are recognized as "immunomodulators." Considering the way they influence the immune system, immunomodulators can modify different cellular functions such as antigen presentation, protein synthesis, apoptosis, inducing transcription of genes, and thereby enhances immune response. Various studies conducted in different experimental models suggested that phytochemicals in spices and herbs have been shown to modulate this signalling, which resulted in improving the immune system.

2.3 Spices/Herbs and Their Active Components

Spices/herbs added to foodstuffs not only impart flavour and taste but also provide enormous nutritional advantages (Opara and Chohan 2014). Numerous findings from research studies on human subjects over the last few decades reported the beneficial effects of spices/herbs and their key phytochemicals components in the prevention and control of various illness, including asthma, arthritis, cardiovascular disease, cancer, diabetes, and neurodegenerative diseases (Opara and Chohan 2014). Commonly used culinary spices that exhibit beneficial biological activity include black pepper, cardamom, cloves, fennel, turmeric, garlic, ginger, onion, cinnamon, rosemary, cumin, thyme, etc. Turmeric (*Curcuma longa*) is a widely used spice in the world for cooking. Curcumin, a yellow-coloured phytochemical of the turmeric (2%–5%) produced from the rootstalk, gives the turmeric a golden colour and was first extracted from turmeric by Vogel in 1842 (Gupta et al. 2013). It has a wide range of health benefits, namely antimicrobial, anti-mutagenic, anti-inflammatory, insecticidal, and anti-cancer activities. In addition to curcumin, turmeric also contains other phytochemicals including bisdemethoxycurcumin, triterpenoids, demethoxycurcumin, diterpenes, and sesquiterpenes (Aggarwal and Kunnumakkara 2009; Gupta et al. 2013). Ginger (*Zingiber officinale*), another widely used spice in Asian countries, has been documented for its various biological activities such as antioxidant, antiproliferative, and anti-inflammatory activities. 6-gingerol is the chief phytochemical of this spice that has many biological activities (Surh 1999). In addition to 6-gingerol, ginger also contains zingiberene, bisabolene, 6-gingerdiol,

cineol, β-phellandrene, 6-paradol, shogoal, gingerdione, zingerone, α-farnesene, etc. (Jolad et al. 2005).

One more frequently used spice is the black pepper (*Piper nigrum*), which is well established for its properties like anti-inflammatory, antioxidant, anti-carcinogenic, anti-ulcer, anti-asthmatic, and immunomodulatory activities (Meghwal and Goswami 2013). Piperine is the principal component in black pepper. In addition to piperine, black pepper also contains α-pinene, terpinolene, limonene, β-pinene, β-caryophyllene, myrcene, α-phellandrene, etc. (Musenga et al. 2007). The red pepper (capsicum) is another spice commonly used worldwide to improve the spice level of the dishes, which predominantly contains capsaicin. Additionally, red pepper also holds lutein, caffeic acid, β-carotene, capsanthin, and zeaxanthin. Another important spice is cardamom, which contains myrcene 1,8-cineole, terpinolene, α-terpinyl acetate, linalool, limonene, and linalyl acetate (Auti and Kulkarni 2019). Garlic (*Allium sativum*) is the most frequently used spice for the Asian medicinal system. It has anti-inflammatory, gastro-protective, and anti-cancer properties due to the phytochemical presence of allicin, ajoene, diallyl disulfides, diallyl trisulfide (DATS), alliin, diallyl sulphides (DAS), S-allylcysteine, cycloalliin, methiin, S-allylmercaptocysteine, and isoalliin (Kimbaris et al. 2006; Srinivasan 2014). In addition to the abovementioned spices, cinnamon (cinnamaldehyde, humulene, cineole, cinnamyl acetate, ethyl cinnamate, coumarin, τ-cadinol, linalool, and β-caryophyllene) (Zare et al. 2019), clove (eugenol) (Aggarwal and Kunnumakkara 2009; Gupta et al. 2013), fenugreek (resins, diosgenin, choline, yamogenin, trigonelline) (Srinivasan 2019), kokum (xanthochymol, garcinol, 1,2-dihydroxy propane-1,2,3-tricarboxylic acid, isoxanthochymol), black cumin (γ-terpinene, 4-dien-7-al, *p*-cymene, cuminaldehyde, 3-diene-7-al, *p*-mentha-1, thymoquinone, β-pinene) (Abd El-Hack et al. 2016), rosemary (camphene, α-pinene, rosmarinic acid, borneol, carnosol, bornyl acetate, limonene, carnosic acid, camphor, cineole, (Z)-linalool oxide), star anise (trans-anethole, estragole, and limonene), and saffron (crocin and crocetin) (Shen et al. 2017) contain sample of beneficial phytochemicals that furnish immense health benefits. Various spices/herbs that improve the immune system have been listed in Table 2.1.

2.4 Role of Spices as Effective Immune Mediators

2.4.1 Turmeric

Turmeric has been widely used for its traditional value as medicine in Southeast Asia. It is not only used as a principal spice for cooking but also is an important component of religious ceremonies. Turmeric contains numerous bioactive compounds, and its most important bioactive compound is curcumin. For decades, curcumin has been used to treat a variety of progressive neurodegenerative problems (multiple sclerosis, neurodegenerative diseases), heart disease, metabolic-related diseases, respiratory-related disorder, rheumatoid arthritis, and autoimmune disorders (Upadhyay et al. 2009). Curcumin is a potent antioxidant that helps to

Table 2.1 Details of other spices/herbs with potential to improve the immune system

Common Name	Scientific Name	Parts Used	Bioactive Compound	Immuno-response	Reference
Aloe vera	*Aloe vera Tourn. ex Linn.*	Leaves gel	Glycosides anthraquinone	Anti-inflammatory, emollient, anti-bacterial, purgative	(Hamman 2008; Sikarwar et al. 2010; AftabUddin et al. 2017)
Piper/ pepper	*Piper longum L.*	Fruits	Alkaloids	Antitumour, anti-inflammatory, anti-seizure, antioxidant, multiple sclerosis	(Chen et al. 2013; Gupta et al. 2015; Gu et al. 2017; Wang et al. 2017a; Kumar and Agnihotri 2019)
Lemongrass	*Cymbopogon citratus*	Whole plant, leaves	Flavonoids, phenols, terpenoids, ketone, tannins	Antibacterial, anti-fungal, anti-malarial, neuroprotective effect, biofilm inhibitory effect	(Chukwuocha et al. 2016; Ilango et al. 2019; Madi et al. 2020; Sahal et al. 2020)
Basil	*Ocimum basilicum*	Flowers, buds, leaves	Polyphenol	Anti-inflammatory, antioxidant, anti-oedematogenic activity	(Rodrigues et al. 2016; Aye et al. 2019)
Coneflower	*Echinacea angustifolia*	Flowers, roots	Polysaccharide	Antioxidant, immunomodulatory	(Gallo et al. 2012; Dall'Acqua et al. 2019)
Wormwood	*Artemisia annua Linn.*	Herb	Artemisinin	Anti-fungal, tumour suppressors	(Krebs et al. 2010; Liu et al. 2019)
Parsley	*Petroselinum crispum*	Leaves, seeds, roots	Phenylpropanoids, tocopherols, flavonoids, carotenoids	Anti-diabetic, anti-fungal, anti-estrogenic property	(Alghamdi 2016; Ghapanchi et al. 2016)
Glasswort	*Salicornia herbacea*	Herb.	Polysaccharides	Antioxidant, antimicrobial, anti-adipogenic activity, immunomodulator	(Kong et al. 2012; Essaidi et al. 2013; Wang et al. 2017b)
Botryllus	*Botryllus schlosseri*	Tunicates	Cytokines	Antioxidant, anti-inflammatory, antimicrobial, and antitumour activity	(Ballarin 2008; Matozzo et al. 2014)
Oregano	*Origanum vulgare*	Leaves	Monoterpenoidic phenols	Anti-proliferative, anti-enzyme, antioxidant, anti-inflammatory	(Han et al. 2017; Avola et al. 2020)

(continued)

Table 2.1 (continued)

Common Name	Scientific Name	Parts Used	Bioactive Compound	Immuno-response	Reference
Mistletoe	*Viscum album L.*	Leaves, berries	Polyphenols	Anti-inflammatory, antitumour properties	(Elluru et al. 2007; de Oliveira Melo et al. 2018; Murthuza and Manjunatha 2018)
Milk thistle	*Silybum marianum L.*	Flowers, seeds	Flavonoid, silibinin	Cardioprotection, anti-diabetic, antibacterial, anti-aging, antioxidant	(Zhu et al. 2017; Vilahur et al. 2018; Arvanag et al. 2019)
Brahmi	*Centella asiatica Linn.*	Herb	Triterpenoid, saponins	Wound healing property, antioxidant	(Mali and Hatapakki 2008; Anand et al. 2010; Choochuay et al. 2016)
Punarnava	*Boerhaavia diffusa*	Herb	Alkaloid	Anti-proliferative and antiestrogenic, anti-diabetic, antioxidant	(Sreeja and Sreeja 2009; Olaleye et al. 2010; Dora et al. 2018; Oyebode et al. 2018)
Tulasi	*Ocimum sanctum Linn.*	Whole plant	Eugenol, apigenin, carvacrol	Antispasmodic, Anti-asthmatic, hepatoprotective, carminative	(Vaghasiya et al. 2010)
Bhringraj	*Eclipta alba L.*	Leaves, seeds	Triterpenoid glucoside	Anti-cancer, antioxidant, anti-diabetic, analgesic, suppress the nephrotoxicity effect	(Dungca 2016; Anitha and Mythili 2017; Vijayakumar et al. 2020)
Asthma weed	*Euphorbia hirta Linn.*	Herb	Myricitrin, quercitol, gallic acid	Anti-inflammatory, antioxidant, hepatoprotective	(Sandeep et al. 2009; Anitha and Mythili 2017)
Creosote bush	*Larrea divaricata DC.*	Herb	Lignans	Anti-inflammatory, immunosuppressor, anti-diabetic, anti-inflammatory	(Dadashpour et al. 2018; Han et al. 2019; Shebbo et al. 2020)
Chamomile	*Matricaria chamomilla*	Flowers	Protein	Anti-cancer, hepatoprotective effect	(de Souza Reis et al. 2008; Dadashpour et al. 2018; Shebbo et al. 2020)
Brahmdaru	*Morus alba Linn.*	Fruits, leaves, Bark	Anthocyanins, flavonoids	Antiviral, anti-inflammatory, antioxidant	(Yimam et al. 2016; Maryam et al. 2020; Paudel et al. 2020)

Ninjin	*Panax ginseng Wall.*	Roots, fruits	Saponins	Adaptogenic properties, anti-diabetic, antiarrhythmic	(Liu et al. 1995; Xu et al. 2017; Jovanovski et al. 2020; Yang et al. 2020)
Yarrow	*Achillea millefolium C. Koch*	Root, leaves	Flavonoids, coumarins, polyacetylenes, triterpenes, alkaloids	Anti-inflammatory, antinociceptive, antimicrobial	(Sharififar et al. 2009; Radulović et al. 2012)
Shatavari	*Asparagus racemosus Wild.*	Roots	Sitosterols, saponins	Antitumour, ulcer healing agent, anti-hepatotoxic, anti-gout	(Bopana and Saxena 2007; Benil et al. 2020)
Celery seeds	*Apium graveolens Linn.*	Leaves, seeds	Coumarins, flavonoids	Anxiolytic, antioxidant, anti-inflammatory, antimicrobial activity, anti-aflatoxigenic	(Tanasawet et al. 2017; Das et al. 2019)

prevent oxidative damage and also improves the body's antioxidant enzymes. Oxidative damage resulted from the action of free radicals to biomolecules, which exhibits central function in the aging process and other diseases. Thus, these kinds of reactions inside the body are very important to understand the defence mechanism of our immune system. Numerous studies have reported that turmeric exhibits anti-inflammatory effects by inhibiting TNF- α production (Kesharwani et al. 2018; Crivelli et al. 2019; Güran et al. 2019; Porro et al. 2019; Shimizu et al. 2019). Besides, curcumin has shown a significant reduction in the p38 mitogen-activated protein kinases (MAPK), a stress-sensitive kinase responsible for the inflammatory responses (Mohammadi et al. 2019).

Studies have shown that curcumin exhibits immunomodulatory effects not only through the interaction with immune cells but also through communication with other signalling proteins such as cytokines and various transcription factors with their downstream signalling pathway. Curcumin inhibits the immunostimulatory action of dendritic cells and impedes the maturation of myeloid dendritic cells. These effects can be achieved by the inhibition of CD80 and CD86 expression, which provides signals for T-cell activation. Furthermore, increased circulatory immunoglobulin G (IgG) and immunoglobulin M (IgM) has been observed in rabbit supplemented with curcumin-rich diet, suggesting that curcumin improves immune function (Alagawany et al. 2016). The Janus kinase (JAK)-signal transducers and activators of the transcription (STAT) pathway through the modulation of a wide range of biomolecules including cytokines and growth factors strongly influence immune responses (Jang and Baik 2013). Studies reported that curcumin inhibits IL-2 production, nitric oxide (NO) generation, T-cell proliferation elicited by phytohaemagglutinin (PHA), nuclear factor-kappa beta (NF-κβ) activation by lipopolysaccharide, and increases NK cell cytotoxicity in multiple cell lines (Yadav et al. 2005). Studies conducted in rats reported that curcumin supplementation increases antibody synthesis and NK cell activity in splenocytes (South et al. 1997; Antony et al. 1999; Singh et al. 2015). IL-12 plays a salient function in the enhancement of the immune system by increasing Th1-type cytokine production against microbes. Curcumin through the inhibition of IL-12 production attenuated Th1 cytokine production in CD4+ T cells in the mouse splenic macrophages (Kang et al. 1999). Furthermore, it is also documented that the upregulation of nuclear transcription factor NF-κβ has been inhibited by curcumin supplementation without influencing the proportion of constitutively expressed NF-κβ (Kang et al. 1999). Thus, from the study, it is evident that curcumin intake has been effectively improving the immune system by affecting various signalling events in our body (Singh et al. 2016; Kesharwani et al. 2018).

2.4.2 Ginger

Ginger is one of the classic spices in cooking but also confines remarkable medicinal significance. Being a root spice, ginger is enriched with a lot of beneficiary compounds especially vitamins, minerals, antioxidants, and nutrients that are

responsible for health-related effects. Several studies have claimed that ginger has bactericidal and anti-inflammatory action. Ginger is enriched with a lot of bioactive molecules including α-farnesene, 6-gingerol, α-curcumene, paradol, β-bisabolene, zingerones, 6-shogaol, α-zingiberene, etc. (Mao et al. 2019). Since ginger is rich in phytochemicals, it has been employed in Chinese and Ayurvedic medicines. Ginger is consumed as a natural immunoregulatory and has been documented in several research publications (Choi et al. 2018). The bioactive components in ginger have the potential in reducing the risk of liver, skin, colorectal, gastric, and ovarian cancers (Semwal et al. 2015). Studies conducted by Zhu et al. (2020) suggested that mixed polysaccharides obtained from ginger along with shiitake mushroom, *Poria cocos,* and tangerine enhance immune efficacy and attenuate lung inflammation in mice immunized with H_1N_1 vaccine (Zhu et al. 2020). It has also been reported that consumption of ginger extract significantly increases IgM levels in non-smokers, thereby showing a powerful antibody response against respiratory infections. Besides, ginger supplementation raises the level of red blood cell (RBC) counts and haemoglobin levels in smokers, which was stated to have favourable effects for smokers with anaemia (Mahassni and Bukhari 2019). Furthermore, supplementation of 3% ginger powder in zebrafish enhances immunological and biochemical responses as well as upregulates genes related to antioxidant and immune systems (Ahmadifar et al. 2019). Numerous research evidence support that constituents of ginger exhibit anti-inflammatory actions in various research models (Habib et al. 2008; Jeena et al. 2013; Jalali et al. 2020).

Ginger exhibits its anti-inflammatory property through multiple pathways including by attenuating arachidonic acid-instigated platelet aggregation; synthesizing thromboxane B; enhancing histone H3 acetylation; inhibiting histone deacetylase 1 expression (Shen et al. 2017); inhibiting IL-8, IL-1β, and TNF-α production; and suppressing cyclooxygenase 2 (COX-2) and inducible NO synthase (iNOS) gene activation by the suppression of NF- κβ. Besides, ginger also exhibits anti-inflammatory effects through the activation of cellular stress-sensitive kinases such as extracellular signal-regulated kinases 1 and 2 (ERK1/2), c-Jun N-terminal kinase (JNK), and MAPK (Semwal et al. 2015). Active compounds present in ginger include gingerol; shogaol has been found to inhibit prostaglandin and leucotriene production by attenuating the expression of 5-lipoxygenase. Furthermore, studies documented that active components in ginger attenuate the production of pro-inflammatory cytokines such as TNF-α, IL-8, and IL-1 β (Tjendraputra et al. 2001). Similarly, 6 and 10-gingerol and 8 and 10-shogaol in ginger suppress I-κBa phosphorylation and COX-2 nuclear factor-κB (NF-κB) activation and thereby downregulate iNOS and TNF-α expression (Oyagbemi et al. 2010; Zhang et al. 2019). Research evidence suggests that ginger extract can attenuate NF-κβ activation and TNF-α synthesis in ethionine-induced hepatoma rats. The upregulation of NF-κβ has been associated with numerous inflammatory diseases like atherosclerosis, osteoporosis, multiple sclerosis, asthma, and human immunodeficiency virus (HIV) infection (Aggarwal and Kunnumakkara 2009). It has been found that lipopolysaccharide-induced COX-2 activation has been inhibited by the

administration of gingerols stating that ginger is capable of attenuating prostaglandin E2 (PGE2) synthesis (Lantz et al. 2007).

Studies reported that intraperitoneal administration of ginger extract lowers eosinophil levels along with the reduction in IL-5, IL-4, and eotaxin levels in experimental models (Choi et al. 2018). Further pieces of evidence have also reported that 6-gingerol (25–50 mg/kg) attenuated formalin-instigated licking time and acetic acid-instigated writhing response in experimental models. However, a huge dose of 6-gingerol (50–100 mg/kg) is needed to retard carrageenin-induced paw oedema. The anti-inflammatory properties of ginger will be supportive to control health problems like respiratory infections, arthritis, allergic diseases, and gout (Yatoo et al. 2018). Thus, from the research evidence, it is crystal clear that ginger and its components can be recommended to improve immunoregulatory response.

2.4.3 Garlic

Traditionally garlic has been widely used as a medicinal agent to improve health (Talib 2017). Garlic has been recommended as a potential candidate for regulating the homeostasis of the immune system including activation of immune-related pathways, diminishes platelet aggregation, and is used in the chemoprevention of cancer (Shang et al. 2019). The biological actions of garlic have been attained mainly due to the presence of organosulfur compounds including DAS, Sallylmercaptocysteine (SAMC), DADS, S-allyl-L-cysteine sulfoxides, non-starch polysaccharides, and δ-glutamyl-S-allyl-L-cysteine (Percival 2016; Shang et al. 2019). Various garlic formulations like aqueous garlic extract, aged garlic extract (AGE), and an oil extract from garlic are being used by different regions for various health benefits. Studies conducted in Balb/c mice post infected with plasmodium stated that garlic administration effectively modulates the Th1 cytokine profile during the initial phase of malarial infection. It has been reported that allicin, an active principle in garlic, has been shown to exhibit reduced parasitemia and prolonged life expectancy due to the increased interferon-gamma secretion (Feng et al. 2012).

Garlic and its extracts can quench reactive oxygen species and thereby exhibit its antioxidant activity. Furthermore, it enhances antioxidant enzyme activity, such as catalase, glutathione peroxidase, glutathione S-transferase (GST), and superoxide dismutase (Fallah-Rostami et al. 2013). Studies reported that garlic usage results in the increase of leucocytes, as well as the characteristics of blood homeostasis in experimental models. Studies reported that garlic usage will help in viral and proliferative diseases by increasing the synthesis and release of nitric oxide and by enhancing IFN-α secretion in humans (Chung et al. 2016). Moreover, garlic attenuates NF-$\kappa\beta$ activation and thereby exerts its anti-inflammatory activity in humans. Studies documented that AGE improves immune response in opposition to implanted tumours in mice. Besides, AGE along with naltrexone-enhanced survival time attenuates tumour growth and interferon-γ production (Fallah-Rostami

et al. 2013). Moreover, some garlic-derived compounds like S-allyl-L-cysteine, caffeic acid (CA), uracil, DATS, and DAS can attenuate the transcription factor NF-κB, a master regulator, thereby downregulating the expression of several cytokine genes related to pro-inflammatory responses, including IL-1β, TNF-α, IL-12, IL-6, and monocyte chemoattractant protein 1 (MCP-1) (You et al. 2013; Ho et al. 2014).

The most significant action of garlic is to protect the human from the risk of cancer (Shang et al. 2019). Studies documented that supplementation of garlic and its formulations protects the skin, colon, prostate, mammary glands, and lungs from the development of cancer. (Shen et al. 2017). Organosulfur garlic compounds inhibit carcinogenic gene activation, improve phase II detoxification mechanism, trigger cell cycle arrest mainly in the G2/M phase, activate the apoptotic mitochondrial pathway, promote histone acetylation, and suppress tumour multiplication, which clearly stated that garlic supplementation reduces the risk of multiple common cancers (Mandal et al. 2019). Formulations like fresh garlic juice and aged garlic extract have a hold tendency to attenuate cell proliferation, thereby leading to apoptosis (Shen et al. 2017). The research documented that the DAS, DADS, and DATS would trigger sequential signalling cascades, which ultimately leads to cancer cell apoptosis (Mandal et al. 2019). Thus, garlic can be believed to be one of the most promising spices for improving the immune system, enhance the body's antioxidant status, and protect the body from free radicals damage, inflammation, and cancer.

2.4.4 Black Cumin

Black cumin seeds were used in herbal medicine to cure and prevent a variety of diseases (Abd El-Hack et al. 2016). Studies conducted in humans documented that supplementation of 1 g of *Nigella sativa* twice a day improves the immune system by modulating the T4/T8 ratio and by improving natural killer cell activity (Elluru et al. 2007). In another study, researchers noticed that *N. sativa* increases the production of IL-1 beta indicating that it influences macrophages (Haq et al. 1999). Moreover, because of the existence of free radical scavenging activity, it has been involved in the protection of the central nervous system's parts such as the medulla spinalis and brain tissues against autoimmune encephalomyelitis (Ghasemi et al. 2014). It was reported that black cumin seed essential oil exhibits a remarkable analgesic effect in acetic acid-mediated writhing and light tail-flick tests. Moreover, the anti-inflammatory effects of black cumin have been observed in paw oedema mediated by carrageenan in rats and ear oedema caused by croton oil in mice (Hajhashemi et al. 2004). Thymoquinone (2-Isopropyl-5-methyl-1, 4-benzoquinone) is an important bioactive principle of *N. sativa*, which exhibits several beneficial activities such as antioxidant, renoprotective, hepatoprotective, antitumour, neuroprotective, and anti-ischemic (Ahmad et al. 2019). Tekeoglu et al. (2008) reported that arthritis induced by adjuvant has been attenuated by thymoquinone (active essential oil ingredient) through the inhibition of leucotrienes (LTs), by modulating the synthase activity of 5-lipoxygenase and leucotriene C_4 (LTC_4) in experimental rats. Another

black cumin active ingredient is α-hederin, which potentially attenuates the metastasis of breast cancer cells by decreasing the mitochondrial membrane potential and by reducing the mitochondrial Apaf-1 and cytochrome c levels in mammary cancer cells. Besides, α-hederin upregulates caspase-3 and caspase-9 activity in mammary cells. Besides, α-hederin also exhibits antitumour activity in B16 cells of melanoma, Lewis carcinoma of the lung, hepatocellular carcinoma (HepG2), and P388 cells of murine leukaemia by scavenging the reactive oxygen species (ROS) (Saadat et al. 2015). Thus, it is evident that the addition of black cumin in the diet may enhance the immune system.

2.4.5 Cinnamon

Cinnamon and its essential oil have been used as a natural food spice and traditional herbal ingredient. It has been reported that its powder, extracts, and essential oil contain trans-cinnamaldehyde, which exhibits anti-cancer, anti-inflammatory, antimicrobial, antioxidant, anti-diabetic, arteriosclerosis, cardiovascular, Alzheimer's disease, and beneficial effects on arthritis (Han and Parker 2017a; Abdel-Tawwab et al. 2018; Shishehbor et al. 2018; Arangannal et al. 2019; Zare et al. 2019). Besides, cinnamaldehyde exhibits anti-inflammatory properties by activating toll-like receptor 4 (TLR4) in gut microbiota, resulting in downregulated cytokine IL-10 and upregulated pro-inflammatory cytokine synthesis (tumour necrosis factor [TNF]-α, IL-6, IL-1β) (Li et al. 2020). Studies have been documented that cinnamon essential oil exhibits the anti-inflammatory effects against inflammatory bowel disease (IBD), and furthermore, it has antimicrobial property (Li et al. 2020). Hence, cinnamon essential oil has been extensively used for intestinal microflora control. Results showed that mice fed orally with cinnamon essential oil highly influence gut microbiota population, which resulted in reduced in *Helicobacter* and *Bacteroides* and a rise in Bacteroidales S24-7 and short-chain fatty acids (SCFA) synthesizing (*Alloprevotella* and *Lachnospiraceae_NK4A136_group*) bacteria. Moreover, the study showed that TLR4 and TNF-α had a positive relationship with *Helicobacter* but a negative relationship with bacterial SCFA (Li et al. 2020).

Several studies conducted in various experimental models documented that cinnamon is effective against arthritis, an autoimmune disorder in which our body's immune system targets joints resulting in inflammation and pain in joints. Clinical conditions of arthritis include rheumatoid arthritis and juvenile rheumatoid arthritis. Many natural foods have been used to treat arthritis, one of which is cinnamon (Catrina et al. 2016). Studies conducted in various rat experimental models stated that polyphenol fraction from cinnamon bark effectively reduces inflammation in rheumatoid arthritis and adjuvant-induced polyarthritis. Likewise, cinnamon supplementation has been shown to be effective against allergy (Shishehbor et al. 2018). Studies conducted in mouse cell lines revealed that treatment with cinnamon polyphenol extract induces tristetraprolin; thereby, it reduces the inflammatory-related genes like TNF-α and COX-2 (Cao et al. 2008). Studies have been performed to examine the role of cinnamon nanoparticles on

antioxidant enzymes and innate immunity of Nile tilapia. Results showed that dietary cinnamon nanoparticles are able to enhance the activities of antioxidant enzymes and enhance the innate immunity in Nile tilapia against pathogenic bacteria (*Aeromonas hydrophila*) infection (Abdel-Tawwab et al. 2018). Besides, supplementation of fish with cinnamon significantly reduces histamine production, which resulted in the protection of fish from allergic reactions (Shakila et al. 1996). Experiments conducted in humans documented that cinnamon supplementation effectively reduces nasal allergy symptoms and prostaglandin D_2 (PG $D_{2)}$ release in seasonal allergic rhinitis (Khan et al. 2003). Thus, cinnamon intake might be effective in the management of inflammation and immune-related disorders.

2.4.6 Cardamom

Cardamom is an essential spice and is considered as a salient source of flavonoids, alkaloids, terpenoids, anthocyanins, and phenolic compounds. It has been widely used for many years in Ayurveda medicine to treat asthma, blood pressure, dysuria, indigestion, and so forth (Aggarwal and Kunnumakkara 2009). It has been reported that cardamom has nutritionally rich metabolites including catechin, myricetin, quercetin, kaempferol, lutein, and β-carotene (Ashokkumar et al. 2020). Experimental pieces of evidence suggest that cardamom exhibits anti-inflammatory, antiproliferative, antioxidant, immunomodulatory, and detoxification properties (Auti and Kulkarni 2019). Experimental pieces of evidence suggest that cardamom exhibits great potential to act as an immunomodulatory agent through the inhibition of inflammation. It has been observed that the treatment of splenocytes and macrophages with aqueous extracts of cardamom in the presence of lipopolysaccharides and interferon-γ significantly inhibited the secretion of IL-6 and TNF-α, suggesting that cardamom exhibits immunomodulatory action by cytokine release (Majdalawieh and Carr 2010). Experiments conducted in skin cells confirmed that cardamom essential oil (CEO) exhibits anti-inflammatory and immunomodulatory activities.

Eucalyptol, an important active principle of CEO, is documented to downregulate the influence of NF-κB and its downstream signalling cascades (Proshkina et al. 2020). A study reported that treatment with eucalyptol attenuates lipopolysaccharide-mediated TNF-α, IL-6, and IL-1β production through the inhibition of NF-κB. Studies conducted in mice reported that attenuation of IL-1β, NF-κB, TNF-α, and IL-6 has been found with the treatment of eucalyptol (Zhao et al. 2014). Another research conducted in human bronchial epithelial cells documented that eucalyptol inhibited *Dermatophagoides pteronyssinus*-induced macrophage colony-stimulating factor (M-CSF) IL-6 and IL-8 production, supporting the anti-inflammatory activity of cardamom (Han and Parker 2017b). Besides, treatment of inflamed human dermal fibroblasts with CEO effectively suppresses vascular cell adhesion molecule 1 (VCAM-1) and M-CSF, which strongly supports the anti-inflammatory and immunomodulatory properties of CEO (Han and Parker 2017b). Thus, the overall anti-inflammatory and immunomodulatory properties of the CEO

were exhibited by attenuating the NF-κB signalling cascade and other signalling pathways. Another study was conducted to evaluate the effect of *Elettaria cardamomum* distillate (ECD) on the immune system based on lymphocyte, CD4+, and CD8+ number in rats. Results suggest that treatment with ECD improves the number of CD4+ and CD8+ cells by increasing the leucocyte, lymphocyte, and neutrophil numbers (Raksamiharja et al. 2012), which strongly supports that cardamom administration greatly modulates the immune cells in experimental models.

2.4.7 Fenugreek

Fenugreek (*Trigonella foenum graecum* L.) is a plant abundantly cultivated in India for its multipurpose. It finds application in culinary and traditional medicine as well. Fenugreek has been used in India for a long time as a seasoning agent and flavouring agent for soups and pancakes. In India, the leaves of fenugreek have been frequently used and consumed as green leafy vegetables, which are a good source of calcium, iron, β-carotene, and other vitamins. Besides, fenugreek contains phytochemicals such as trigocoumarin, alkaloid trigonelline, trigomethyl coumarin, diosgenin, and steroidal saponin such as gitogenin and traces of trigogenin, which result in its beneficial action as herbal medicine.

In the past few decades, researchers have focussed on the potential benefits of fenugreek seeds in humans. Studies have reported that fenugreek has been used as hypoglycaemic, anti-ulcerogenic, hypocholesterolaemic, and antihypertensive agents (Kirtikar et al. 1975; Sharma et al. 1996; Zia et al. 2001). Besides, experimental evidence strongly suggests its immunomodulatory actions in various experimental models. Studies have reported that *T. foenum* administration stimulates lymphocytes and bone marrow haematopoietic cells and thereby increases thymus mass in experimental mice. The stimulatory effect of the extract might be due to the presence of saponins, which has a mitogenic effect that may elicit stimulatory effects on immune-competent cells (Liu et al. 1995). Studies conducted in macrophages harvested from male albino rats reported that fenugreek galactomannan with alkali-extracted polysaccharide B exhibits phagocytic activity along with the secretion of IgM in HB4C5 cells (Ramesh et al. 2002). Some previous studies documented that there was an increase in the immune system and growth in fish after supplementation of a diet enriched in fenugreek. It was observed that there was an upregulation in the level of immune-related genes (IL-8, colony-stimulating factor 1 receptor (CSF-1R), and major histocompatibility complex 1 (MHC1)) and antioxidant enzyme genes (superoxide dismutase and catalase) in fish supplemented with fenugreek diet.

Furthermore, there was an increase in the secretion of IgM and WBC in experimental fish models (Awad et al. 2015). Additionally, dietary supplementation of fenugreek seeds improves mucosal immune parameters by increasing IgM levels and by modulating some enzymatic activities (peroxidase, anti-protease, protease, esterase) and ceruloplasmin after 8 weeks of feeding in gilt-head sea bream (Guardiola et al. 2018). Moreover, researchers have claimed that fenugreek seed has been shown to modulate intestinal microbiota and immunological responses in piglets after

Fig. 2.1 Various spices to support the immune system

weaning. The supplementation of fenugreek greatly influences immunological variables such as increased relative concentration of the T-cell population in the circulation with a concomitant depletion of antigen-presenting cells (Hossain et al. 2015). Studies showed that the treatment of crude fenugreek seeds greatly increases haemoglobin, lymphocyte percentages, and the total leucocyte count and was found to have low mortality in Nile Tilapia with or without cadmium contamination. Moreover, there was an increase in IL6 and IL8 gene expressions in the groups treated with crude fenugreek seeds (Yao et al. 2019). Thus, from the experimental results, it has been confirmed that fenugreek could be also considered as a promising plant to be used as an immune booster for humans. Figure 2.1 depicts various spices involved in the improvement of the immune system.

2.4.8 Guduchi

Guduchi (*Tinospora cordifolia*) is shrub that is native to India, which has great importance in Ayurveda since ancient times, and is also termed as 'devine nectar' or 'heavenly elixir' in traditional medicine. It has various health benefits such as antioxidant, anti-diabetic, cardioprotective, neuroprotective, anti-ulcer, radio-protective, anti-anxiety, anti-inflammatory, anti-diarrheal, antimicrobial, anti-cancer, and thrombolytic agent. Different plant body parts are reported to contain numerous bioactive compounds belonging to various classes like alkaloids, steroids, diterpenoid lactones, aliphatics, and glycosides (Upadhyay et al. 2010). Additionally, nutritionally, it is a rich source of macro and micronutrients such as polysaccharides, protein, iron, zinc, copper, calcium, phosphorus, and manganese (Saeed et al. 2020). The presence of these metabolites exhibits various

immunomodulatory properties in multiple ways. Studies reported that the ethyl acetate in water fraction from *T. cordifolia* upregulates the phagocytic function of human neutrophils. Experimental evidences showed that ROS generation has been significantly increased in polymorphonuclear leucocyte while using ethyl acetate fraction. Furthermore, it was confirmed that the synergistic effect of a group of compounds is responsible for the immunomodulatory activity (Sharma et al. 2012). Studies conducted by Gupta et al. (2017) revealed that the polysaccharide G1-4A extracted from *T. cordifolia* exhibits the immunomodulatory action through the activation of various intracellular signalling molecules. The study confirms that incubation of G1-4A polysaccharide with murine macrophages upregulates nitric oxide production and MHC-II and CD-86 cells through the activation of p38, ERK1/2, and SAPK/ JNK MAPK signalling events. It has been documented that herbal extract from *T. cordifolia* showed a significant increase in various immunomodulatory cells like IFN-γ, IL-2, IL-4, and IL-1 levels in the peripheral blood mononuclear cells (PBMCs) of chickens developed with infectious bursal disease (Sachan et al. 2019). Kalikar et al. (2008) reported that the root extract of *T. cordifolia* improves the immune system of HIV-infected subjects. The stem extract of the plant lowers the eosinophil count and increases B lymphocytes, macrophages, haemoglobin, and polymorphonuclear leucocytes. Experimental evidences reported that alcoholic extract of *T. cordifolia* exhibits wound healing effects through collagen synthesis (Shanbhag et al. 2005). *T. cordifolia* has also been shown to treat endocrine and metabolic disorders and is well known for its immune booster property (Dhama et al. 2016).

2.4.9 Panax Notoginseng

Panax notoginseng (common name: sanqi), is a perennial herb belonging to the family Araliaceae and has been widely used in traditional Chinese medicine. With progress in research, several polysaccharides from the plant have been identified to possess immunomodulatory activity through modulation of immune cells like lymphocytes, macrophages, dendritic cells (DCs), and natural killer (NK) cells. Liu et al. (2020) reported that a novel polysaccharide (PNPS-0.3) isolated from *P. notoginseng* residue exhibits strong immunomodulatory activities towards bone marrow dendritic cells (BMDCs) by inducing their maturation. This can be visualized by changes in the morphology of cells, promotion of expression of surface phenotypic markers (CD40, CD80, CD86, and MHC II), and stimulated secretion of TNF-α and IL-12 proinflammatory cytokines. The mechanism behind this reaction involved binding of PNPS-0 with TLR4 of BMDCs, which in turn activates the TLR4/Myd88/NF-κB signalling pathway resulting in the maturation of DCs, which has been illustrated in Fig. 2.2 (Liu et al. 2020).

In another report a heterogalactan polysaccharide isolated from *P. notoginseng* was evaluated for immunomodulatory properties. In vitro analysis revealed the ability of the polysaccharide to activate RAW264.7 macrophages resulting in increased macrophage phagocytosis, release of nitric oxide, and secretion of

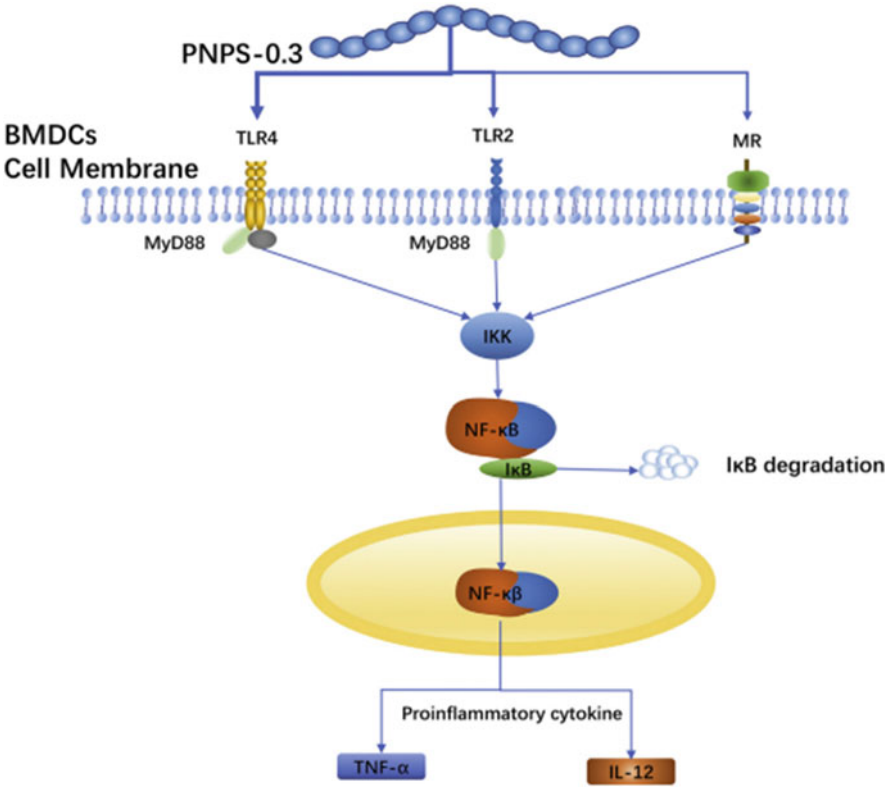

Fig. 2.2 The immunomodulatory mechanism of PNPS-0.3 isolated from *P. notoginseng* responsible for maturation of BMDCs (Source: Liu et al. 2020)

TNF-α, IL-6, IFN-γ, and IL-1β cytokines. Further, studies conducted in mice model documented that this compound resulted in enhancement of immunity in cyclophosphamide (CTX)-induced immunosuppressed mice (Cui et al. 2021). Li et al. (2016) studied the antitumour activity of polysaccharide from *P. notoginseng* on the proliferation of H22 live cancer cells. The incorporation of polysaccharide resulted in an increase in activated CD4+ T cells and elevation of serum IL-2 level, which ultimately showed antitumour activity with increase in survival rate of mice. In a study on mice, Choi et al. (2017) confirmed the ability of *P. notoginseng* root water extract against influenza A virus infection. This was mediated through elevated secretion of pro-inflammatory cytokines TNF-α and IL-6. Along with this, stimulation of NK cell activity plays a major role in providing protection against the virus. Additionally, the treatment also inhibited viral proteins and viral mRNA. Apart from these properties, these polysaccharides also possess antioxidant and anti-ageing properties as well (Feng et al. 2019).

2.4.10 Ashwagandha

Withania somnifera is an important herb and traditionally is being used as a medicine in many South Asian countries for millennia. The root of the herb forms part of more than 200 formulations in Ayurveda, Siddha, and Unani medicine (Palliyaguru et al. 2016). *Withania somnifera* possesses a unique blend of diverse phytochemicals including steroidal alkaloids and lactones. The pharmacological properties of the plant are attributed to bioactive compounds such as withanolide A, withanolide D, withaferin A, and withaniamides. Apart from these, proteins such as *Withania somnifera* glycoprotein and withania lectin like-protein are associated with antimicrobial and anti-snake venom properties (A Dar et al. 2016).

Studies documented that a marked increase in the level of total leucocyte count has been observed in normal Balb/c mice and in gamma-irradiated mice upon the administration of 75% methanolic extract (Kuttan 1996). The pharmacological effect of the roots is mainly attributed due to presence of withanolides, which are naturally occurring C-28 steroidal lactones and plays a major role in its immunomodulatory effect (Girme et al. 2020). Studies on giant freshwater prawns *Macrobrachium rosenbergii* (de Man) have shown that *W. somnifera* extract enhances the innate immune response against *Aeromonas hydrophila*. This caused increase in phenoloxidase enzyme activity resulting in melanization, which is a sign of cellular defence reactions. Apart from this, superoxide anion production and superoxide dismutase activity were also enhanced (Harikrishnan et al. 2012). Study has shown that *Withania somnifera* increases total platelet count in animals treated with cyclophosphamide. In a study on mice, attenuation in delayed type hypersensitive reactions and significant elevation in phagocytic potential of macrophages were observed (Agarwal et al. 1999). The plant extract is reported to provide strong immunity against intracellular bacterial proliferation through its influence on T cells. Moreover, oral administration of extract resulted in increased neutrophil count and stimulated phagocytosis. *Withania somnifera* extracts also increases IL-7 in IEC-6 expression in the intestinal cells, which in turn resulted in immunoprotection (Siddiqui et al. 2012).

In another study, supplementation of the root extract of the plant showed immunoenhancing effects in broiler chicken infected with *E. coli* (Kumari et al. 2020). A group supplemented with root extract showed higher *E. coli*-specific antibody titer and enhanced lymphocyte proliferation response than that of the non-supplemented group. Along with this, cellular and humoral immune responses were enhanced, which resulted in reduced severity, mortality, and recovery period of the infection (Kumari et al. 2020). Increase in nitric oxide production was also reported, which enhances the cell-mediated immune response, which ultimately increases the pathogen-destroying ability of the immune cell. This is mediated through the upregulation of iNOS expression through NF-κB transactivation in macrophages (Iuvone et al. 2003). These immunoenhancing properties are crucial considering the increased antibiotic resistance of pathogens. Along with these, *W. somnifera* is also linked with many immunosuppressing properties, which mainly

include anti-inflammatory action. Another study on Balb/c mice reported the role of *W. somnifera* in protection against zinc oxide nanoparticle-mediated toxicity. The administration of *W. somnifera* extract and Withaferin A in experimental models reduces the toxic effect through the suppression of *TLR6* overexpression and restoration of phagocytic activities (Kumar et al. 2019). It also proved that by treating animals with *W. somnifera*, there is an outstanding growth of the bone marrow cell (Davis and Kuttan 2000). Withanolides contain many activities like anti-inflammatory and analgesic because of cyclooxygenase-2 diffidence behaviour. In *W. somnifera*, a glycol protein known as WSG (glycowithanolides) is also liable for antimicrobial activities (Girish et al. 2006). Apart from all these, *W. somnifera* has been found to be useful in treating many brain disorders (Zahiruddin et al. 2020), cancer (Palliyaguru et al. 2016), tuberculosis (Kumar et al. 2018), and HIV-AIDS (Maurya et al. 2019). Based on the coronavirus disease (COVID-19) trend, a recent study has indicated that the *Withania somnifera*, a medicinal plant, contains a major compound called Withanoloid. A docking study points out the importance of the *W. somnifera*; among the number of Withanoloid, D, G, M, and Q were found to have the highest drug-likeness score inhibiting multiple proteins via multi-compound-multiprotein interaction necessary for boosting up the immunity and inhibition of COVID-19 infection (Khanal et al. 2020b).

2.5 Other Herbs and its Immune Response

Marjoram (*Origanum majorana*), sage (*Salvia officinalis*), thyme (*Thymus vulgaris*), oregano (*Origanum vulgare*), and rosemary (*Rosmarinus officinalis*) have been employed as herbal remedies for years especially in the management of inflammation and immune-related disorders. In ancient times, Greeks and Romans employed these herbs to manage dermal problems like skin sores and muscle-related complications like relieve aching muscles. Furthermore, it has been used as antiseptics (Han et al. 2017; Marrelli et al. 2020). Herbs like sage and thyme are commonly meant to manage acute inflammation and to treat obesity-related complications. A lot of research evidence strongly supports that apart from culinary applications many herbs and spices are associated with multiple health benefits due to their pharmacological properties. An independent study has shown the potential effect of commercial herbal medicines in immune stimulation with the inhibition of platelet reactivity. The suppression of platelets immensely acts on the risk of bleeding (Mothibe et al. 2019). Ginseng stem-leaf saponins (GSLS) have been identified as an immune booster and supplemented into the oil phase of adjuvant CV13 which has an excellent potential for foot and mouth disease (FMD) vaccine production (Xu et al. 2020). Ginseng roots and leaves act as herbal medicine and help improve fatigue syndrome, heal bronchial disorder and chronic fatigue, and boost the immune system and its anti-cancerous property (Kathal and Rawat 2016). *Echinacea purpurea*, well-known herb in America, contains alkamides, CA derivatives, and polysaccharides, which is used to treat respiratory infections including chemotherapy for upper and lower respiratory infections. Additionally,

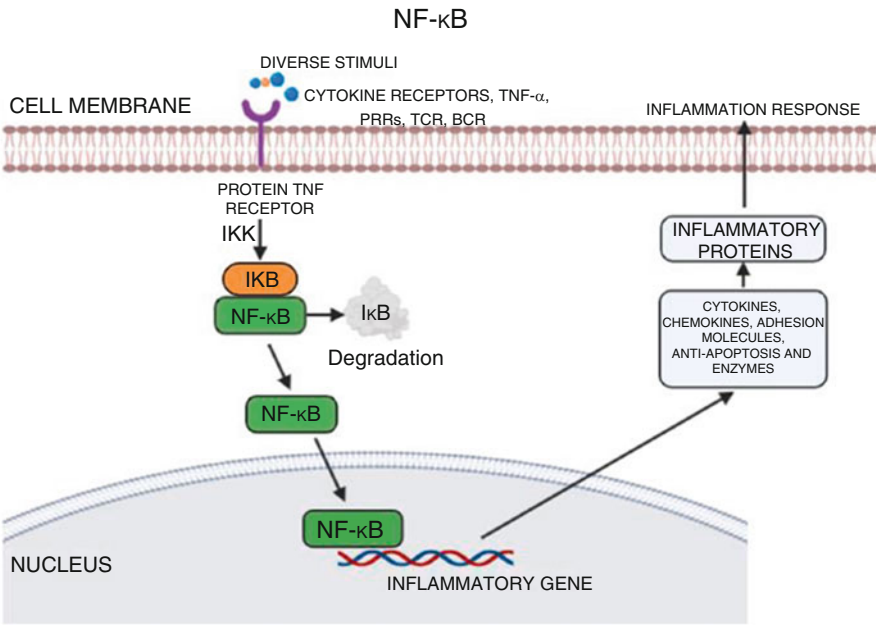

Fig. 2.3 Representation of the immunomodulatory mechanism of BAK (Kumar et al. 2021)

alkamides are known for their immune-stimulant effect, and polysaccharides are known for their anti-inflammatory effect (Samuel and Priyadarshoni 2019). Astragalus is found as an edible immunomodulatory herb and is a good source of polysaccharide constituent. This will play a critical role in exhibiting a strong immune response and thereby stabilizing the immune system against severe infections (Chen et al. 2020).

The immunomodulatory activity of *Psoralea corylifolia* is mainly attributed to the compound Bakuchiol (BAK) phenol, which is primarily found in plant seeds. Chemically BAK is a meroterpene and consists of aromatic ring in its structure. The compound is found to have numerous applications in treating several diseases in traditional Indian and Chinese medicine (Kumar et al. 2021). It has also been reported to provide protection for the human red blood cells and retina from oxidative damage. A recent study demonstrates the immunosuppressive action of BAK where the expression of proinflammatory cytokine is significantly downregulated, which further resulted in delayed hypersensitivity (Kumar et al. 2021). Its immunoregulatory mechanism is elucidated in Fig. 2.3. On the other hand, in another research, the immunoenhancing effect of *Psoralea corylifolia* is reported. A polysaccharide (PCp-I) isolated from the plant is able to upregulate the expression of iNOS, TNF-α, and IL-6 mRNA in RAW264.7 macrophages resulting in enhancement of NOS, ROS, TNF-α, and IL-6 levels and increase in phagocytic activity (Wang et al. 2021).

Another computational analysis indicated the importance of *Astragalus membranaceus* and *Panax ginseng* in the immune system (Liu et al. 2019). According to the study, in *Astragalus membranaceus* quercetin, kaempferol and formononetin were found to be a major regulate compound to immune response and rules cytotoxic activity of NK cells, thymus, and spleen index enhancement and proliferation of lymphocytes (Liu et al. 2021). In *Panax ginseng*, ginsenoside Ra1, ginsenoside Rh1, and kaempferol play a critical role (Sharma and Lee 2020).

2.6 Dietary Intake and Bioavailability

The quantity of spices consumed and the bioavailability of the bioactive compounds present in it strongly determines their health benefits. Usually, the spices are consumed in small amounts as part of a diet, and the consumption patterns are also region and cuisine dependent (Fairweather-Tait and Southon 2003). However, it also has to be noted that the bioactive compounds are present in higher concentration in spices. Though these compounds are characterized by low bioavailability, which is significantly influenced by several factors, they exert their beneficial health impact. For example, consumption of 10–100 mg of a polyphenolic compound results in its plasma concentration of maximum 1 μM.

Besides, the bioavailability varies from one compound to another. Therefore, even the most abundant bioactive compound in spices may not result in its higher levels in target tissues (Bi et al. 2017). Moreover, having these compounds as being heat sensitive, food processing operations such as cooking may result in a significant loss in their bioactivity and potency. Suresh et al. (2007) studied the effect of thermal treatment on bioactive compounds, namely, curcumin from turmeric, capsaicin from red pepper, and piperine from black pepper. Curcumin and capsaicin reported the thermal loss of 27%–35% and 18%–36%, respectively, whereas piperine reported a loss of 16%–34%. Nonetheless, there is a possibility of adverse effect, which depends on the amount consumed and drug interactions (Suresh et al. 2007).

Although these natural spices are Generally Recognized as Safe (GRAS), researchers have conducted safety assessment in order to define the recommended dietary intakes. In the case of curcumin, a dose of up to 12 g/day was known to be a safe consumption and did not show any side effects in clinical trials (Gupta et al. 2013). Ginger is generally considered as safe, and several studies conducted on human and animals did not report any adverse effects (Rong et al. 2009). Similarly, there have been no adverse effects reported for garlic. Overconsumption of cinnamon is reported with many adverse health effects, and it is concluded that larger doses for longer duration must be clinically monitored (Hajimonfarednejad et al. 2019). In a safety assessment study of *Withania somnifera* extract on rats, acute and sub-acute toxicity studies were conducted. In both studies, no adverse effect was reported even at the highest dose of 2000 mg/kg of body weight (Patel et al. 2016). For *Tinospora cordifolia*, Chandrasekaran et al. (2009) performed safety assessment for four genotoxicity tests on Balb/c mice. Oral administration of *T. cordifolia*

extract did not display clastogenicity and DNA damage in bone marrow erythrocytes and peripheral blood lymphocytes, respectively.

2.7 Market Share Insights of Spices/Herbs

The global spice industry includes diverse subsectors and different applications of herbs and spices involving many domestic and international players. In 2019, the market size was worth 13.77 billion USD and is forecasted to grow with CAGR of 6.3 from 2020 to 2027. The Asia Pacific region asserted dominance in global spices and herbs market sharing more than 30% of the revenue. High demand and consumption have been observed in Southeast and South Asian countries, which can be attributed to their traditional practices of extensive application of spices in daily cuisine for flavour purpose and medicinal applications as well. India recorded the highest production, consumption, and export of the spices across the world, accounting more than 65% of volume followed by other countries such as Bangladesh, Turkey, China, Ethiopia, Sri Lanka, Jamaica, and Pakistan. Apart from this, North America is emerging as a potential market in upcoming years with USA and Canada as major players. Increased demand of salad dressings and sauces in these countries is the reason behind this. Furthermore, consumer interest towards ethnic foods and easy availability of spices will be a key factor in the growth of this market (Sharma and Lee 2020). Increased awareness about the therapeutic benefits of spices is mainly responsible for increasing the demand in the near future. Furthermore, several other efforts by major industrial players such as new convenient products, improved export portfolios, and geographical expansion mergers and acquisitions are responsible to fuel the growth of the market.

2.8 Conclusion and Future Directions

Traditional spices and herbs provide several remedies for improving the body's resistance against diseases by modulating the immune system components. Unlike allopathic medicines like antibiotics, which can have serious side effects, most of these herbs and spices are relatively safe. These plant products contain a variety of phytochemicals, which not only kick off the flavour but also improve our immune system. Some compounds of spices and herbs exhibit immunomodulatory functions in cells and animal models, indicating that they could be effective in improving the immune system. Several components of spices show their effects on other health-related complications, indicating that these plant materials could be a healthy dietary means for preventing other diseases. Although the margin of safety for these spices and herbs are large, more scientific experiments are needed to understand the advantage of using herbs to improve immune-related infections.

Conflict of Interest The authors declare that there is no conflict of interests.

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Immune Boosting Activity of Nutraceuticals and Functional Foods

3

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Abstract

The key to good health is to have a functional and strong immune system. A strong immune system is characterized by the ability to protect the body from infection and any form of invasion by foreign objects. The strength of an immune system is also exhibited by the ability to prevent allergy and autoimmune diseases. The two major components of immune responses are innate and acquired immune responses which work together in synergy. Recent advances in medicine use diverse immunomodulators of natural origin, which can evoke biological reactions and reinforce body's natural defense mechanisms. A lot of studies in dietary and food biotechnology are ongoing about new alternatives for disease prevention. The need to improve health and quality of life has led to the discovery of certain food substances, which have nutritional value as well as biological activities. These are classified as either nutraceuticals or functional foods. Extensive studies, which have established that there is a relationship between gut microbiome and immunity, have spurred many research and studies on functional foods. Majority of the nutraceuticals and functional foods have been known to possess multiple therapeutic benefits against a variety of disease conditions. Their biological activities include antioxidant, anti-inflammatory, antimicrobial, anti-tumor, hepatoprotective, immunomodulatory, and many more. This chapter aims to discuss immune boosting activities of functional foods and nutraceuticals as well as narrate the mechanism of the immune modulation.

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Keywords

Nutraceutical · Functional food · Dietary supplement · Immune system · Immunomodulation

3.1 Introduction

Scientific evidence have established the fact that diet and eating habits have a lot of influence on health at every stage of life. Specific bioactive compounds in food can help to prevent diseases by modifying the pathological mechanisms that lead to disease development (Panico et al. 2014). Fundamentally, all foods can be regarded as functional because they provide one nutritional benefit or the other. However, nutritional science has gone beyond eating for nutritional benefits alone. Nutritional studies is now paying a lot of attention to improving health and quality of life through diet.

A lot of times, the two words, functional foods and nutraceuticals are used interchangeably. The term "functional foods" was not commonly used until the early 1990s (Ramesh and Jamuna 2012), and several definitions have been proposed for them. Functional foods are those that contain nutrients with biological activities (Olaiya et al. 2016). Martirosyan and Singh (2015) defined functional food as those that contain bioactive compounds, which not only supply food nutrients but are also very useful for maintaining good health. This means that functional foods have a potentially positive effect on health beyond basic nutrition when they are consumed as part of a varied diet on a regular basis. Functional foods include conventional foods such as whole grains, fruits, vegetables, etc. They also include modified foods such as fortified or enhanced foods like yogurt.

Nutraceuticals are the food components (e.g., polyphenols, flavonoids, carotenoids, saponins, sulfides, etc.) with potential health benefits in addition to their nutritional values. In many countries, nutraceuticals are taken as part of dietary supplements.

The immune system is vital for protecting the body from most of the disease conditions, including cancer, cardiovascular, neurological, microbial infections, and many more. Unfortunately, the immune functions are often affected when there is malnutrition, physical stress, and mental stress. Immunity is also depleted by aging and unwholesome lifestyle. Hence, consuming foods that can boost immune activities will help to prevent myriads of infections and also help to prevent cancer (Kaminogawa and Nanno 2004). Since it is important to correct nutritional deficiency or insufficiency in order to boost immune functions, many recent studies have suggested that increased intake of some nutrients above recommended levels may improve immune function and at the same time maintain tolerance (Wu et al. 2019). In addition to the six nutrients, there are myriads of phytochemicals and other functional foods that can also help with immune system optimization. These phytochemicals are not necessary for normal cell function and metabolism, so there is no recommended daily intake yet.

Several studies have established the beneficial effects of nutraceuticals and functional foods against different disease conditions. One of these is their role in maintaining and improving immune function, which helps with prevention and treatment of diseases.

3.2 Nutraceuticals and Functional Foods

The concept of functional foods and nutraceuticals is being promoted globally due to their overall nutritional value and biological activities. Some nutraceuticals are registered in many countries as dietary supplements or pharmaceuticals, while many others which are not registered yet are being used by people for self-medication.

Nutraceuticals are a sector of dietary supplements made only from whole foods to promote good health. Functional foods on the other hand are similar in appearance to natural food. Some of them are foods that are regularly consumed and have been established to contain bioactive compounds that can prevent chronic diseases. The difference between functional food and nutraceutical is that nutraceutical contains defined bioactive compounds isolated from food and put in a simple matrix, while functional food contains bioactive compounds, often not well defined, in a complex food matrix. Any functional foods that assist in preventing or treating diseases (beyond deficiency condition) are classified as nutraceuticals.

3.2.1 Nutraceuticals

The concept of nutraceuticals is the midpoint for food and drugs. What makes the difference between nutraceuticals and pharmaceuticals is their origin. Pharmaceuticals have synthetic origins, while nutraceuticals have natural origins. Nutraceuticals have powerfully claim their own legal space in medical sciences, since their characteristics and therapeutic potential are getting defined (Keservani et al. 2015; Aronson 2017). Nutraceuticals are therefore used for the purpose of improving health, of which prevention of chronic diseases is paramount. Additionally, they help to delay the aging process and increase life expectancy (Zhao 2007).

Hence, nutraceuticals are those substances that are carefully extracted from nature so as to prevent denaturation. Biological properties of the extracts are determined and documented through in vivo and in vitro studies before they are registered for marketing. These may be marketed as dietary supplements, isolated nutrients or specific diets, processed foods, beverages, genetically engineered foods, and herbal products (Prakash et al. 2004; Keservani et al. 2017). Most of the nutraceuticals help with the maintenance of optimal immune response. Scientific studies have also demonstrated the potentials of nutraceuticals in preventing arteriosclerosis (Madihi et al. 2013)), metabolic diseases (Khosravi-Boroujeni et al. 2012; Baradaran et al. 2013), cancer (Shirzad et al. 2013), as well as neurological disorders (Keservani et al. 2010a, b; Roohafza et al. 2013).

Nutraceuticals are classified based on:

(i) **Food source**: Plant, animal, or microbial food source.
(ii) **Mechanism of action**: Nutraceuticals can have anticancer activity, hypolipidemic activity, anti-inflammatory activity, etc.
(iii) **Chemical nature**: Nutraceuticals may be carotenoids, collagen hydrolysate, dietary fibers, fatty acids, flavonoids, etc.

3.2.2 Functional Foods

Health Canada has defined functional food as those with similar appearance to a conventional food or a regular food, which have physiological benefits and prevent chronic diseases (Corzo et al. 2020). These foods are designed in such a way that their presentation to consumers is close to their natural state (Keservani et al. 2017). They are prepared to provide the required amount of macronutrients and micronutrients for the body. To be classified as functional food, the following characteristics must be present in such foods:

(a) They exist in their natural form.
(b) They are an essential part of the daily diet.
(c) They are able to initiate mechanisms that modulate the physiological systems that prevent or control diseases (Keservani et al. 2010a, b; Patil et al. 2016).

3.2.3 Types of Functional Foods

Functional foods are generally categorized into three:

(i) **Conventional:** These are natural food items that have health benefits and the ability to prevent diseases in addition to their primary function of delivering nutrients. They are rich in important nutrients like vitamins, minerals, antioxidants, and polyunsaturated fatty acids (PUFA). Some examples of conventional functional foods are fruits, vegetables, nuts, seeds, legumes, whole grains, seafood (e.g., salmon, mackerel), fermented foods, herbs and spices, beverages, etc. (Keservani et al. 2010a, b).
(ii) **Manipulated (via biotechnological processes):** This involves production processes that convert standard food to functional food. They are usually fortified with additional ingredients, such as vitamins, minerals, probiotics, or fiber in order to increase the food's health benefits. One example is that of eggs rich in omega-3 which were produced by modifying the feed of chickens. Another example is the production of genetically modified tomato strain with a higher lycopene content. Lycopene has been demonstrated to have anticancer properties, and it can also reduce the incidence of cardiovascular diseases.

(iii) **Processed or modified:** This has to do with the modification of foods during production processes in food factories. This may be by adding or extracting certain compounds or microorganisms. The dairy factory is particularly dominated by artificially enriched functional products. For example, yogurt has been successfully transformed into a functional food through the addition of a range of health beneficial bacteria (probiotics) that can be consumed to improve body functions.

Prebiotics and probiotics are functional foods obtained from plant sources like vegetables, cereals, dairy, etc., as well as meat products (Ashaolu 2020; Das et al. 2020; Green et al. 2020; Silva et al. 2020). Prebiotics and probiotics confer many health benefits on the host, and this may include reduced macular degeneration and decreased risk of prostate cancer and colon cancer. These functional foods also demonstrate antioxidative properties, which promotes reduction in the incidence of certain chronic diseases (Silva et al. 2020). Prebiotics and probiotics have the capacity to boost the immune status of consumers (Guimarães et al. 2020). Prebiotics and probiotics have immunomodulatory and immune boosting benefits.

There are many types of prebiotics, and majority of the these are oligosaccharide carbohydrates. Prebiotics are not available in large quantities in foods, so they are largely manufactured industrially. Lactose, sucrose, and starch are usually the preferred raw materials for such industrial processes (Bouhnik et al. 2006). Organisms from *Bifidobacteria*, *Enterococci*, *Lactobacilli*, *Leuconostoc*, and *Saccharomyces* species are among the common probiotics that are often used for producing functional foods (Wan et al. 2019). The market for probiotics has risen spontaneously due to their immune-enhancing benefits.

3.3 The Immune System

The immune system is a complex network of defense systems that protect the host from invading microorganisms and malignant cells. Different types of cells, tissues, and organs are involved in the immune system. Cells of the immune system are referred to as lymphocytes (T-cells, B cells, and NK cells), neutrophils, and monocytes or macrophages. The immune system is broadly classified as innate immunity (nonspecific) and adaptive immunity (specific).

3.3.1 Innate Immunity

Innate immunity is the body's first line of protection against invading pathogen. This type of defense mechanism is not specific in action, rather it is characterized by physical and biochemical barriers that evolve immediately or within hours of an attack by an antigen. It includes macrophages and other cells that engulf and destroy foreign material. It also includes various mechanisms such as inflammation, coagulation, and complement (Kumar et al. 2020). This depends on the ability of the body

to recognize certain molecules found in the pathogens, which are absent in the host. In vertebrates, such pathogen-specific molecules are recognized by complement proteins. Complement proteins act by disrupting the membrane of the invading pathogen and target them for phagocytosis. This is what produces inflammatory responses.

3.3.2 Adaptive Immunity

Adaptive immunity is a specific immune action which provides long-term defense against invading pathogens. This long-term defense is made possible through the activities of immunological memory cells that are created from B and T lymphocyte in response to infection. Immunological memory is the ability of the immune system to swiftly recognize an antigen that the body has previously destroyed and swiftly generate an efficient and dramatic immune response (Pancer and Cooper 2006). Immunological memory can also be artificially achieved through vaccination. Vaccines are made with antigens that imitate the activities of a pathogen. The vaccination will activate an adaptive immunity (Kumar et al. 2020). Moreover, T lymphocytes can generate cellular immunity as a result of the activation by cytokines, which are discharged from helper T cells (Moser and Leo 2010).

Many substances derived from food can activate either innate or acquired immunity. What is desirable for optimal health is a balance of innate and acquired immunity (Kaminogawa and Nanno 2004).

3.3.3 The Gut Immune System and Its Microbiota

The intestinal immune system is very important since gut microbiota generally reside in the lower region of the GIT. The function of the intestinal immune system is to protect the gut from several types of antigens that may get into the body through foods, commensal, and pathogenic bacteria. Additionally, several intestinal cells help to activate the production of IgA isotypes that also increase gut immunity (Atarashi et al. 2011).

At birth, the human gut is predominantly inoculated, but colonization of the baby's gut by microbiota begins immediately after birth through contact with the environment and breastfeeding. Depending on certain feeding and dietary patterns, the diverse microbiome in children continues to develop until a child reaches 3–5 years of age, when it would be similar to what is found in adults (Rodríguez et al. 2015). These microbiota disturb the production of many cells and tissues within the gut so as to confer immunity on the system. However, aging, diet, infection, or indiscriminate use of medications may upset this microbial makeup and also affect their fermentation products. Disruption of the gut microbiota is responsible for many acute and chronic disorders like obesity, inflammatory bowel disease (IBD), etc. (John et al. 2018; Yoshida et al. 2018). These days, gut microbes are continuously being engineered into commercially available probiotics.

3.3.4 Immunomodulation

Optimal functioning of the immune system is determined by the action of biological or synthetic molecules that modulate, suppress, or stimulate elements of the immune system. These molecules are termed immunomodulators (Jantan et al. 2015). Byrne et al. (2020) define immunomodulation as changes that occur in the immune system after exposure to any substance that either induces or suppresses immunity. Immunomodulation is therefore a very broad term, which includes any process that modify or regulate the immune response therapeutically. Hence, immunomodulation includes processes such as:

- Reduction of inflammation that accompanies immune response to injury or infection
- Combating diseases arising from microbial infections or cancer

Immunomodulation may involve induction, enhancement, amplification, or suppression of any part or phase in the immune response toward a particular disease state. Hence, it may involve strengthening or suppression of the indicators of cellular and humoral immunity. Immunomodulators are natural or artificial agents which could stimulate, suppress, or modulate any of the elements of the immune system, whether innate or adaptive immune response (Wen et al. 2012). There are three (3) types of immunomodulators:

- Immunoadjuvants
- Immunostimulants
- Immunosuppressants

3.3.4.1 Immunoadjuvants

Adjuvant is an agent that stimulates the immune system by enhancing immune response to vaccines without producing specific antigenic effects. They act as adjuvant to pharmacological treatments, most importantly in viral infections and cancers (Hui et al. 2018; Temizoz et al. 2016).

Adjuvants perform any of the following three functions:

(a) Serve as the place where antigens are stored and from which they are slowly released.
(b) Assist in targeting antigen to immune cells and thereby facilitate phagocytosis.
(c) Enhance and exert a modifying influence on the type of immune response induced by the antigen (Feng et al. 2020).

Sometimes, adjuvants may give warning signal required by the immune system in order to be responsive to an antigen just like it would respond to an active infection. Additionally, immunoadjuvants have been proposed for solving a critical challenge to vaccine designers by choosing between cellular and humoral immune responses;

Th1 and Th2; immunoprotective and immunodestructive; and immunoglobulin E versus immunoglobulin G type of immune responses (Shantilal et al. 2018).

3.3.4.2 Immunostimulants

Immunostimulants are also known as immunoenhancers since they enhance the body's resistance to infections, allergy, autoimmunity, or cancer. Immunostimulants work with both innate and adaptive immune response. Immunostimulants can serve as prophylactic agents, i.e., as immune potentiators in healthy individuals. Immune potentiator activates innate immune cells directly through pattern recognition receptors (RRRs). In individuals with compromised immune conditions, immunostimulants work as immunotherapeutic agent (Chandua and Kailash 2011). However, these agents have no effect on immunological memory cells which makes their pharmacological effect to fade away quickly. Hence, immunostimulants need to be renewed by administering the drug either periodically or continuously (Seyed 2019). On the other hand, immunostimulants boost endogenous immune defenses, which helps the body to restore or maintain homeostasis (Wen et al. 2012).

Immunostimulants can be successfully used as immunotherapeutic agents for people with comprised immune system. Additionally, they can serve as suitable prophylactic treatment in healthy individuals especially those who easily succumb to viral infections (Wen et al. 2012). For instance, the trained immunity by vaccines, which induce heterologous protection, has been proposed as a logical strategy to boost antiviral defenses and reduce susceptibility to COVID-19 infection Furthermore, immunostimulants can be used as adjuvant anticancer treatments, to counteract the immunosuppressive side effects of cancer therapy (Mohamed et al. 2017).

3.3.4.3 Immunosuppressant

Immunosuppressants restore normalcy either by inhibiting immune response activation or decreasing the activities of its components. Immunosuppressants are used to control pathological immune response that may occur in instances like organ transplantation or autoimmune diseases (Manu and Kuttan 2009; Wen et al. 2012). Rejection processes are the major cause of morbidity in organ transplantation, and it also leads to graft loss. For this reason, immunosuppression in organ transplantation serves the purpose of blunting the immune response of the patient to the allograft and at the same time maintain adequate resistance that will prevent opportunistic infections and malignancy.

The effect of a compound on dendritic cells (DCs) activity is one important factor that is usually considered in immunomodulation because DCs are the link between innate and adaptive immunity (Wen et al. 2012). Any compound that can stimulate DC activities will have great therapeutic potential because they will be able to strongly enhance T cell responses. Such compounds will be immunostimulatory and can serve as good vaccine adjuvants. Meanwhile, compounds that downregulate DC function can induce immune tolerance. Such compounds will be of therapeutic purpose in treating autoimmune diseases and allergies as well as promote transplant tolerance.

Acute inflammation is a key component of immune response which is usually initiated by immune-associated disorders such as autoimmune diseases, microbial infections, and other chronic diseases (Pawelec and Gupta 2019). Unfortunately, chronic inflammatory responses can impact the immune function negatively. Innate immune cells including T-lymphocytes and B-lymphocytes are usually affected by chronic inflammation. For this reason, anti-inflammatory immunomodulators are very important and have attracted a lot of attention as potential chemopreventive agents (Pawelec and Gupta 2019). These immunomodulatory substances have been documented to ameliorate chronic inflammation, which is responsible for the transition of normal cells to cancer cells (Mohamed et al. 2017).

Anything that disrupts gut microbiome may stimulate diseases as they play an important role in maintaining immune function (Zheng et al. 2020). The implication of this is that immune defense can be strengthened by promoting activities of the gut microbiome through supplementation with prebiotics or probiotics. Supplementation with prebiotics or probiotics is an appropriate preventive measure to disruption of microbial communities, which represents an alternative immunomodulatory strategy (Michel et al. 2018).

3.3.5 Modulation of Immune Function by Foods

The function of food is beyond growth and development; food is also essential for the maintenance of a good state of health and a host's ability to resist detrimental changes by foreign and pathogenic substances. Much like any other cell, cells of the immune system require appropriate nutrition for proper functioning (Childs et al. 2019). Through feeding, individuals are frequently exposed to foreign proteins and other substances that are immunologically significant (Farias et al. 2014). Therefore, it may be expected that the influence of food components on the immune system may be beneficial or detrimental.

A healthy immune system does not only involve an adequate response exclusively to pathogens but also a moderation of that response to prevent damage to innocuous proteins (Jeurink et al. 2019). During an immune response, there is an increased demand for both nutrient and energy, and adequate nutrition is essential to meet these demands (Childs et al. 2019). The beneficial effect of food on the immune system could either be by stimulating a host's defenses (against infection) or suppressing immune response (in allergies and chronic inflammation) (Hachimura et al. 2018). In addition to stimulating the host's defenses, appropriate food intake also helps to resolve the immune response quickly in order to prevent chronic inflammations that may have further deleterious effects (Childs et al. 2019).

In order for the body to maintain cell homeostasis and for the cells to perform their respective functions, there is a need for sufficient supply of various nutrients to the body. One of the key purposes of immunology research in nutrition is to establish dietary factors that are needed for maintaining strong immunity that will strengthen the body's defense for protection against pathogen. It is also important to define what their optimal intake should be. The immune system can therefore be regulated by

functional foods, and this can be either through immunostimulation or immunosuppression.

3.4 Immunomodulatory Properties of Probiotics

Probiotics are beneficial living microorganisms, which can be administered through diet or pharmaceuticals to boost the health of the host (Hill et al. 2014). That means that the benefits of probiotics are beyond mediation of gut microbiota. Probiotics are now being used in health management as alternative therapy. They are also used to complement foods and pharmaceutical agents in lifestyle medicine (Sanders et al. 2019). An important indicator for measuring the effectiveness of probiotics is its ability to stick to the gastrointestinal tract without passing out via gut motility. This makes it possible for the probiotics to multiply and eventually colonize the gut, thereby modulating the immune system in every part of the body through the provision of competitive restraint on the pathogens (Guimarães et al. 2020).

3.4.1 Mechanism of Probiotics' Action

Probiotics usually interfere with the composition and function of gut epithelial cells including that of immune cells. Probiotics can boost human immunity by inhibiting the activities of pathogens in the gastrointestinal system. The mechanisms by which they exert their activities on their host include the following:

- Release of antimicrobial compounds
- Stimulating intestinal barrier function
- Competitive exclusion for adhesion sites and nutritional sources
- Immunomodulation (Wan et al. 2019)

Probiotics are very potent for stimulating the production of sIgA (secretory immunoglobulin A), which enhances barrier function (Wang et al. 2016). sIgA acts by promoting the clearance of antigens and pathogenic microorganisms from the intestinal lumen. This is done in three steps:

(i) Hindering the access of antigens and pathogenic microorganisms to epithelial receptors
(ii) Entrapping antigens and pathogenic microorganisms in mucus
(iii) Facilitating the removal of antigens and pathogenic microorganisms by peristaltic and mucociliary activities

Nevertheless, probiotics can also interact with the intestinal immune system and other specific immune cells leading to production of selected cytokines.

Selected species of *Lactobacilli* and *Bifidobacteria* have outstanding probiotics coupled with anti-inflammatory properties. They suppress pro-inflammation by

increasing interleukin 10 (IL-10) and Th1-type cytokines. The administration of probiotics can induce both T cell and B cell hyporesponsiveness and can downregulate Th1, Th2, and Th17 cytokines without causing apoptosis.

Inflammatory immune disorders can be treated with probiotics, which stimulates the generation of regulatory DCs and T-regs (Kwon et al. 2010). Probiotics have the potential to suppress intestinal inflammation through the following mechanisms:

(i) Downregulation of Toll-like receptors (TLRs) expression
(ii) Secretion of metabolites that can prevent tumor necrosis factor-α from entering blood mononuclear cells
(iii) Inhibition of NF-κB signaling in enterocytes (Wells 2011)

The underlying mechanisms through which probiotics can combat allergies include modulating the lymphocyte Th1/Th2 ratio to favor Th1 response. Hence, there will be decreased secretion of Th2 cytokines. Moreover, IgE concentrations are decreased, and production of C-reactive protein and IgA is increased (West et al. 2015).

3.4.2 Production of Antimicrobial Substances by Probiotics

Probiotics produce different types of antimicrobial substances that can inhibit both Gram-positive and Gram-negative bacteria. Some of the substances produced by probiotics include bacteriocins, short-chain fatty acids (SCFA), and hydrogen peroxide. Many strains of Lactobacillus spp. secrete both low-molecular-weight bacteriocins (LMWB; molecular weight $\leq$ 1000 Da) and high-molecular-weight bacteriocins (class III) (molecular weight $\geq$1000 Da). The LMWB have been documented to have antimicrobial activities. The mechanism of action of LMWB involves either destruction of the target pathogenic cells through pore formation or inhibition of cell wall synthesis (Hassan et al. 2012).

Another mechanism for the production of antimicrobial substances involves the production of short-chain fatty acids, which helps with acidification and reduction of the intestinal pH. Reduced intestinal prevents the growth of pathogens. Consequently, the intracellular pH is reduced, and the cytoplasm becomes acidic, leading to the collapse of proton motive force. Ultimately, these will lead to inhibition of nutrient transport which have bactericidal effects (De Keersmaecker et al. 2006). Another factor is that probiotic bacteria release microcins, which binds iron siderophore receptors for cell entry. Once they are inside the cell, probiotic bacteria may also produce harmful substances. The overall effect of all these probiotic activities often lead to inhibition of many intracellular enzyme activities. Consequently, the functions of these enzymes are also inhibited, e.g., mRNA translation. All these will lead to the pathogen's cell death.

The role of probiotics is reinforced by the changes in fecal short-chain fatty acid (SCFA) or branched-chain fatty acid (BCFA) concentrations as observed in children with diarrhea (Hemalatha et al. 2017). As a result of the roles that probiotics play in

the modulation of the immune system, they have the potential to prevent certain childhood diseases like eczema and allergies.

3.5 Immunomodulatory Properties of Prebiotics

Prebiotics are nondigestible food substances, which encourage the growth of probiotics and induce their action (Gibson et al. 2017). This means that prebiotics include noncarbohydrate materials, such as polyphenols, and their action goes beyond the gastrointestinal tract. Prebiotics are usually obtained from the nondigestible fiber in certain plant-based foods. There are many types of prebiotics, and the key compounds in prebiotics are galactooligosaccharide, oligosaccharides, and inulin.

Probiotics can make use of dietary fiber and polyphenols, which are present in food to produce health-promoting short-chain fatty acids (SCFA) as well as phenolic acid metabolites. This is the microbiota-mediated activity that is responsible for the prevention and management of chronic diseases. The composition of gut microbiota can change gut barrier and affect regulation of energy metabolism and adipose tissue proliferation. Several metabolic dysregulations that often lead to inflammation of the brain, liver, and intestine can be traced to the gut microbiota (Geurts et al. 2014). With the proper use of prebiotics, this inflammation can be prevented because prebiotics have the ability to lower endogenous pathogens found inside the gastrointestinal (GI) tract and maintain homeostasis of the immune system. This encourages the immune system to keep pathogens under control. This is in addition to swift reaction to eliminate infections from external sources (De Sousa et al. 2011).

The details of well-known prebiotic effects on the immune systems are discussed below:

Oligofructose and inulin mixture: Oligofructans and inulin mixture can stimulate antibody responses toward viral vaccines (Chen et al. 2017).

Fructo-oligosaccharides (FOS): FOS consumption improves antibody response to influenza vaccine and also reduces the side effects that accompany the vaccine (Ford et al. 2018; Pandey et al. 2015). This category of prebiotics can also reduce the diarrhea-associated fever in infants and reduce the need for use of antibiotics. FOS consumption can also shorten duration of diseases and reduce the incidence of febrile seizures in infants (Carroll et al. 2012).

Galacto-oligosaccharides (GOS): Consumption of GOS can increase the level of interleukin 8 (IL-8), interleukin 10 (IL-10), and C-reactive protein. However, it decreased IL-1β in the blood of adults. Similarly, consumption of GOS improves the function of natural killer cells (Joossens et al. 2011).

Acidic oligosaccharides (AOS): AOS can reduce the possibility of atopic dermatitis in low-risk infants.

3.5.1 Mechanism of Prebiotics' Action

Prebiotics encourage the growth of probiotics that compete with species which are detrimental to energy sources. Prebiotics exclude such detrimental organisms by promoting the production of beneficial fermentation substances, such as SCFAs. SCFAs have immunomodulatory properties (Van der Beek et al. 2017).

A novel mixture of GOS, prepared from a probiotics plus GOS produced by industrial β-galactosidase (β-GOS), plays a significant role in immune modulation. β-GOS supplementation has the potential to increase the immunoregulatory cytokine IL-10 while reducing IL-1β expression (Vulevic et al. 2015). β-GOS is also able to improve natural killer (NK) cell activity and increase the blood level of interleukin 8 (IL-8) and C-reactive protein (Vulevic et al. 2015).

3.6 Immunomodulatory Properties of Nutraceuticals from Selected Plants and Phytochemicals

Plants produce diverse secondary metabolites, which belong to different phytochemical classes. Plants produce these metabolites and use them for their protection from predators. They are responsible for the characteristic color, flavor, smell, and texture, which has been linked to many biological activities. Phytochemicals, which have nutraceuticals importance, are bioactive constituents of plants that have various biological activities. One of the attributes of these phytochemicals is that they display immunomodulatory properties that may include immunosuppression, immunostimulation, and tolerogenicity through dynamic regulation of the target immune systems (Spelman et al. 2006).

These phytochemicals, either alone and/or in combination, have enormous medical benefits. They have various biological activities that are of pharmacological significance in human health. They also prevent unhealthy aging, cancer, DNA damage, diabetes, osteoporosis, and heart diseases. Phytochemicals also exhibit robust immunomodulatory and carminative effects (Chen et al. 2005; Kure et al. 2017). Diterpenoid alkaloids from *Aconitum laciniatum* (Ranunculaceae), terpenes and flavonoids isolated from *Ajania nubigena* (Asteraceae), isoquinoline alkaloids isolated from *Corydalis crispa* (Fumariaceae) and *Corydalis dubia* (Fumariaceae) have all demonstrated immunomodulatory bioactivity in dendritic cell line (Wangchuk et al. 2018).

Epidemiological data have established the fact that intake of foods rich in certain phytochemicals can protect the onset of many chronic diseases (Lu and Zhao 2017). These epidemiological data also confirmed that there is a potential effect of phytochemicals on immune function.

3.7 Bioactive Polysaccharides

Polysaccharides are found naturally in plants (seeds, stems, and leaves) as well as in animal body fluids, extracellular fluids, bacteria cell walls, yeast, and fungi. Various polysaccharides such as heteroglycans and proteoglycans can modulate both innate and adaptive immune responses. Polysaccharides such as glucans, mannans (glucomannan, galactomannan, and galactoglucomannan), pectins, fucoidans, galactans (arabinogalactans, carragenans), fructans (inulin, levan), and xylans are the most studied polysaccharides (Ferreira et al. 2015; Oyedepo and Kayode 2020). Naturally derived polysaccharides have specific broad-ranged immunomodulatory properties. Many of these polysaccharides do have interactions with the immune system to upregulate or downregulate specific parts of the host's immune response. This is the reason why they are classified as immune modulators (Wang et al. 2013). Chemical structure, molecular weight, branching, conformation, and the presence of functional groups are some of the structural features that are responsible for the immunostimulatory properties of these polysaccharides. Another major advantage of plant polysaccharides is that they have low toxicity (Albuquerque et al. 2020; Oyedepo and Kayode 2020). Therefore, they are an ideal alternative for immune modulation.

The immunomodulatory activity of polysaccharides includes activation of the following:

- Macrophages/monocytes
- Natural killer (NK) cells
- Lymphocyte- activated killer cells
- Dendritic cells (DC)
- Tumor-infiltrating lymphocytes and other lymphocytes

Polysaccharides also stimulate the release of various cytokines such as interferons, interleukins, tumor necrosis factor (TNF), and colony-stimulating factors. For this reason, polysaccharides are considered to be multicytokine inducers due to their potentials to elicit gene expression for diverse immunomodulatory cytokines as their receptors (Novak and Vetvicka 2008).

β-glucans are polysaccharides with potent immunomodulatory activities, which can affect both innate and adaptive immunity (Tian et al. 2013). Glucans can have either linear or branched chain because of the different glycosidic bonds such as (β1 → 4), (β1 → 3), and (β 1 → 6) or (α1 → 3), (α 1 → 4), and (α 1 → 6) present in the polysaccharide. The (β1 → 3)-D-glucan are the moiety, which are particularly involved in immunostimulatory activity (Ferreira et al. 2015). A type II transmembrane protein receptor known as dectin-1 can bind β-1,3 and β-1,6 glucans and thereby initiate as well as modulate the innate immune response (Schorey and Lawrence 2008). Dectin-1 is expressed on many of the cells that are responsible for the innate immune response (Schorey and Lawrence 2008). Dectin-1 recognizes β-glucans present in bacterial and fungal cell wall. This has a lot of health benefits for humans because β-glucans are absent in human cells. This results in effective

immune responses such as phagocytosis and production of pro-inflammatory factor that eliminates infectious agents in humans (Oyedepo and Kayode 2020).

The immunomodulatory activities of polysaccharides have a direct relationship with their structures. Generally, bioactivities of polysaccharides have a relationship with their composition, molecular weight, tertiary structure, or conformation. Polysaccharides with.

β-1-3, 1–4, or 1–6 branch chains have substantive biological activities, but complex branch-chained polysaccharides with higher molecular weights and anionic structures have higher immunostimulating activities (Kim and Kim 2017). These differences in bioactivity may be as a result of their differences in receptor affinity or receptor-ligand interaction on the cell surface (Li et al. 2016, 2017). Numerous studies have confirmed the immunostimulatory activities of many polysaccharides in animals and humans (Tian et al. 2013).

3.7.1 Mechanism of Action

Immune modulation by plant polysaccharides can be through direct or indirect mechanisms. Direct mechanism is by activating the immune cells, while the indirect mechanism has to do with formation of short-chain fatty acid (SCFA).

Plant polysaccharides can activate the macrophages by interacting with specific receptors on cells. These receptors are known as pattern recognition receptors. Macrophages are involved in immune regulation, and they play a critical role in all phases of host immune response (Zhao et al. 2015a, b). They play a vital role in various types of complex microbicidal functions on the target organisms (Niu et al. 2017). They could bind and interact with polysaccharides through toll-like receptor 4 (TLR4), CD14, dectin-1, mannose receptor, etc. (Hollmig et al. 2009). After the activation of the receptors, downstream signal and production of pro-inflammatory factors will usually follow.

Hence, the immunomodulatory action of plant polysaccharides on macrophages can be through any of the following:

(i) Production of reactive oxygen species (ROS) and reactive nitrogen species (NOS)
(ii) Modulation of cytokines secretion
(iii) Enhancement of cell proliferation
(iv) Activation of macrophage phagocytic activity (Yin et al. 2019)

The immunomodulatory activity of plant polysaccharides is achieved by modulating cytokine release from intestinal dendritic cells. For instance, pectin was documented to hinder the release of IL-6 and IL-10 through induction by synthetic lipopeptide P3CSK4 (Sahasrabudhe et al. 2018). Similarly, β-glucan, arabinoxylan, inulin, and pectin can also increase IL-10/IL-12 ratio and slow down the expression of IL-1, IL-6, IL-8, IFN-γ, IL-12, monocyte chemoattractant protein

(MCP)-1, macrophage inflammatory proteins (MIP)-1α, RANTES, and TNF-α by dendritic cells (Bermudez-Brito et al. 2016).

Another important process that contributes to the immunomodulation activity of polysaccharides is the activation of natural killer (NK) cells. Polysaccharides of *Astragalus membranaceus* (a popular immunomodulatory herb in Chinese medicine) can intensify the activity and killing effects of NK cells. The herb can also promote the proliferation of NK cells (Li et al. 2009). Polysaccharides were also able to boost CD3-CD4-$CD8^+$ NKs in peripheral blood lymphocytes (Li et al. 2011).

Plant polysaccharides have also been documented to modulate adaptive immunity. Polysaccharides from *Astragalus membranaceus* was documented to significantly upregulate the proliferation of B lymphocytes. This action may be through their interaction with immunoglobulins that are found on the surface of B cells (Fan et al. 2012; Hong et al. 2018). Furthermore, *A. membranaceus* polysaccharides also enhanced the number of $CD3^+CD4^+CD8^+$ memory T helper (Th) cells and $CD3^+CD4^-CD8^+$ cytotoxic T cells (Li et al. 2011). The polysaccharides also improved $CD4^+/CD8^+$ T cell ratio (Abuelsaad 2014). An in vitro study by Novak and Vetvicka (2008) established the fact that β-glucan microparticles enhance T-cell activation and proliferation. Polysaccharides are now being explored as a suitable adjuvants for vaccines due to their ability to induce Th1 and/or Th2 type of immune response (Aguilar and Rodriguez 2007).

Some of the immunomodulatory properties of polysaccharides are exhibited indirectly. For instance, dietary fibers are metabolized anaerobically by intestinal bacteria in the cecum and colon to generate SCFA (Luu and Visekruna 2019). These SCFA molecules can cross the gut epithelium and have interactions with surface receptors on immune cells. They interact with surface receptors like G-protein-coupled receptors (GPRs) 41 and 43 (Koh et al. 2016). This activation of GPRs by SCFA is responsible for the modulation of inflammatory signaling pathways, e.g., NF-κB, ERK, and p38 MAPK (Kim et al. 2013).

SCFA are also able to reach the nucleus of T lymphocyte and modulate many of their functions by histone deacetylase (HDAC) inhibition. SCFA can also induce metabolic alterations in T cells through the stimulation of mTOR complex activity. Upon absorption into T cells, SCFA may stimulate activity of mTOR complex and promote conversion of pyruvate into acetyl-CoA. Additionally, the acetyl groups from SCFA are usually linked to CoA and then enter tricarboxylic acid cycle. Subsequent increase in the levels of citrate from TCA cycle is exported from the mitochondria into the cytoplasm. In the cytoplasm, the enzyme ATP citrate lyase converts citrate into acetyl-CoA, which is then used by histone acetyltransferases (HATs) for histone acetylation in addition to the regulation of cytokine gene expression (Luu and Visekruna 2019).

β-Glucans also have anticarcinogenic potentials. They inhibit oncogenesis through their action against potent genotoxic carcinogens (Novak and Vetvicka 2008). Antiangiogenesis may be one of the pathways through which β-glucans reduce tumor proliferation and disrupt tumor metastasis. Hence, β-glucan can serve as an adjuvant to cancer chemotherapy and radiotherapy (Moreno-Mendieta et al. 2017). Additionally, the mechanism of immunotherapy using monoclonal

antibodies (a novel strategy of cancer treatment) could be elicited in the presence of β-glucans (Carvalho et al. 2016).

3.8 Immunomodulatory Properties of Mushrooms

Immunostimulating activities, which is one of the numerous health benefits of mushrooms, have been documented in many Eastern countries (Friedman 2016). Mushrooms are good prebiotic source since they are rich in bioactive polysaccharides (Singdevsachan et al. 2016). These nondigestible polysaccharides in mushrooms have the potential to induce the growth of probiotics bacteria in the gut resulting in the inhibition of pathogens' proliferation (Bhakta and Kumar 2013). They also boost host immune defense through the activation of complement system, enhancement of macrophages, and promotion of natural killer cell function (Friedman 2016). Mushrooms therefore have a significant role in immune response during the treatment of respiratory diseases, atherosclerosis, cancer, and other metabolic diseases (Varshney et al. 2013).

Compounds of mushrooms in crude and pure form have effectively demonstrated antitumor and immunomodulatory activities (Krishnamoorthy and Sankaran 2016). Many polysaccharides, which have been isolated from mushrooms, have been classified as biological response modifiers (BRM), which modify immune responses (Kim et al. 2006). Biological response modifiers (BRMs) have been prepared from the fruiting bodies of mushrooms as well as stalk, spores, and mycelium. They can also be isolated from fermentation broth that is cultivated in submerged culture. Some studies applied BRMs simultaneously with conventional cancer treatments like chemotherapy and radiotherapy to increase the efficiency of treatment (Oyedepo and Morakinyo 2020). Mushroom BRMs have been classified into four major categories going by their chemical structure:

(i) Lectins
(ii) Terpenoids
(iii) Polysaccharides
(iv) Fungal immunomodulatory proteins (FIPs) (El Enshasy 2010)

Immunological alterations which are induced by mushroom polysaccharides include:

(i) Inhibition of prostaglandin synthesis
(ii) Reduction in pro-inflammatory cytokines
(iii) Activation of immune cells
(iv) Increased antibody production
(v) Increased interferon production
(vi) Increased immune activity against a range of cancers,
(vii) Inhibition of tumor metastasis (Saman et al. 2016).

Mechanism of action: Mushrooms produce chemical compounds, which are able to boost the activities of the immune system (El Enshasy 2010). They induce cellular responses through their specific interaction with different cell surface receptors such as dectin-1, complement receptor 3 (CR3; CD11b/CD18), lactosylceramide, and other selected scavenger receptors. Mushrooms polysaccharides (mainly α- or β-glucans and glycoproteins) demonstrated immunomodulatory activities through:

(a) Activation of cytotoxic lymphocyte, i.e., natural killer (NK) cells
(b) Regulation of cytokines production by dendritic cells
(c) Increased production of TNF-α, IL-1, IL-6, IL-8, IL- 12p40, and NO
(d) Expression of iNOS by macrophages (Borchers et al. 2008)

3.8.1 Immunomodulatory Properties of Polysaccharopeptides Extracts from Coriolus Versicolor

The bioactive components of *Coriolus versicolor* mushroom extracts include two polysaccharopeptides (PSPs) derived from two different strains of *C. versicolor*:

(a) COV-1 (PSP): used in China
(b) Polysaccharide Krestin (PSK): used in Japan

These protein polysaccharides have a molecular weight of about 100 kDa. The carbohydrate component of each compound includes mannose, xylose, galactose, and fructose (in PSP) or arabinose and rhamnose (in PSK).

3.8.1.1 Mechanism of Action

3.8.1.1.1 Induction of a Predominantly pro-Inflammatory Cytokine Profile

This is the most common immunomodulatory effect of PSP that has been reported (Saleh et al. 2017). PSP produces remarkable effect on both in vivo and in vitro expressions of tumor necrosis factor (TNF)-α, which induces apoptosis and tumoricidal activities (Bradley 2008). Human peripheral blood mononuclear cells (PBMCs) demonstrated large TNF-α expression and protein production resulting from PSP intake (Lee et al. 2006). This effect is not disrupted by blockade of toll-like receptor 4 (TLR4) and is an indication that PSP does not depend on TLR4 activation (Wang et al. 2013). PSP is also able to induce cytokines related with TNF-α through its stimulation of IL-12 (Ho et al. 2004; Wang et al. 2013). Meanwhile, IL-12 is an established inducer for interferon (IFN)-γ, which is an active potent immunostimulatory cytokine. That is the reason why PSP always promotes IFN-γ expression especially when used with phytohemagglutinin (PHA), a mitogen (Lee et al. 2006). Furthermore, *C. versicolor* can increase the sensitivity of cells to other

stimuli and work in synergy with other factors to enhance immune response (Singdevsachan et al. 2016).

3.8.1.1.2 Effect of PSP on Immune Cell Populations

The stimulatory effects of polysaccharopeptide on many immune cells are diverse, and these include enhancing their proliferation and cytokine release (Ho et al. 2004). PSP is also able to increase the number of monocytes and macrophages such as $CD14^{+}CD16^{-}MHCII^{+}$ monocytes (Sekhon et al. 2013; Sze and Chan 2009). In vitro treatment of purified murine splenic B cells with *C. versicolor* extract resulted in significant proliferative response. The response can, however, be inhibited by BCR blocking antibody, indicating that it has a role in *C. versicolor*-mediated B cell activation (Yang et al. 2015).

3.8.1.1.3 Effects on Adaptive and Innate Immune Responses

PSP enhances immune responses by inducing the production of immunoglobulin and the activities of diverse pattern-recognition molecules (Yang et al. 2015). PSP can also be used as an adjuvant to conventional cancer treatments. They mediate humoral responses via T cell-dependent stimulation of B cell activity and generation of nonspecific polyclonal antibody response (Sze and Chan 2009). β-glucan polysaccharides from *C. versicolor* were documented to activate many pattern-recognition receptors (PRRs), and this is very important for initiating the innate immune response when there is an encounter with a pathogen-associated molecular pattern (PAMP) (Barsanti et al. 2011). Dectin-1 is another PRR that is usually stimulated by β-glucans and expressed by monocytes, macrophages, dendritic cells, and certain T cells (Kang et al. 2013).

3.8.1.1.4 Induction of Superoxide Dismutase (SOD)

SOD is the enzyme that catalyzes the generation of oxygen or hydrogen peroxide from superoxide radicals ($O_2^{\bullet}$). This activity of this enzyme is often disrupted in the tumor microenvironment following myelotoxic regimens. Myelotoxicity leads to a decreased production of cells responsible for providing immunity (leukocytes). Extracts from *Coriolus versicolor* can therefore modulate immune responses and control tumor progression by reducing the stress generated by superoxide radicals in the tumor environment (Parascandolo et al. 2017). The Japanese strain of *C. versicolor* extract demonstrated significant radical scavenging activity similar to that of SOD in vitro (Saleh et al. 2017; Kotsafti et al. 2020). Treatment with PSK (or SOD) can restore the activity of NK cells, which normally would decrease in the presence of free radicals (Kotsafti et al. 2020). Under oxidative stress, lymphocyte surfaces are thought to become anionic, pointing to a potential mechanism by which SOD reverses this surface charge imbalance and rescues their ability to bind targets (Kotsafti et al. 2020). Finally, many of the PSP-induced cytokines (TNF-α, IFN-γ, IL-1, and IL-6) have been linked to increased SOD activity (Saleh et al. 2017).

3.9 Immunomodulatory Compounds from Microalgae

Microalgae are photosynthetic microorganisms, which are naturally living in both marine and freshwater habitats. They can also survive in extreme weather conditions. This ubiquitous capability is the reason why there are able to produce so many interesting natural and bioactive substances (Plaza et al. 2009) with industrial and pharmaceutical interest (Mimouni et al. 2012; Riccio and Lauritano 2020). Extracts, fractions, and pure compounds obtained from microalgae have established biological activities, and immunomodulation is one of such activities (Manzo et al. 2017). Their immunomodulatory effect has two major mechanisms:

(a) Modification of macrophage activation
(b) Release of pro- and anti-inflammatory mediators (Kong et al. 2016)

Diet supplementation with microalgae has been associated with immunostimulatory activities. A diet supplemented with commercially prepared *D. salina* in mice led to greater NK and macrophage activation, and the survival rate of the leukemic mice increased (Kwak et al. 2012).

Immunomodulatory compounds that have been obtained from microalgae include:

(i) **Sulfate polysaccharides:** These are responsible for the stimulation of macrophage cells (Bahramzadeh et al. 2019).
(ii) **Sulfolipids:** Sulfolipids can potentiate the immune system and are thus used as vaccine adjuvants (Manzo et al. 2017).
(iii) **Polyunsaturated fatty acids (PUFAs):** In addition to using microalgae as substitutes for fishery and seafood resources, they have been identified as sustainable and eco-friendly PUFA producers (Khozin-Goldberg et al. 2016). PUFAs are well known for their immunostimulatory and anti-inflammatory properties.
(iv) **Astaxanthin:** Astaxanthin (ASX) is a carotenoid pigment. ASX-containing products are now broadly used as human health food supplements. The commercially available astaxanthin approved by the Food and Drug Administration (FDA) is mainly produced from the microalgae *Haematococcus pluvialis*. ASX can enhance immune response through increased NK cell cytotoxic activity and increased total T and B cell subpopulations (Park et al. 2010; Davinelli et al. 2019).

3.10 Immunomodulation Activity of Functional Fatty Acids

Among all the classes of fatty acids, short-chain fatty acids (SCFA) are produced by gut microbiota enzymes such as propionate-CoA transferase and propionaldehyde dehydratase. These enzymes produce SCFA during the metabolism of polysaccharides or peptides containing branched-chain amino acids (Feng et al.

2018). Bacteroidetes are the gut bacteria that solely produce acetate and propionate, while Firmicutes produce butyrate. Meanwhile, some other bacteria such as *Lactobacillus* and *Bifidobacterium* spp. can also produce SCFA (Feng et al. 2018).

Long-chain fatty acids and not SCFA are responsible for immune-modulating properties. Oleic acid, which represents 49% to 83% of total fatty acids in olive oil, is a widely distributed fatty acids, but unfortunately it is not available in appreciable quantity among other oils (Servili et al. 2013). Essential PUFA include linoleic acid (LA) and alpha-linolenic acid (ALA). Omega-3 and omega-6 fatty acids have been found in several natural sources, although in different amounts (Saini and Keum 2018). Certain vegetable oils (e.g., rapeseed and sunflower oils) contain higher amount of linoleic acid than alpha-linolenic acid. This same trend is also seen in soybean, corn, dried walnuts, and Brazil nuts. On the other hand, higher alpha-linolenic acid to linoleic acid ratio are reported in flaxseed oil (Saini and Keum 2018). Interestingly, green leafy vegetables are good sources of ALA (Kim et al. 2018). Another good source of omega-3 PUFA is fish oil. They are especially rich in DHA (docosahexaenoic acid) and EPA (eicosapentaenoic acid) with lower amounts of docosapentaenoic acid (omega-6 PUFA) (Saini and Keum 2018). However, marine species from the wild habitat are richer in ω3 PUFA when compared to farmed ones, which may be as a result of the feed composition (Saini and Keum 2018).

The immunomodulatory potential of fatty acids is mainly due to their ability to get incorporated into the cell membrane. By this action, they are able to alter membrane composition and change membrane fluidity. These two action lead to modulation of membrane-protein interaction and signal transduction (Di Sotto et al. 2020).

3.10.1 Monounsaturated Fatty Acids (MUFA)

MUFA, especially oleic acid, can modulate the immune system by influencing both innate and adaptive immune response (Yaqoob 2002). It can diminish NK cell activity and also reduce expression of leukocyte adhesion molecules (Jeffery et al. 1997). Leukocyte adhesion molecules are responsible for certain pathophysiological conditions like rheumatoid arthritis (Jeffery et al. 1997). For the adaptive response, oleic acid can inhibit the proliferation of immune cells, probably through cell cycle regulation (Kim et al. 2017; Llado et al. 2010). Furthermore, oleic acid can activate proapoptotic effects in T lymphocyte and B lymphocyte cells. The possible mechanism of this action may be mitochondrial depolarization and ROS production (Llado et al. 2010).

3.10.2 Polyunsaturated Fatty Acids (PUFA)

A conjugated PUFA (18:3) known as punicic acid found in pomegranate seed oil has been shown to improve the immune system development through the stimulation of

$CD4^+$ and $CD8^+$ lymphocyte-mediated immunity. Punicic acid can also increase the immune response against viruses (Zhao and Wang 2018).

Omega-6 PUFAs can increase neutrophil function thereby promoting innate immunity. However, their action may promote inflammation coupled with increasing ROS levels (Radzikowska et al. 2019). Arachidonic acid, which is an omega-6 PUFA, is a precursor of other fatty acids such as prostaglandins, leukotrienes, etc., which function as regulators of inflammation. When there is substantial addition of long-chain omega-3 PUFA to diet, there will be a partial replacement of arachidonic acid in cell membranes by eicosapentaenoic and docosahexaenoic acids. Effectively, there will be a reduction in the production of arachidonic acid-derived mediators. This action is responsible for the anti-inflammatory potential of omega-3 fatty acids. Omega-3 fatty acids can also suppress the production of pro-inflammatory cytokines (Calder 2005).

Dietary omega-3 PUFA can activate macrophage function, by activating G-protein-coupled receptors (GPR) and promoting anti-inflammation (Radzikowska et al. 2019). This action can also affect leukocyte function. Furthermore, omega-3 PUFA can inhibit pro-inflammation responses in dendritic cells and T cells (Radzikowska et al. 2019).

Omega-3 PUFA can positively affect the microbiota composition, which will increase the production of anti-inflammatory compounds, especially short-chain fatty acids (Costantini et al. 2017). Another anti-inflammatory benefit of omega-3 PUFA is their ability to restore impaired barrier function and reduce the production of pro-inflammatory mediators in epithelial cells during inflammation (Radzikowska et al. 2019). Moreover, a strong relationship has been reported between omega-3 fatty acids, gut microbiota, and immunity. This tripartite relationship is an essential factor for maintaining the integrity of intestinal wall.

3.11 Conclusions

When there is nutritional inadequacy, there will be an impairment of the immune function. Conversely, adequate nutrient intake will positively modulate immune function, reduce chronic inflammation, eliminate autoimmune conditions, and decrease the risk of infection. Functional foods and nutraceuticals can maintain or improve immune function. They also improve communication between the innate and adaptive immune systems.

Even though many of these natural immunomodulators are cheap and quite effective, many of them lack proper standardization of the active ingredients. Moreover, lack of analytical test for efficacy and qualitative and quantitative changes in preparations have all contributed to inconsistencies in published results and proper documentation. Randomized, double-blind clinical studies should be conducted on many of the functional foods and nutraceuticals as well as their bioactive compounds. This will help to provide more evidence on the mechanisms responsible for their biological/pharmaceutical activity and document clinical efficacy and safety of these products. In order to have a complete understanding of these functional

foods/nutraceuticals as well as their implications for therapeutic purposes, their mechanism for cellular signaling networks and the nonlinear relationship between dose and effectiveness have to be explored.

Competing Interest The authors declare that there are no competing interests.

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Antioxidants and Immunomodulation

4

Shriya Gururani, Kanchan Gairola, and Shiv Kumar Dubey

Abstract

The immune system, one of the most sophisticated defence systems of the body, is capable of recognizing and eliminating the unlimited varieties of foreign and undesirable agents. A strong defence mechanism is needed for a balanced and disease-free body. However, modern lifestyles and stress generate changes in the endogenous system and physicochemical circumstances, which causes damage and modifies immunity, resulting in the beginning of free radicals, which causes diseases such as cancer, ageing, neurological and cardiovascular disorders. The simple remediation to these perturbations might be progressively used antioxidants which possess the strong potential to scavenge these free radicals. Several compounds like vitamin A, E, C along with lipoic acid and various enzymes possess rich antioxidant properties aiding and improving our body's immune system from several diseases and ageing. The health-promoting capacity of the antioxidants along with its immunomodulatory effects makes them suitable for use in developing antioxidant-based therapeutics. This chapter focuses on the mechanism of antioxidant immunomodulation, as well as the sources, incidence, classification, and potential health implications.

Keywords

Immune system · Free radicals · Immunomodulators · Antioxidants

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4.1 Introduction

Every human wish to have a long, youthful, and disease-free health and disease-free life. When confronted with physical, emotional, or social challenges, being healthy is a state in which one is more likely to cope and adapt (Huber et al. 2011). Having a strong immune system in good working order is necessary to sustain good health, and a remarkably sophisticated defence mechanism is the key for a balanced state and satisfactory biological defence against infection, any harmful biological invasion, and diseases, with tolerance to evade allergies and autoimmune diseases. It is hard to believe that some indispensable elements participating in the immune system can be the basis for severe deleterious effects on the body. The immune system basically involving innate or adaptive immunity majorly plays the protective role in preventing the body from any foreign pathogen invasion and inflammations. The immune system functions such as phagocytosis clues the generation of several reactive molecules which if left unchecked may cause severe harm by inducing oxidative damage followed by a chain reaction profoundly occurring in ageing and inflammatory cells. This problematic situation generated by the immune system can be fixed with the help of antioxidants exerting immunomodulatory effects at the molecular level.

Antioxidants, along with a variety of other oxidising chemicals, have the ability to destroy free radical intermediates; they can block a variety of undesired oxidation reactions by oxidising, effectively halting the chain reaction. Numerous enzymes that scavenge free radicals are found in antioxidants, while glutathione (GSH), tocopherols, ascorbic acid, and thioredoxin are well known for repairing and preventing immunological processes (Devasagayam et al. 2004).

Various vitamins, such as vitamins A and D, ascorbic acid and tocopherols, along with micronutrients like Zn and Se, pro-oxidant metal as iron, copper and low- and high-molecular-mass agents are documented to prevent several immune diseases. Various studies have shown that catalase helps in H_2O_2 detoxification and ethanol metabolism. Glutathione activates several transcription factors, such as AP-1 and NF-κB (Smith et al. 2004). Ascorbic acid plays a critical role in shielding the thiol protein group from oxidation by raising intracellular glutathione levels and decreases ROS and DNA damage along with decrement of tumour necrosis factor-α and interleukin-6 in complete blood cells preventing disease like community-acquired pneumonia (Naziroglu and Butterworth 2005). Being fat-soluble, the α-tocopherol plays a significant role in lipid oxidative degradation chain events in the cell (Pryor 2000). Zinc and selenium both reduce the damage by counteracting ROS produced during oxidative stress and prevent any harm to antioxidant proteins (Maggini et al. 2008, 2018). Antioxidant molecules, both non-radical and free radical quenchers, guard the body from any oxidative destruction by lowering, inhibiting or entirely removing the free radicals, thus preventing any cellular damage and providing a disease-free immune system (Lobo et al. 2010).

4.2 Free Radical Generation

Free radicals and other oxidants have gained prominence in recent years due to their critical involvement in a variety of biological functions and their link to a variety of diseases. Endogenously, reactive species are created as a consequence of regular metabolism in different cell organelles and exogenously as a result of other events. These can be either positively or negatively charged but neutralize themselves by reacting with another molecule resulting in oxidation and reduction (Cheeseman and Slater 1993). Reactive oxygen species (ROS), reactive nitrogen species (RNS), and reactive sulphur species (RSS) are the most common reactive species (Fig. 4.1).

The free radicals from ROS/RNS/RSS include several radicals (Halliwell 2001), which are weak, short-lived and highly reactive resulting in a chain reaction that damages the living cell (Bahorun et al. 2006). Acids, organic peroxides, aldehydes, oxygen-rich molecules, nitrogen-rich molecules, and sulphane from reactive sulphur species are among the non-radical species (Kohen and Nyska 2002; Halliwell 2001). These reactive species show a dual character in being beneficial as well as toxic for the living system. At low or moderate levels, they show the beneficial effects in immune function in the cellular signalling pathways, mitogenic response, redox regulation, apoptosis of affected or defective cells, detoxification of xenobiotics by cytochrome p450 and in oxygenases for the generation of prostaglandins and leukotrienes (Valko et al. 2007; Nordberg and Arner 2001). However, at greater concentrations, they produce severe damage, resulting to oxidative stress and damage to the stability of numerous biomolecules such as lipids, proteins and DNA (Yla-Herttuala 1999; Marnett 2000; Stadtman and Levine 2000), further resulting in various disorders like cancer, cardiovascular disorder, diabetes, liver damage, nephrotoxicity, rheumatoid arthritis, neurological disorders, inflammation and ageing (Patel and Patel 2011; Vana 2017).

Endogenous sources reported to produce reactive species are the mitochondria, peroxisomes, endoplasmic reticulum and phagocytic cells. Intracellular ROS are generally produced from the mitochondria and result in the formation of superoxide ($O_2^{-\bullet}$) via electron transport chain by the fast intake of oxygen and NADPH oxidase activation. Other enzymes, which can produce superoxide, comprise of

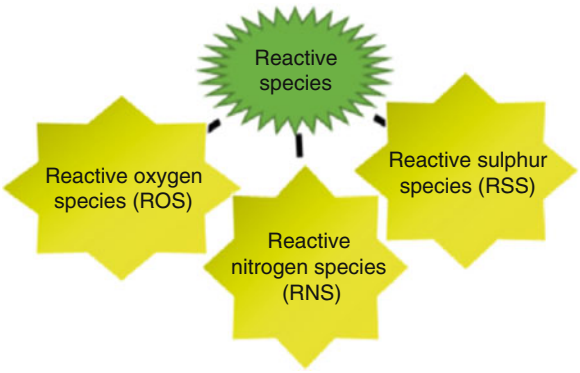


Fig. 4.1 Types of reactive species

lipoxygenase, cyclooxygenase, xanthine oxidase and NADPH-dependent oxidase (Granger 1988) (Eqs. (13.1) and (13.2)).

$$2O_2 + \text{NADPH} \xrightarrow{\text{Oxidase}} 2O_2^{-\bullet} + \text{NADP}^+ + \text{H}^+ \tag{13.1}$$

Hydrogen peroxide is formed as a result of dismutation of superoxide in the presence of superoxide dismutase (SOD).

$$2O_2^{-\bullet} + 2\text{H}^+ \xrightarrow{\text{SOD}} H_2O_2 + O_2 \tag{13.2}$$

The respiratory route in peroxisomes produces H_2O_2 by β-oxidation of fatty acids and various enzymes such as acyl Co-A oxidases and urate oxidase (De Duve and Bauduhuin 1966). The reactive species can be generated by myeloperoxidase (MPO)-halide-H_2O_2 system. MPO is an enzyme found in the neutrophil cytoplasmic granules. In the presence of a chloride ion and MPO, hydrogen peroxide is transformed to hypochlorous acid (HOCl), which is a powerful oxidant that kills microorganisms in the airways and is a rich antimicrobial agent (Babior 1984; Klebanoff 2005). Research show that hypochlorous acid (HOCl) is a causative agent for diseases like atherosclerosis (Van der Veen et al. 2009), Alzheimer's disease and arthritis (Wyatt et al. 2014) (Eq. (13.3)).

$$\text{Cl}^- + H_2O_2 + \text{H}^+ \xrightarrow{\text{MPO}} \text{HOCl} + H_2O \tag{13.3}$$

Haber–Weiss and/or Fenton Reactions can also produce ROS from H_2O_2 and superoxide produced by respiratory burst (Haber and Weiss 1934) (Eqs. (13.4) and (13.5)).

$$H_2O_2 + Fe^{2+} \rightarrow {}^{\bullet}OH + OH^- + Fe^{3+} \ (\textbf{Haber} - \textbf{Weiss reaction}) \tag{13.4}$$

$$O_2^{-\bullet} + H_2O_2 \rightarrow {}^{\bullet}OH + OH^- + O_2 \ (\textbf{Fenton reaction}) \tag{13.5}$$

($NO^{\bullet}$), an important RNS, is created enzymatically from arginine by nitric oxide synthase (NOS) (Eq. (13.6)).

$$\text{L-Arginine} + O_2 + \text{NADPH} \xrightarrow{\text{NOS}} NO^{\bullet} + \text{L-Citrulline} + \text{NADP}^+ \tag{13.6}$$

$NO^{\bullet}$ can be transformed into nitrate, nitrogen dioxide, trioxide and a range of other reactive nitrogen compounds since it is a lipophilic free radical diatomic gas (Lamattina et al. 2003). $NO^{\bullet}$ can also produce RSS when it reacts with thiols. By interacting quickly with superoxide radicals, $NO^{\bullet}$ can generate the highly reactive $ONOO^-$ (Eq. (13.7)).

$$NO^{\bullet} + O_2{}^{\bullet -} \rightarrow ONOO^- \tag{13.7}$$

$ONOO^-$ is recognised to play a role in the aetiology of a variety of disorders, since it causes the synthesis of nitrotyrosine by interacting with aromatic amino acid residues, resulting in enzyme deactivation and immediate cell death in *Escherichia*

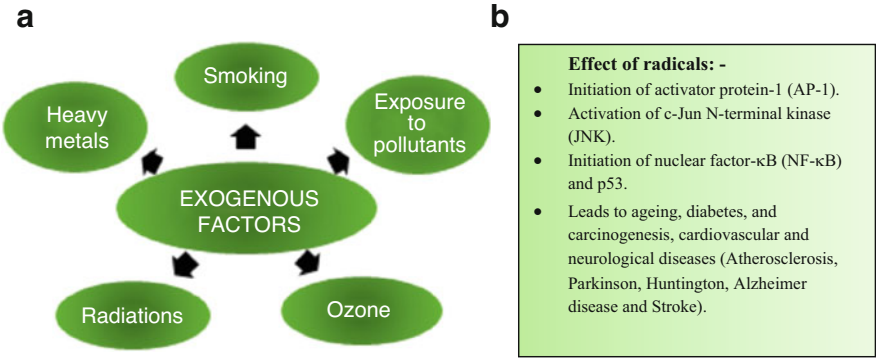


Fig. 4.2 Several exogenous factors (**a**) and the effects of radicals (**b**)

coli (Ischiropoulos et al. 1992; Zhu et al. 1992). $NO^{\bullet}$, on the other hand, is a cytotoxic effector molecule that protects against fungi, helminths, mycobacteria, and tumour cells.

Several enzymes in the endoplasmic reticulum produce ROS, including cytochrome p-450, b5 enzymes, diamine oxidase, thiol oxidase and thiol reductase (endoplasmic reticulum oxidoreductin 1enzyme, Ero1). Mental stress, infection, inflammation, prostaglandin synthesis, immune cell activation, cancer, ageing and ischaemia are some of the other variables that contribute to ROS formation (Cheeseman and Slater, 1993). Because reactive species have been linked to the genesis of a number of degenerative diseases, it is vital to control their activities so that they do not contribute to the increase in oxidative stress.

Exogenous causes such as smoking, pollution or ozone exposure, radiation and hyperoxia deplete enzyme activities and induce cellular damage, resulting in the activation of numerous activator proteins-1 (AP-1), c-Jun N-terminal kinase (JNK), nuclear factor-B (NF-B) and p53 (Fig. 4.2). Heavy metals such as lead and arsenic can cause cellular damage by depleting enzymatic activities via reactions with nuclear proteins and DNA via lipid peroxidation, whereas metal-catalysed reactions generating ROS can modify DNA bases, causing diseases such as cancer, ageing, diabetes and cardiovascular, neurological and autoimmune diseases.

4.3 Antioxidants as Immunomodulators

To cope up with pro-oxidant production, humans have evolved complex immunomodulatory strategies. Immunomodulatory activity normalises or modifies pathophysiological processes by activating or inhibiting, and ultimately changing, an organism's immune system. Immunostimulants stimulate the immune system by improving vaccination response, whereas immunosuppressants suppress it. Immunoadjuvants stimulate the immune system by enhancing vaccine response without having any exact antigenic effect (Alfons and Patrick 2001).

Immunopharmacology, a new and rapidly growing discipline, is gaining popularity as a result of its implications in the search for immunomodulators. Many research studies have identified the critical role of immunomodulatory mechanisms in controlling oxidative damage caused by endogenous and exogenous sources. In a healthy body system, the normal health status is maintained intelligently by upregulating and downregulating immuno-oxidative responses. As part of the defence system, antioxidant molecules can assist in decreasing, reducing or inhibiting the oxidation of other molecules. They protect the cell at various levels from free radical damage by inhibiting or completely scavenging the action of oxidants and free radicals to protect the body from any oxidative damage (Lobo et al. 2010). They either directly destroy the oxidising agents or indirectly degrade it by increasing antioxidant substances. By contributing an electron to the free radical and converting it to a harmless non-reacting molecule through a chain-breaking mechanism, or by healing damage and reconstituting membranes, they either lower free radical energy or inhibit radical formation (Yang et al. 2018).

4.3.1 Mechanism of Antioxidants

The body has two defence mechanisms against oxidative damage:

- The first technique is to use electron donors like glutathione (GSH), vitamins E and C and thioredoxin to scavenge free radicals, and the second is to use enzymes like catalase, glutathione peroxidase and superoxide dismutase (SOD) to eliminate free radicals and reactive species. Metal ion binds to certain metallic proteins such as transferrin, metallothionein, ceruloplasmin and haptoglobin (Wang et al. 2019).
- Antioxidants can counterpoise the effects of oxidants before they attack the cells. In doing so, the antioxidants themselves become oxidised by the process of oxidation. It can be done by breaking the chain cascade with the help of various antioxidants such as vitamin A, ascorbic acid and tocopherols.

4.3.2 Classification of Antioxidants

Antioxidants can be natural, plant based, or synthetic in nature. Enzymatic, non-enzymatic, low-molecular-weight and high-molecular-weight proteins are all types of natural antioxidants. Glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD) are the most common enzyme antioxidants. Enzymatic activity is the initial line of antioxidant protection, and it plays a critical part in the host biological system's overall defence mechanisms as well as the entire antioxidant defence grid (Ighodaro and Akinloye 2018; Yang et al. 2018). Vitamins A and D, tocopherols and ascorbic acid, as well as peptide and some minerals and ions (zinc, copper, selenium), are the most common non-enzymatic antioxidants. Both low- and high -molecular-weight protein and antioxidants help in binding and

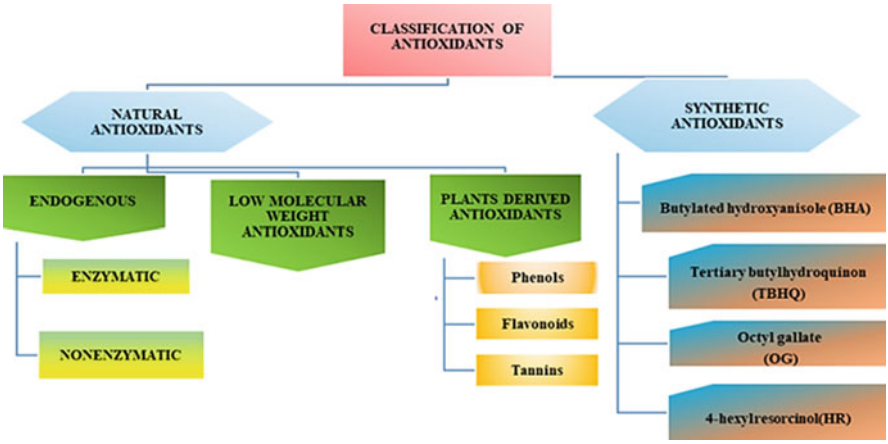


Fig. 4.3 Classification of antioxidants

capturing the free radicals. Several research findings suggest that medicinal plants and spices are good sources of antioxidants (Agarwal 1999). Synthetic antioxidants have been widely utilised in recent years to inhibit unsaturated fatty acid oxidation and prevent cellular damage by free radical scavenging, owing to their superior performance and wide range of availability.

A detailed classification and sub-classification of antioxidants have been displayed in (Fig. 4.3).

4.3.2.1 Natural Antioxidants

4.3.2.1.1 Enzymatic Antioxidants

The human body consists of a set of antioxidant enzymes that serve as the primary line of defence in destroying free radicals. Enzymes including biliverdin reductase, glutathione reductase (GRx), catalase (CAT), glutathione peroxidase (GPx), haeme oxygenase, superoxide dismutase (SOD) and thioredoxin reductase play important roles in the host biological system's defence mechanisms.

1. **Catalase (CAT):** It is found in the blood and helps in the decomposition of H_2O_2 to H_2O and O_2 (Aslani and Ghobadi 2016). CAT acts catalytically whenever the H_2O_2 concentration is higher and acts peroxidically when H_2O_2 concentration is lower. The hydrogen donating atoms (ethanol, methanol and phenol) removes H_2O_2 and oxidises the substrate through peroxidation reaction (Eq. (13.8)).

$$2H_2O_2 \xrightarrow{CAT} 2H_2O + O_2 \tag{13.8}$$

2. **Superoxide dismutase (SOD):** Superoxide dismutase is a putative enzyme discovered by Irwin Fridovich in 1968. Superoxide dismutase (SOD) reduces superoxide anions ($O_2^{-\bullet}$) to hydrogen peroxide (H_2O_2) and helps in repairing cells from any kind of damages (McCord and Fridovich 1969) (Eq. (13.9)).

$$2O_2^{-\bullet} + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2 \quad (13.9)$$

3. SOD has several families that are found in the cytosol and mitochondria. Cu-Zn-SOD is a unique and significant free radical scavenger that exists in both prokaryotes and eukaryotes, including higher plants, and plays a crucial part in the defence system against the toxic effects of oxygen radicals. It protects enzymes and proteins from oxygen toxicity (Peskin et al. 1977; Scandalios 1992). It is an apoptosis inducer, and conjugating it with polyethylene glycol (PEG) has resulted in a stronger and more visible defence against low oxygen levels.
4. **Glutathione peroxidases (GSHPx) and glutathione reductase (GRx)**
   It consists of a group of enzymes that are selenium dependent. The selenocysteine (Sec) residue is found in four of its isoforms (Lubos et al. 2011).
   (a) Cytosolic GSHPx
   (b) Plasma GSHPx
   (c) Phospholipid hydroperoxide PHGSHPx
   (d) Gastrointestinal GSHPx-GI

All secondary glutathione peroxidases enzymes, such as glutathione reductase (GRx) and glucose-6 phosphate dehydrogenase (G-6-PDH), require GSH as a cofactor for effective action. G-6-PDH uses GSH as a cofactor to catalyse the reduction of H_2O_2 to H_2O and alcohols and then creates NADPH to recycle the GSH (Birben et al. 2012). Glutathione reductase is a cytosolic protein that reduces oxidised glutathione by using NADPH (GSSG). It also aids in the maintenance of the GSH/GSSG ratio, as large levels of GSSG inside the cell can cause protein denaturation, DNA breakage and lipid peroxidation (Zitka et al. 2012). With the help of the enzyme glutathione peroxidase (GPx), Lipid hydroperoxides (LOOH) are converted into corresponding alcohols (LOH) (Eqs. (13.10) and (13.11)).

$$LOOH + 2GSH \xrightarrow{GPx} LOH + GSSG + H_2O \quad (13.10)$$

$$GSSG + NADPH + H^+ \rightarrow NADP^+ + 2GSH \quad (13.11)$$

4.3.2.1.2 Nonenzymatic Antioxidants

1. **Glutathione**
   Glutathione, also known as glutamyl-cysteinyl glycine, is a non-enzymatic antioxidant made up of cysteine, glycine and glutamic acid that is found throughout the body. It supports intracellular redox equilibrium in either reduced (GSH) or oxidised form (GSSG). GSH scavenges reactive oxygen species (ROS) such as H_2O_2, O_2, and OH (Misak et al. 2018). It aids in the restoration of vitamin C via the ascorbate-GSH cycle, protects cells by neutralising (i.e., reducing) ROS through increased metabolic detoxification and immune system control and acts as a barrier against hydroperoxide-induced oxidation (Noctor and Foyer 1998).

2. **Vitamin A**
   Vitamin A aids in the regulation of pro-inflammatory TNF and the creation of IL-2, boosting macrophage microbial action in the oxidative burst and phagocytic activity. These macrophages are primarily active during inflammation, Th1 and Th2 cell growth, and differentiation. It keeps the usual antibody-mediated Th2 response going by preventing Th1 cells from producing IL-12, IFN and TNF (Maggini et al., 2008).
3. **Vitamin C**
   Vitamin C (ascorbic acid) is a six-carbon lactone that works as a reducing agent to keep proteins, lipids and DNA from oxidising (Harats et al. 1998; Niki 1987). During oxidative bursts, vitamin C maintains redox equilibrium inside the cells and defends against reactive species (ROS, RNS) (Maggini et al. 2008). It can repair and sustain vitamin E levels in the cell membranes, as well as the antioxidant glutathione, which is important for controlling cytokine production and lowering histamine levels (Wintergerst et al. 2006). It is an effective $ONOO^{-}$, $NO^{\cdot}$ free radical scavenger that quenches $O_2^{-\cdot}$, $OH^{\cdot}$ with HOCl, and reduces H_2O_2 to H_2O through the ascorbate peroxidase reaction.
4. **Vitamin D**
   Calcitriol upsurges macrophage anti-inflammatory cytokine expression, oxidative burst potential and superoxide production (Wishart 2017; Sly et al. 2001; Tanaka et al. 1991; Maggini et al. 2008). It inhibits B cell antibody synthesis and reduces the generation of proinflammatory cytokines (Lin and Li 2016; Zhang et al. 2012; Topilski et al. 2004).
5. **Vitamin E**
   The lipophilic vitamin E is a strong antioxidant that shields cells from free radicals by acting as a chain reaction breaker in lipid peroxidation of cell membranes (Bayani et al. 2009). It increases the synthesis of IL-2 and decreases the production of prostaglandin E2 (PGE2), which protects T cell activity indirectly (Droge 2002; Haryanto et al. 2015). Nuts, seeds, whole grains and vegetable oils are the best sources of vitamin E.
6. **Zinc (Zn)**
   Zinc is an important mineral with anti-inflammatory and antioxidant properties that defend against reactive oxygen and nitrogen species (ROS and RNS), as well as affecting antioxidant protein activity (Jarosz et al. 2017; Maggini et al. 2008). By limiting the production of pro-inflammatory Th 9 and Th17 cells, it regulates the creation and control of cytokine release such as IL2, IL6 and TNF (Kitabayashi et al. 2010; Foster and Samman 2012; Wessels and Rink 2019).
7. **Iron (Fe)**
   It is one of the most significant ions in a biological system, as it is involved in the control and production of cytokines, as well as the generation of pathogen-killing ROS by neutrophils during an oxidative burst (Haryanto et al. 2015). It boosts cytotoxic T cells by activating NF-B if reactive oxygen intermediates are produced (Bubici et al. 2006). It reduces the activity of natural killer (NK) cells, decreases lymphocyte bactericidal activity and compromises cellular immunity by reducing T helper cells (Calder et al. 2007) (Maggini et al. 2008).

8. **Copper (Cu)**
   Copper homeostasis is important for IL2 synthesis, response and oxidative burst, and it accumulates at the inflammatory sites (Saeed et al. 2016). It keeps the intracellular antioxidant balance in check and aids in the inflammatory response (Maggini et al. 2008).
9. **Selenium (Se)**
   It acts as redox regulator and cellular antioxidant. It is essential for the function of seleno-proteins, which potentially counteract ROS produced during oxidative stress (Maggini et al. 2018). Numerous studies show that Se helps in the enhancement and proliferation of activated T cells (cytotoxic lymphocytes) (Rayman 2000). Selinium along with the combination of glucan activates the immune system but inhibits the immunomodulatory effects of glucan in tumourigenesis. It reduces NK-cell cytotoxicity, improves T cell differentiation and proliferation and maintains the ratio of cytotoxic T cells to T helper cells (Haryanto et al. 2015). Furthermore, it improves the immunological response to virus infection in those who have weak immune system (Maggini et al. 2008; Calder et al. 2007).
10. **Thiol Antioxidants**
    Thioredoxin, also known as "moonlighting protein" (Jeffery 1999), is a disulphide reductase protein that functions as an oxidative stress biomarker and a rich electron source for various enzymes including ribonucleotide reductase, methionine sulfoxide reductase, and thioredoxin peroxidase. It maintains thyroxin levels by controlling redox reactions in signal transduction pathways (Arnér and Holmgren 2000).

    The primary thiol-disulphide redox, glutathione (GSH), is an intracellular soluble antioxidant that interacts with pro-apoptotic and anti-apoptotic signalling pathways, protecting cells against apoptosis. It controls and activates activator protein 1 (AP-1), nuclear factor kappa B (NF-kB) and specificity protein 1 (Sp-1) (Masella et al. 2005). It may also enhance vascular endothelial growth factor (VEGF) synthesis by stimulating hypoxia-inducible factor-1 (HIF-1), tumour angiogenesis and treatment resistance by increasing hypoxia-inducible factor-1 (HIF-1) (Welsh et al. 2002).
11. **Carotenoids (β-Carotene)**
    The basic colouring pigments present in plants and microorganisms consist of carotenoids (β-carotene). Its antioxidant activity is due to their delocalized unpaired electrons (Mortensen et al. 2001). It helps regulate many transcription factors (Niles 2004), protects lipophilic compartments from damage and suppresses oxidant-induced NF-kB activation, interleukin (IL-6) production and tumour necrosis factor (TNF-alpha) production (Stahl and Sies 2003).

4.3.2.1.3 Low-Molecular-Weight Antioxidants

Water-soluble antioxidants and fat-soluble antioxidants are two types of antioxidants. Vitamin C, uric acid and other polyphenols are water-soluble antioxidants, whereas fat-soluble antioxidants include vitamins E and A, quinones and bilirubin, among others. They scavenge free radicals, which slows or prevents

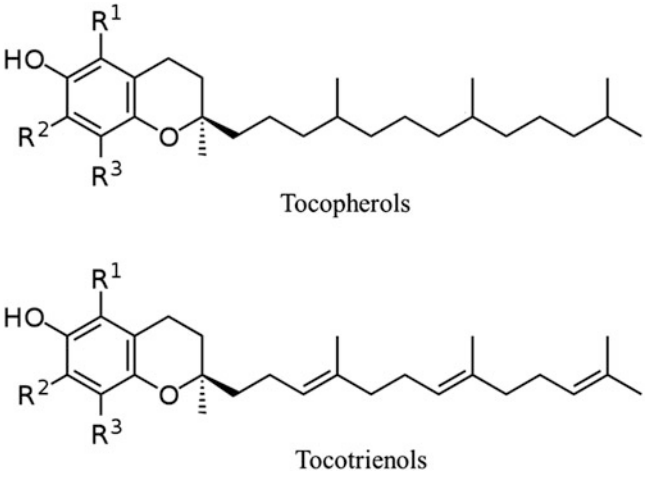


Fig. 4.4 Structure of tocopherols and tocotrienols

cellular damage. Vegetable oils are high in tocopherols and tocotrienols and have a high level of oxidative stability (Fig. 4.4). Tocopherols (200–2000 ppm) decompose the hydroperoxides and may potentially undergo spontaneous oxidation at greater quantities.

4.3.2.1.4 High-Molecular-Weight Proteins

Albumin, ceruloplasmin, transferrin and haptoglobin are plasma proteins that establish bonds with redox metals and regulate metal-catalysed free radicals. Haptoglobin molecules binds with haeme-containing proteins permitting them to circulate in the body. Copper particles can tie to albumin, and ceruloplasmin and transferrin bind to free iron. By quenching the free radicals, these molecules reduces the danger of production and liable movement of reactive radicals throughout the body.

4.3.2.2 Plant-Derived Antioxidants

Various in vivo studies revealed that dietary phytochemical antioxidants can remove free radicals. Phenolics are large, varied categories of secondary plant metabolites found across the plant kingdom. Free radicals can be removed by dietary phytochemical antioxidants, according to many in vivo investigations. Several studies have established that natural phenolic compounds have a high antioxidant capacity, e.g. combining tocopherols with citric acid or isopropyl citrate improves antioxidant characteristics. Phenolic antioxidants (PhH) react with $ROO^{\bullet}$ to produce ROOH and an unreactive phenoxyl radical ($Ph^{\bullet}$) (Eqs. (13.12) and (13.13)).

$$ROO^{\bullet} + Ph\ H \rightarrow ROOH + Ph^{\bullet} \tag{13.12}$$

$Ph^{\bullet}$ undergoing chain termination reactions with $ROO^{\bullet}$ (Hashim et al. 1993).

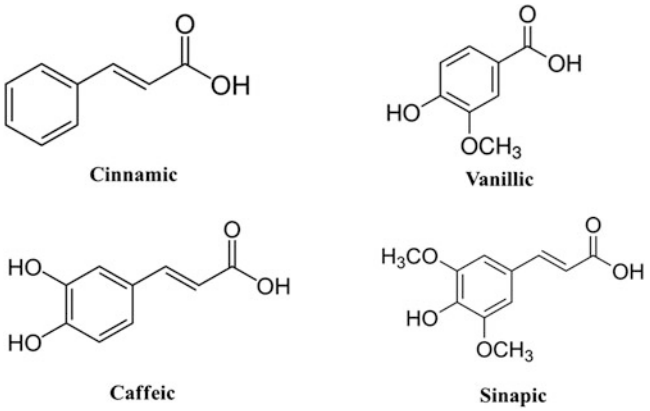


Fig. 4.5 Structure of various phenolic acids

$$ROO^{\bullet} + Ph^{\bullet} \rightarrow Non - radical\ products \tag{13.13}$$

Cinnamic acids, vanillic acids, sinapic acids and caffeic acids are all monohydroxylated phenolic acids that compete with the substrate (RH) for the chain carrying peroxyl radicals and operate as a chain-breaking antioxidant (Taruscio et al. 2004). The structure of different phenolic acids is depicted in the diagram (Fig. 4.5).

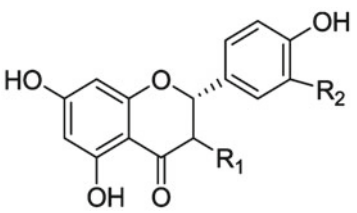
One of the main phenolic compounds consists of flavonoids and tannins (Rababah et al. 2005). Polyphenolic chemicals found in edible plants, such as flavonoids and catechin, have been discovered to have powerful antioxidant properties in previous research (Fang et al. 2002). They aid in the removal of reactive free radicals such as hydroxyl, peroxyl and superoxide radicals (Hopia and Heinonen 1999). Their presence in blood plasma reduced LDL cholesterol oxidation and inhibited the activity of lipoxygenase and cyclooxygenase enzymes (Wang et al. 2005).

These findings suggest that antioxidants derived from plants could be used as an effective immunomodulator for preventing chronic diseases. Several structures resulting from substitutions in the R1 and R2 groups of flavonoids are depicted in (Fig. 4.6) (Bors et al. 1992).

4.3.2.2.1 Medicinal Plants and Spices Having Antioxidants

Exploration of rasayana (medicinal plants) has always been an interesting area in Indian traditional medicinal system, i.e. ayurveda and also for all scientist worldwide as these rasayanas or medicinal plants exhibit a wide range of activities like antioxidant, anti-inflammatory, hepatoprotective, anti-asthmatic, hypocholesterolemic, antifungal, cardiotonic, diuretic and other medicinal activities.

One of the magnificent herb of Lamiaceae family *Ocimum sanctum* commonly known as tulsi helps in delaying hypersensitive response and possesses stimulatory effect on humoral immunity, and research proves that due to prolonged exposure to



a

Flavonoids	Substitution of R1	Substitution of R2
1.Isoquercitrin	O-Glucose	H
2. Luteolin	H	H
3.Myricetin	OH	OH
4.Myricitrin	O-Rhamnose	OH
5. Quercetin	OH	H
6.Rutin	O-Glucose	H

b

Fig. 4.6 (**a**) Flavonoid structure. (**b**) Derivatives of flavonoids based on R1 and R2 substitutions

acute and chronic stress, it exhibits changes in plasma levels of corticosterone along with its antispasmodic, anti-asthmatic, hepatoprotective, hypocholesterolemic, and diuretic functions (Vaghasiya et al. 2010; Singh et al. 2007; Khare 2008; Nadkarni and Nadkarni 2007). *Ganoderma lucidum* belonging to the Polyporaceae family commonly called Reishi mushroom shows antioxidant activity (Habijanic et al. 2001), and its extract enhances the enzymatic antioxidant activity in different body parts of mice (Hasnat et al. 2013). It has anti-tumour immune responses (Pan et al. 2013), and it could become a popular food supplement for cancer patients and others undergoing treatment. It is currently being employed in the research and development of new nutraceutical and pharmaceutical formulations.

Asparagus racemosus of the Liliaceae family commonly called shatavari is used as an ulcer healing agent, nervine tonic and an anti-gout (Nadkarni and Nadkarni 2007; Bopana and Saxena 2007). Being a strong immunomodulatory agent and antioxidant, it provides defence from all kinds of stress (biological, physical and chemical). It also shows myelosuppressive effects when given with different doses of cyclophosphamide (Nadkarni 2005). It demonstrates the immunoadjuvant impact of the diphtheria, tetanus and pertussis vaccine (Gautam et al. 2004). *Eclipta alba* of the Compositae family commonly called bhringraj possesses anticancer, antileprotic, analgesic, antioxidant and antimyotoxic properties (Jayathirtha and Mishra 2004). *Moringa oleifera* of Moringaceae family, commonly called sahijan, is a rich source of vitamins A, B and C, carotenoids and saponins and acts as a strong immunomodulator (Gupta et al. 2010; Kumar et al. 2005). *Piper longum*, commonly called pippali of the Piperaceae family, shows extraordinary antioxidant property (Sunila and Kuttan 2004). Being an ayurvedic herbal medicine, it is used in the treatment of chronic dysentery and worm infestation, increases macrophage migration and shows a phagocytic activity (Sunila and Kuttan 2004).

Chamomile is one of the world's oldest medicinal plants, with a wide range of healing properties ranging from inflammation to serious wounds (Reis et al. 2008). Apoptosis induction in cancer cells is a path paved by Chamomile (Srivastava and Gupta 2007). The plant is often used to treat toothaches, neuralgia and earaches, as

well as external swelling (Hamon 1989). It reduces cyclooxygenase (COX-2) enzyme activity without affecting cyclooxygenase-1 activity and suppresses prostaglandin E2 production generated by lipopolysaccharide (LPS) (Srivastava et al. 2009). Studies revealed that it inhibits the growth of skin, prostate, breast and ovarian cancer (Way et al. 2004; Birt et al. 1997; Patel et al. 2007; Gates et al. 2007; Shukla et al. 2005).

Curcuma longa is a well-known spice from the Zingiberaceae family, commonly referred to as haldi. The essential oil extracted from the plant rhizomes contains phenolic components, which contribute to its antioxidant activity and greater scavenging characteristics (Singh et al. 2010; Maizura et al. 2011). It prevents inflammation by inhibiting numerous ROS (reactive oxygen species) enzymes such as COX (cyclooxygenase), LOX (lysyl oxidase) and XDH (xanthine dehydrogenase). It protects against oxidative species (OS), mitochondrial and protein malfunction, and inflammation (Kim et al. 2012). Curcumin has been found to be beneficial in a number of investigations. Curcumin decreases intracellular ROS and oxidative damage and activates nuclear factor erythroid 2-related factor (Nrf2) targeting genes in primary spinal cord astrocytes, according to numerous research (Jiang et al. 2011).

4.3.2.3 Synthetic Antioxidants

Butylated hydroxyanisole (BHA), tertiary butyl hydroquinone (TBHQ), propyl gallate (PG), nordihydroguaiaretic acid (NDGA), 2,4,5-trihydroxy-butyrophenone (THBP), octyl gallate (OG) and 4-hexylresorcinol (4HR) are the most common synthetic antioxidants used in edible vegetable oil and cosmetics (Fig. 4.7) (Guan et al. 2005; Sindhi et al. 2013; Guo et al. 2006). The suppressive activity of propyl

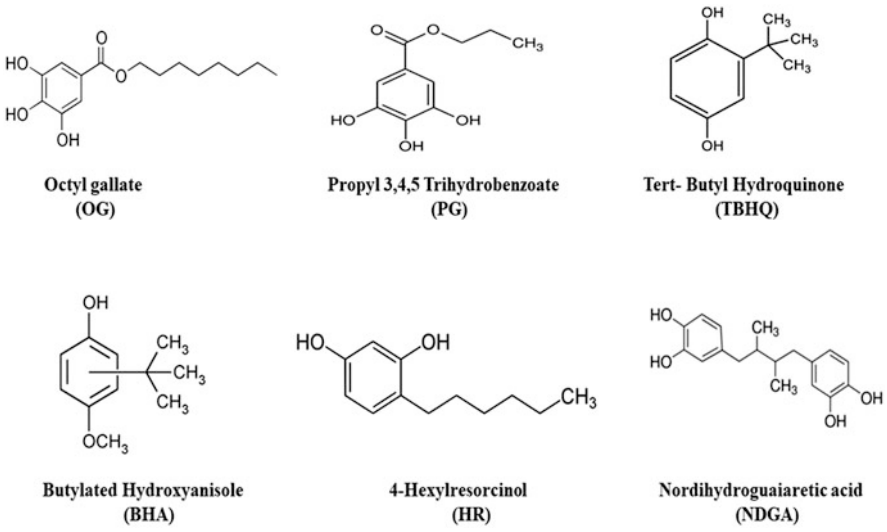


Fig. 4.7 Structure of different synthetic antioxidants

gallate and butylated hydroxyanisole in chain initiation and production of oxidised unsaturated fatty acids is the highest. Most fats and oils are dissolved by BHA. TBHQ is one of the most effective antioxidants for delaying the oxidation of unsaturated lipids.

4.4 Medicinal and Immunomodulatory Applications of Antioxidants

4.4.1 Significance of Antioxidants in Red Cells

According to studies, having an uneven mixture of enzymes and antioxidant system increases oxygen free radical formation. The defective haemoglobin in sickle cell anaemia (HbS) has a good sensitivity for the red blood cell membrane and is prone to membrane lipid peroxidation. They reveal decreased glutathione levels, reduced catalase function and enhanced superoxide dismutase and glutathione peroxidase activities, which are all typical. A stringent diet, supplementation and treatment of antioxidants, as well as immunomodulation, are essential to control this condition (Li Bing 2009).

4.4.2 Treatment of Acute Central Nervous System Injury Using Antioxidants

The rise in reactive oxygen species production results in cell and tissue damage via a variety of cellular and molecular processes. Acute central nervous system (CNS) impairment caused by oxidative stress can result in ischemia or haemorrhagic stroke or trauma. Furthermore, acute brain injury raises glutamate levels, which leads to the production of reactive oxygen species (ROS), which promotes destruction. Antioxidants have been proven to increase survival and related neurological outcomes in various animal models and modest clinical investigations, and they play a significant role in overall wellness and therapeutic ageing.

4.4.3 Use of Antioxidants in Cancer Therapy

In a multifactorial disease such as cancer, tumour cells show elevated levels of ROS. Increased level of oxidative stress created via ROS in cancer cell as compared to normal cells causes an alteration in pro-oncogenic signalling pathways ultimately leading to activation of the oncogenes of cells. ROS being the main culprit for causing carcinogenesis alters various signalling pathways leading to genetic instability, DNA damage and the development of drug resistance (Kumari et al. 2018). Several studies have revealed that combining tannins (a type of polyphenol) with doxorubicin reduces the drug's cardiotoxicity while maintaining the antioxidant's anticancer activity. In recent research, combining a nitro-oxide

(3-carbamoylpyrroline nitroxyl derivative pirolin) with docetaxel and doxorubicin to reduce oxidative stress was found to be helpful (Stanner et al. 2004). Several studies proved that quercetin acts as an adjuvant with cisplatin in ovarian tumour cells and showed similar results when taken with other antioxidants 5-FU (5-fluorouracil), taxol and pirarubicin (Ferda et al. 2016). Selenium is also important in the prevention of cancer and the management of heart failure (Hamid et al. 2010). Lycopene, a pigment present in tomatoes, has been demonstrated to reduce and prevent cancers of the prostate, pancreatic, rectum, oesophagus, cervix and mouth. It also aids in the prevention of heart disease and solar damage to the skin (Sharoni et al. 2000). Thus, appropriate antioxidant intake can quench free radicals in the body, lessening the risk of cancer, atherosclerosis and numerous neurological and autoimmunological illnesses.

4.5 Conclusion

Many crucial biological molecules lose their form and function via free radical production. Such adversarial changes lead to severe disease conditions. Antioxidants are important in this disturbance because they scavenge reactive species (ROS, RNS, RSS), chelate and inactivate non-heme-containing lipoxygenase, prevent lipid peroxidation and quench photosensitizers. Dietary sources rich in vitamins A, C, D and E and Zn, Cu and lipoic acid serves as a strong antioxidant and can act as stimulant, suppressant or an adjuvant exerting immunomodulatory activities. Ischemia, atherosclerosis, diabetes, neurodegenerative disorders, autoimmune illnesses and cancer are just a few of the complicated diseases that antioxidants are being used to regulate and cure. Scientific studies claim that antioxidants are the potent stimulator of the immune system, thereby enhancing immunomodulation. However, much more research is needed to fully comprehend the mechanism of these antioxidants' immunomodulation in order to use them as a viable therapeutic treatment.

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Part II

Nanotechnology and Cancer

Nanotechnology and Immunomodulators in Cancer

5

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Abstract

In the last years, immunotherapy represents a promising strategy for treatment in cancer without massive damaging normal cells, by reprogramming and activating antitumor immunity. However, the adverse events of immunotherapy related to the low specificity in tumor cell targeting represent limits of immunotherapy efficacy. In this regard, nanotechnologies implemented in medicine can represent new opportunities to deliver different immunotherapeutic drugs with high responses and low side effects for specific tumors. The potential of nanotechnologies is represented by the possibilities of carrying immunotherapeutic agents by nanoparticles with various material types, with different shapes, sizes, coated ligands, loading method, hydrophilicity, elasticity, and biocompatibility.

In this review are summarized different types of cancer immunotherapy already approved for cancer treatment or currently studied in clinical trials, which can be possibly correlated with nanotechnologies. Also, the immune-editing process, nanoparticles design strategy in cancer immunotherapy, and types of most promising nanoparticles, including lipid nanocarriers, dendrimers, polymeric and inorganic nanoparticles, magnetosomes, virus-like particles, and carbon nanomaterials, will be discussed. The influences of nanoparticles on enhancing the efficacy of immunotherapeutics in cancer and nanoparticle mediation of immune chemotherapy or combination of immunotherapy with other

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medical procedures are presented also. Various immune regulation mechanisms were described, revealing a complex network consisting of different immune cells, which can be engineered to work cooperatively to destroy tumor cells, taking account of the metabolic status of cancer cells and immunosuppressive tumor microenvironment (TME) as factors that influence immunomodulation using nanosystems. Furthermore, the possibilities of nanotechnologies to influence the local immune tolerance and many steps of the metastatic cascade process are presented.

Keywords

Nanotechnology · Nanomedicine · Cancer immunotherapy · Immune checkpoint blockade · Exosomes

5.1 Introduction

The somatic mutation theory (SMT) is the most accepted theory of carcinogenesis that considers cancer a genetic disease and tumor cells are being initiated by mutations that stimulate oncogenic drivers (Sigston and Williams 2017). It is supposed that one cell can experience more than 20,000 damaging events of DNA and more than 10,000 replication errors per day. The replication errors are usually repaired by specific DNA repair pathways (Lindahl and Wood 1999, Preston 2005). A few of not repaired cells acquire potential malignant changes and are recognized and destroyed by the immunosurveillance system as nonself. A cancer cell can express almost 11,000 genomic mutations and, also, can have new tumor-associated antigens (TAA), including products of overexpressed tumor suppressor genes or proto-oncogenes, antigens produced by oncogenic viruses, altered glycoproteins, or glycolipids (Stoler et al. 1999).

These new tumor-associated antigens can be presented with their major histocompatibility complex molecules on the cell surfaces. The antigen-MHC complexes are recognized by the T cell receptor, but for the activation of naïve T cells, additional co-stimulatory signals are required. These are represented by a so-called immunological synapse, represented by CD28 receptor from the surface of T cell and B7 ligand molecules from antigen-presenting cells (APC). The functions of the immune system in cancer were underestimated for a long time because tumor cells suppress the immune response by enhancing negative regulatory pathways (checkpoints) involved in immune homeostasis or adopting features that prevent detection by the immune system. The two well-known checkpoints are CTLA4 (cytotoxic T lymphocyte protein 4) and PD-1 (programmed cell death protein 1). CTLA4 is a negative regulator of T cells involved in the control of T cell activation, being in competition with CD28 and CD86, that are co-stimulatory factors. PD-1 binds to ligands PD-L1 or PD-L2 and represents a cell surface receptor expressed by T cells. These ligands are currently expressed on a diversity of cells, but PD-L2 is found to be expressed especially on dendritic cells in normal tissues. An antitumor

response in mice was demonstrated by blocking CTLA4 and PD-1(Iwai et al. 2002; Blank et al. 2004). It was demonstrated that the inhibition of the interaction PD-L1-PD-1 by some antibodies can obtain clinical tumor responses in various malignancies (Zou et al. 2016). These clinical responses frequently for a long time suggest that many patients with cancer have T cells already suppressed by PD-L1/PD-1. However, the axis PD-L1/PD-1 is not the single mechanism of inhibition of immune response. There are intrinsic features (genetic factors, cytokine secretion) and extrinsic factors (microbiota, exposure to sunlight) of the tumor that are combining to develop a cancer-immune set point, defined as an equilibrium established between promotors or suppressor factors of immunity. These factors are involved in various responses of treatment in patients with similar tumors. Immunotherapy is a dynamic process, targeting simultaneously different abnormalities specific to cancer cells.

5.1.1 Immune Cell Functions in Cancer

The presence of a significant number of leukocytes within a tumor described by Virchow has supposed a possible correlation between inflammation and cancer (Grivennikov et al. 2010). Actually, it is confirmed that the inflammation is the hallmark of cancer, including the presence of inflammatory cells and mediators similar to those reported in chronic inflammatory responses (Mantovani 2018).

The tumor microenvironment (TME) includes immune cells from innate and adaptive immunity. T cells identified in TME from different tumors are represented by tumor-infiltrating lymphocytes (TILs) and have an essential role in tumor initiation and progression (de Visser et al. 2006). TILs can have both pro-tumoral and antitumoral activity. As an example, inhibition of tumor growth is achieved by CD4+ T helper 1(Th1) and T helper 2 (Th2), CD8+ T cells, and natural killer (NK) T cells, by stimulating the production of interferon gamma (IFN-γ), which is activating macrophages for cancer cell phagocytosis. Macrophages are producing interleukin-2 (IL-2) that enhance Th1 cell differentiation (Lin and Karin 2007). The balance of Th1 and Th2 cells is critical in various immune responses and also in antitumor immune responses. Th1 cells stimulate IFN-γ and IL-2 production, which are essential for the induction of cellular immunity eradicating the tumor mass, whereas Th2 cells play a major role in stimulating the humoral immunity by inducing tumor necrosis (Nishimura et al. 1999).

The antigen-presenting cells (APC) are stimulated by the released IFN-γ and activate CD8+ cytotoxic cells, followed by immediate recognition of peptide antigens presented by the tumoral MHC class I molecules. The majority of tumors are positive for MHC class I and negative for MHC class II. It was demonstrated by some studies that Th2 can also exhibit, together with T regulatory (Treg) cell's pro-tumoral functions, by repressing CD8+ cytotoxicity. Th2 releases IL-4, IL-5, IL-10, and IL-13, inducing anergy to T cells and Tregs that inhibit the CD4+ and CD8+ synthesis (DeNardo and Coussens 2007; Mailliard et al. 2002).

A various group of immature myelomonocytic cells is represented by myeloid-derived suppressive cells (MDSCs) that have enhanced immunosuppression on T cells.

ARG-1 (arginase-1) and IDO (indoleamine 2,3 dioxygenase) are enzymes expressed by MDSCs, producing depletion of tryptophan, cysteine arginine in TME. These enzymes generate T cell receptor complexes with lack of expression on Ag-activated T cells (Srivastava et al. 2010; Gabrilovich et al. 2012) and also are involved in the production of TGF-β, oxygen species, IL-10, and nitric oxide, responsible for suppressing antitumoral immunity (Gabrilovich and Nagaraj 2009).

Other major players in cancer progression are macrophages that have different types of activation related to different signals: 1. The classical activation of macrophages (M1) is correlated with the production of proinflammatory cytokines, which generate reactive oxygen species with cytolytic activity on cancer cells (Mantovani and Sica 2010). and 2. Alternative activation of macrophages (M2) is associated with the production of anti-inflammatory cytokines that promote tissue repair and angiogenesis, favoring tumor progression (Solinas et al. 2009). IFN-γ is stimulating M1 and IL-4 is stimulating M2, leading to the description of a bipolar axis.

The macrophage activation along the bipolar M1/M2 axis involves the participation of several factors as prostaglandin, IL-10, and free fatty acids (Nielsen and Schmid 2017). TME immunosuppression can be promoted also by CTLA-4 and PD-1, both expressed by activated T cells. PD-1 impedes the activation and functions of the effector T cell, preventing the interaction with its ligands PD-L1 or PD-L2. Also, CTLA-4 binds CD80 and CD86 (on the surface of APCs) with more affinity than CD28 and sends an inhibitory signal to T cells (Buchbinder and Desai 2016).

5.1.2 Immunoediting: The Response of the Immune System to Tumor Growth

Cancer immunoediting is a framework consisting of three distinct stages: elimination, equilibrium, and escape. This concept was developed during the experimental observations that the immune system can find and eliminate cancers during their development, maintain some cancer cells after tumor destruction in a dormancy state, and reduce the immunogenicity of cancer cells, providing a mechanism of escape. Understanding these characteristics of the immune system is followed by the recent development of successful tumor immunotherapies.

5.1.2.1 Elimination Phase

The elimination phase represents a modern landscape of cancer immunosurveillance, where the cells of innate and adaptative immunity are working together to identify the presence of a tumor and eliminate it. Sometimes, variants of tumor cells may not be completely destroyed but enter another phase, specifically equilibrium phase, where the immune system is controlling the tumor cell growth. The components of

the immune system that participate in the elimination phase include cytokines (IFN-α/β, IFN-γ, IL-12, and TNF), dendritic cells, macrophages, cells of innate immunity (NK, NKT), cells of adaptive immunity (CD4+ and CD8+ T cells), and immune effector molecules (perforin, TRAIL).

The alert mechanism of the immune system by the presence of a tumor is not fully known. It is supposing that a developing tumor stimulates the production of "danger signals," which are cytokines, such as type I IFNs, that activate the dendritic cells, natural killer, and macrophages. The cells of the immune system recognize the presence of a growing tumor, causing local tissue damage and stromal remodeling. There are generated inflammatory signals to recruit various immune cells as natural killer, macrophages, and dendritic cells are directed to the tumor site. However, various molecules are released from dying tumor cells (e.g., high-mobility group box) or damaged tissues (fragments of hyaluronan). The immune cells are recognizing the presence of a growing tumor, causing local tissue damage and stromal remodeling. This process is generating inflammatory signals to recruit immune cells (NK, macrophages, dendritic cells) to the tumor site.

Another possibility for the activation of the immune system is the expression of stress ligands (e.g., MICA/B), which are activating the receptors of innate immune cells and, as a result, are releasing pro-inflammatory and immunomodulatory cytokines (Schreiber et al. 2011) that stimulate NK T cells and NK cells to produce IFN-γ, which induces partial tumor death by inhibiting the tumor angiogenesis, and are activating the production of chemokines (CXCL11, CXCL10, CXCL09). Dead tumor cells are ingested by dendritic cells, which are migrating in lymph nodes, recruiting new immune cells. Tumor-specific dendritic cells from regional lymph nodes trigger the differentiation of T helper cells, which improve the development of cytotoxic CD8+ T cells (Vesely et al. 2011). In the elimination phase, highly antigenic cancers are frequently destroyed before clinical detection. The tumor cells, which are less immunogenic, are able to escape.

5.1.2.2 Equilibrium Phase

In the second phase of cancer immunoediting, the innate immune system cannot completely eliminate the cancer cells but keeps them in a state of immune-mediated tumor dormancy. Tumors in the equilibrium phase represent a category of dormant tumors. These tumors are selectively controlled by some components of the immune system. The tumor cells and immune system exist in a dynamic balance, where the immune system does not completely eradicate the heterogeneous tumor. Some of the tumor cells are evading from immune-mediated recognition and destruction (Vesely et al. 2011).

There is experimental evidence that the balance between IL-23 and IL-12 decides which immune cell is present at the tumor site and dictates the transition of occult tumors from the equilibrium to the escape phase. High IL-12 increases the stability of tumors developing in the equilibrium phase, allowing cancer cells to maintain the state of immune-mediated dormancy and preventing cancer cell elimination. High IL-23 is suppressing both innate and adaptative antitumor effector responses (Wu et al. 2013). In conclusion, equilibrium represents a model of tumor dormancy

where the immunity of the host controls the tumor growth, both for primary tumors and metastasis for a long time, sometimes for the life span of the individual (Loeser et al. 2007; Eyles et al. 2010). Also, it was demonstrated that the most resistant tumors are staying longer in equilibrium (Wilkie and Hahnfeldt 2013).

5.1.2.3 Escape Phase

Tumor escape from the immune control represents a dramatic result of immunoediting. The escape phase can be considered as a miscarriage of the immune system to destroy or to control the cancer cells, enabling the survival of cell variants, in an unrestricted manner.

The genetic and epigenetic changes of cancer cells can be followed by critical modifications developed to mislead the immunity. The immune system contributes to tumor progression in one of the following ways: selection of the more aggressive tumor types, suppression of the antitumor immune response, or promotion of tumor cell proliferation. A multitude of mechanisms have been reviewed elsewhere (Poschke et al. 2011), but a frame of how tumor cells translate into the phase of immunological escape can be described including two main possibilities: (1) tumor cells achieve cellular modifications that allow the avoidance of immune detection and destruction, and (2) tumor cells are generating an immunosuppressive tumor microenvironment with a substantial slowing effect on immune cells.

It is generally accepted that the immune cells directly suppress the tumor recognition or cytolysis, having demonstrated a little impact on tumor progression due to central or peripheral tolerance induced by tumor cells. In the central tolerance mechanism, self-reactive T cells from the thymus are eliminated or converted to a regulatory phenotype (Kyewski and Klein 2006). As a result of this process, tumors may not be recognized by the adaptive immune system. Peripheral mechanisms of tolerance involve the deletion or nonresponsiveness of T cells in the periphery (Willimsky and Blankenstein 2005). Also, tumor cells can express defects in the pathways of processing and presentation of antigens, enhancing the evasion from adaptive immune recognition. The defects in MHC class I antigen presentation are frequently found in human tumors. The loss of TAP1 (transporter associated with antigen processing) and MHC class I molecules and the inhibition of IFN-γ or IFN-α/β are some examples of these defects. It has been hypothesized that the escape mechanism is due to the camouflage of malignant cells against the immune cells and is considered a consequence of malignant transformation. One of the dreadful cancers, malignant melanoma, was reported two types correlated with the response to immunotherapy according to the pattern of HLA class I expression. The first type, which progresses after immunotherapy, was described as low levels of HLA class I antigens, and the second type, with regression lesions after immunotherapy, was reported with high levels of HLA class I molecules. A plausible hypothesis would be that the tumor microenvironment exerts selection pressure on malignant cells (Carretero et al. 2008). The majority of MHC class I defects were described in carcinomas. Breast cancer, where there is little evidence of immunosurveillance, is an example of high levels of MHC class I defects and also damages in antigen processing and presentation (Georgopoulos et al. 2000).

5.1.3 The Importance of TME (Cancer Immunity Phenotypes)

The immune phenotypes of the TME (tumor microenvironment) have a major influence on immunotherapy and can be classified into three principal phenotypes:

5.1.3.1 Immune-Desert Phenotype

This phenotype has some specific characters like immunological tolerance (losing the response to antigen presentation), ignorance (lack of antigen), and lack of T cells priming (Chen and Mellman 2017). These tumors have a low response to ICI with a worse outcome comparing with other phenotypes, due to the lack of preexisting cytotoxic T cells and a poor clonal collection of T cell receptors. The mechanisms of this phenotype include the inhibition of dendritic cell maturation by the angiogenic growth factor and thereby are reduced the extend of antigen presentation (Veglia and Gabrilovich 2017). Hypoxia is stimulating the expression of chemokines responsible for recruiting regulatory T cells (Tregs) that suppress anticancer immunity and promoting tumor development and progression (Togashi et al. 2019).

5.1.3.2 Immune-Excluded Phenotype

Within this phenotype, the immune cells from the tumor periphery or stroma are hampered by extravascular stroma and immature vessels. Also, the expression of TGF-β and the density of CAFs are enhanced (Chen et al. 2019a, b, c; Chauhan et al. 2019). The tumors with this phenotype are more responsive to immune checkpoint inhibitors than those tumors with immune-desert phenotype due to CD8 + T-effector cell phenotype existing in the stroma, which can proliferate and become active. The major cause of this phenotype is desmoplasia, represented by the secretion of TGF-β and stromal cell-derived factor-1α by CAFs, which prevent the antitumor immunity by restricting cytotoxic T cell to migrate to malignant cells (Mariathasan et al. 2018). A Treg phenotype is developed in naive CD4+ T cell precursors, induced by the TGF-β. Regarding angiogenesis, the expression of adhesion molecules on the vessel walls is dysregulated by angiogenic signals, hampering the leukocyte binding and the migration into hypoxic regions of the tumors (Fukumura et al. 2018). Desmoplasia and angiogenesis are demonstrating to cause hypoxia and to slow down the approach of leukocytes to tumor cells (Hatfield et al. 2015; Rytelewski et al. 2019).

5.1.3.3 Inflamed Phenotype

In the inflamed phenotype, pro-inflammatory cytokines are expressed by T cells from parenchyma, representing a failure of the activity of immune response (Chen and Mellman 2017). They present a large amount of T cells with receptors against tumor-associated antigens but also many immune cells suppressed by hypoxia. This phenotype is considered to have the most potential for sensitivity to ICI (Mariathasan et al. 2018).

Immunosuppressive cells as Treg cells, myeloid-derived suppressor cells, and M2-like tumor-associated macrophages (TAMs) are recruited by VEGF signaling and hypoxia. Immune checkpoint molecule expression is an essential feature of the

inflamed phenotype. The expression of these molecules on immune checkpoint receptors or their ligands on myeloid-derived suppressor cells, DCs, TAMs, and cancer cells are upregulated by VEGF and hypoxia. The pro-tumoral factors like acidity, CAFs, and collagen density are factors that decrease the cytotoxic effect of T cells from parenchyma (Calcinotto et al. 2012). Angiogenesis promoted by tumor cells in all cancer-immune phenotypes can enhance immunosuppression, but certain immune cells can induce also angiogenesis. Monocytic or granular myeloid-derived suppressor cells (M-MDSCs and G-MDSCs, respectively), TAMs (tumor-associated macrophages), and TANs (tumor-associated neutrophils) are common and often associated with increased intratumoral vessel density. Various proteases are expressed by TAMs and TANs, including matrix metalloproteinase-9 (MMP9), which is responsible for releasing extracellular matrix-sequestered VEGF or alternative mechanisms for enhancing VEGF activity (Mazzone and Bergers 2019).

The three main phenotypes of TME described above can be superposed on a new proposed classification of tumors into two categories, "hot" and "cold" tumors, referring mainly to T cell infiltration, and recently a classification of tumors in four categories was suggested: hot, altered, excluded, and cold (Galon and Bruni 2019). This concept for patient stratification is related to the type, density of immune cells within the tumor site, and location that can provide a more accurate information than the classical TNM system in any type of cancer (Mlecnik et al. 2011). The classification in "hot" and "cold" tumors was followed by the development and implementation of the Immunoscore, which is a consensus, standardized scoring system based on the evaluation of two populations of lymphocytes – CD8 and CD3 (Galon et al. 2013; Angell and Galon 2013). The ranges of Immunoscore are from 0 to I4.

Expanding this classification, a new concept of "immune contexture" was developed, which combines the immune parameters that correlate the density, nature, immune functional distribution, and orientation of immune cells within the tumor. These variables are associated with the prediction of response to treatments and long-term survival. Three main coordination profiles were described recently—hot, altered, and cold—that can be translated to the three TME phenotypes in close accordance with the balance between tumor escape and immune coordination, based on the cytotoxic T cell types within a tumor. The altered phenotype was separated into two distinct types—"immunosuppressed" and "excluded" (Camus et al. 2009).

There is a great variety in the TME composition within various cancer types and among patients with the same cancer and even in different tumor sites of the same patient (Mlecnik et al. 2018). This diversity of TME is a result of various factors: deregulation of oncogenes, driver mutations, type of passenger mutations in tumor cells, and the presence or absence of immunosuppressive components of TME (TGF-β, PD-1, PD-L1, IL-15) that interfere with cytotoxic T cells, being suggested that TME has different development according to disease progression and recurrence (Yoshida et al. 2016).

It was demonstrated that the tumor cells and their microenvironment are in close communication represented by stimulating and repressive signals. Distinct

metastatic sites have different anatomically and temporally features and exhibit different clinical responses, genomic architectures, and immune characteristics. It was suggested that immune editing, tumor burden, and Immunoscore represent three major factors that have an essential impact on metastatic disease progression. The metastases with the highest probability of recurrence had, as main characteristics, a large size, low Immunoscore, and no immunoediting. On the contrary, the lowest risk group comprised of immune-edited metastases with high Immunoscore and small burden (Angelova et al. 2018).

5.2 Overview on Actual Immunotherapy in Cancer

There is certain evidence that tumors can escape from the immune system attack and the modulation of the functions of immune cells represents the main way of immunotherapy to recognize and destroy the tumor cells (Mellman et al. 2011). Cancer immunotherapy is focused on developing agents that promote the strategies of recognition and destroying tumor cells by the immune system and represents a new option to classical therapies (Sharma et al. 2011).

A classification of cancer immunotherapy can be the following:

(a) Synthetic immunotherapy, programmed to generate new immune responses directed toward targets expressed by tumors, such monoclonal antibodies (MoAbs) and chimeric antigen receptors (CARs)
(b) Molecules designed to enhance the natural immune responses, such as immune checkpoint inhibitors (ICIs) (Majzner et al. 2017)

5.2.1 Cytokines

In the early years, several cytokines were investigated, leading to US Food and Drug Administration (FDA) approval of IFN-α for the treatment of hairy cell leukemia and high-dose IL-2 for the treatment of advanced renal cell carcinoma and metastatic melanoma (Waldmann 2018).

However, the use of cytokines in cancer treatment as a single treatment did not meet expectations because of high systemic toxicities, low intratumoral concentrations for cytokines administered parenterally, the inducing humoral or cellular checkpoints, and activation of MDSCs and Tregs (Conlon et al. 2019). An IL-2 pathway agonist, bempegaldesleukin (NKTR-214 or BEMPEG), was found to induce activation of CD4+ and CD8+ T cells and NK cells over Tregs in blood and TME, increasing the expression of PD-1 on the malignant cell surface. In a phase I study with the combination of BEMPEG and nivolumab in solid advanced tumors, manageable and generally reversible adverse events (AEs) were found, compared with IL-2 treatment. BEMPEG also improves the CD8+ T cell-mediated tumor elimination induced by PD-1 blockade. In the phase II study (PIVOT-02) of bempegaldesleukin plus nivolumab in advanced melanoma, NSCLC, urothelial,

and kidney tumors, a response rate (RR) of 53% in the melanoma cohort was obtained (Adi Diab et al. 2020). BEMPEG plus nivolumab was evaluated in separate phase II and III pivotal studies [PIVOT-10 (urothelial cancer; NCT03785925), PIVOT-09 (RCC; NCT03729245), PIVOT IO 001 (melanoma; NCT03635983), and PIVOT IO 009 (bladder cancer; NCT04209114)].

IL-6 is another cytokine characterized by overexpression in some aggressive cancers (Kumari et al. 2016) that activate the JAK/STAT3 signaling pathway, which has an inhibiting effect that promote an immunosuppressive TME (Johnson et al. 2018). Chemotherapy agents frequently upregulate IL-6 and determine therapeutic resistance to anticancer therapy. Consequently, the downregulation of IL-6 can be a potential therapeutic approach for treating cancer. There are three molecules approved by the FDA for Castleman disease, chimeric antigen receptor (CAR) T cell-induced cytokine-release syndrome, and myelofibrosis/polycythemia vera: siltuximab (IL-6 inhibitor), ruxolitinib (JAK1/JAK2 inhibitor), and tocilizumab (IL-6 receptor inhibitor).

5.2.2 Immune Checkpoint Inhibitors (ICIs)

The discovery of immune checkpoint inhibitors (ICIs) represented an opportunity for an important breakthrough in cancer immunotherapy. Immune checkpoints are receptors of cell surface which modulate the immune system, inducing T cell-mediated antitumor responses against antigenic peptides, which are existing in cancer cells. Inhibitory checkpoints have an essential role in the downregulation of the immune system and overexpression of inhibitory checkpoints in T cells (Simpson et al. 2013). PD-1 and CTLA-4 are the most known of the class of immune checkpoints, which suppress T cell response to cancers and target the tumors to enable antitumor immunity. The success of CTLA-4 and PD-1/PD-L1 inhibitors in treating cancer has developed an extensive area of preclinical and clinical investigations. James Allison demonstrated that cancer immunotherapy can target the suppressive signal mediated by CTLA-4 (Leach et al. 1996). Also, Tasuku Honjo had proved that the mechanism of activation-induced cell death in lymphocytes is mediated by PD-1, which is an important negative regulator of T cell function (Ishida et al. 1992). For these revolutionary concepts, Allison and Honjo were awarded the Nobel Prize in 2018.

5.2.2.1 Mechanism of Action of Immune Checkpoints (ICs)

T cell function combines in a perfect balance the positive and negative signals followed by the elimination of transformed cells. The identification and destruction of damaged cells imply the binding of the T cell receptor (TCR) to peptide-major histocompatibility complexes (MHC) on tumor cells and antigen-presenting cells. IC molecules are involved in the equilibrium of the individual's immune homeostasis, by adjusting the level of physiological immune responses. Various ICs are involved in limiting tissue damage and enhancing self-tolerance, slowing down the inflammatory activity of T cells. Coinhibitory pathways that control the magnitude and

duration of response of T cells to avoid tissue damage and to maintain self-tolerance were developed.

5.2.2.1.1 PD-1

PD-1 is modulating the limit of antigen responses and maintains peripheral tolerance, actioning as an adjustment mechanism of the immune response. In this context, PD-1 (programmed cell death 1, CD279) is an important regulator of programmed cell death of lymphocytes and has a critical role in improving peripheral self-tolerance through its ligands, PD-L1 (CD274) and PD-L2 (CD273) (Freeman et al. 2000). Also, PD-1 is a key coinhibitory receptor expressed on T cells upon T cell activation. PD-L1 is found in different tissues, including lymphoid organs and non-hematopoietic tissues, in contrast with PD-L2, expressed only in lymphoid organs (Hori et al. 2006). T cell activity is disturbed by the interaction between PD-1 and PD-L1, resulting in the inhibition of T cell, cytokine production, altered functions of cytotoxic T lymphocytes killer, metabolic dysfunctions, and finally death of activated T cells (Butte et al. 2007). PD-1 is found in the subsets of T cells, B cells, myeloid cells, natural killer (NK) cells, and cancer cells (Sharpe and Pauken 2018). Coinhibitory pathways that control the response of T cells to reduce tissue damage and to preserve self-tolerance were developed by hosts. There are various pathways involved, including PI3K, VAV, RAS, phospholipase Cγ, and ERK pathways (Riley 2009). Immunocompetent T cells are hijacked by tumor cells to prevent host immune surveillance, enhancing the expression of PD-L1. This is the reason for the clinical use of checkpoint inhibitors in oncology (Ai et al. 2020).

The aberrantly overexpression of PD-L1 in the TME provides multiple activations of oncogenic signals and also an induction of IFN-γ. T cell functions have different responses to PD-1 signaling. The high expression of PD-1 slows down the production of the inflammatory protein 1β by the macrophage, while a low-level expression of PD-1 is blocking IFN-γ production, and a very low level is inhibiting IL-2 and TNF-α synthesis. It was observed that the high expression of PD-L1 on P815 tumor cells slows down the cytolytic activity of CD8+ T cells (Iwai et al. 2002). PD-L1+ cells stimulate PD-1+ T cells, which determine the overexpression of interleukin-10 (IL-10) and the apoptosis of T cells. PD-L1 is generating a "don't eat me" signal sent to the immune system that protects PD-L1+ cancer cells from the destruction mediated by CD8+ T cells. Also, T cell malfunction was described as a hallmark of the majority of cancers (McLane et al. 2019; Syn et al. 2017). Also, PD-L1 can send back to T cells and tumor cells, affecting their survival (Azuma et al. 2008). These observations were the scientific basis to develop drugs to inhibit the PD-1 pathway. Several drugs targeting the PD-1 pathway are approved until now by the FDA, to treat various tumors: monoclonal antibodies nivolumab (anti-PD-1), pembrolizumab (anti-PD-1), atezolizumab (anti-PD-L1), durvalumab (anti-PD-L1), and avelumab (anti-PD-L1).

5.2.2.1.2 CTLA-4

(CD152) is a T cell surface glycoprotein, a member of the CD28 immunoglobulin family (Ig) and can interact with antigen-presenting cell-derived B7–1 and B7–2.

The proliferation of IL-2 secretion by T cells can be reduced by cross-linking of CTLA-4 and CD28. These are delivering opposing signals to T cells, influencing the response to activation. CD28 through co-stimulatory signals enhances the antigen receptor of T cells, while CTLA-4 promotes inhibitory signals (Krummel and Allison 1995). CTLA-4 is in competition with CD28 to bind to the same ligands, CD86 and CD80, with a greater affinity than CD28 and blocking immune responses against self. It was described as a dampening effect of CTLA-4, through which it becomes a fundamental regulator of T cell self-tolerance and homeostasis.

There are two inhibitory mechanisms performed by CTLA-4: a cell-intrinsic mechanism that affects the cells expressing CTLA-4 and a cell-extrinsic mechanism that affects the secondary cells. Early researches have demonstrated that CTLA-4 was shown to remove CD86 and CD80 from the membranes of APCs by trans-endocytosis, inhibiting the CD28 co-stimulation (Qureshi et al. 2011). Intrinsic CTLA-4 signaling is responsible for T cell regulation of CTLA-4. Furthermore, it was demonstrated that CTLA-4 using intrinsic mechanism inhibits early T cell activation, being expressed by regulatory T cells (Tregs), which are stimulating the PI3K/Akt pathway, thereby promoting the activation of mTOR (Syn et al. 2017, Ai et al. 2020).

This function of CTLA-4 to influence the T cell activity was followed by the genesis of the concept of immune checkpoint blockade. Ipilimumab is found as a CTLA-4 inhibitor and was approved by the FDA for the treatment of advanced melanoma in 2011, following durable clinical responses and improved median overall survival (OS) (Hodi et al. 2010). The approval of ipilimumab was the first step in the development of other T cell inhibitory molecules. Strong evidence obtained from a variety of clinical trials led to evaluate the efficacy of PD-1/PD-L1 blockade by other monoclonal antibodies (Brahmer et al. 2015), and the FDA granted accelerated approvals of more immune checkpoint inhibitors. The current estimation of ICI benefit is $<13\%$ of patients with cancer, and a part of patients receiving immune checkpoint blockade (ICB) therapies will develop immune-related adverse events (Haslam & Prasad 2019).

5.2.2.1.3 Clinical Trials with Checkpoint Inhibitors

Various ICB therapies are currently used in clinical trials in different stages of neoplasms. Camrelizumab, pidilizumab, sintilimab, BMS-936559 (MDX-1105), and toripalimab (JS001) are undergoing clinical trials, being investigated for their efficacy and safety profiles (Huang et al. 2019; Fried et al. 2018; Ishizuka et al. 2019). Efforts are being made in finding and determining new immune targets, dosage regimens, and strategies for combining of ICBs with targeted therapy, chemotherapy, radiotherapy, and other immunotherapeutic modalities, to obtain an improvement of therapeutic efficacy. Possible obstacles in obtaining therapeutic efficacy of ICB include tumor resistance, found when cancer cells decay the bioactivities that are connected with cell signaling, immune recognition, gene expression, and DNA damage and/or extrinsic resistance (Fares et al. 2019).

5.2.2.2 New Immune Checkpoints

Two types of novel immune checkpoints were described: one type is represented by co-stimulatory T cells (e.g., GITRL), and the second type includes molecules that have functions of suppressive factors (e.g., VISTA).

5.2.2.2.1 Co-Stimulatory Targets

GITR and GITRL

Glucocorticoid-induced tumor necrosis factor receptor-related protein (GITR) demonstrates a high expression on Foxp3+ Tregs. GITRL represents the specific activate ligand of GITR. Human (h)GITR is described as a type I transmembrane protein that has a sequence identical to murine (m)GITR. GITR has a low expression on effector CD4+ and CD8+ T cells but is overexpressed when these cells are activated, being enriched on Tregs. But GITR also can be expressed on macrophages, DC, and NK cells. The expression of GITRL is reported on APCs. A major factor of GITR regulation on Tregs is represented by Foxp3, but NF-κB, NFAT, and CD28 signaling are also regulators of GITR in T cells (van Beek et al. 2019; Zhan et al. 2008). MAPK and NF-κB pathways are majorly involved in modulating GITR signaling and growing of Bcl-xL expression on CD8+ cells and can suggest a possible role for GITR in promoting cell survival. GITR and GITRL expressions are found on hematopoietic cells, epidermal keratinocytes, and osteoclast precursors, and the axis GITR/GITRL is involved in various cytological functions other than immune modulation (Xuan 2020).

GITRL-Fc demonstrated an effective antitumor immunity in vivo and in vitro. The therapy with GITR agonists could activate the T cells by enhancing IFN-γ synthesis. There are three molecules related to GITR/GITRL axis under phase I clinical trials. TRX518 is a fully humanized Fc-dysfunctional glycosylated IgG1κ monoclonal antibody that regulates hGITR signaling. There are two phase I trials – NCT01239134 study in melanoma and NCT02628574, where TRX5018 is associated with an anti-PD-1 drug in patients with advanced refractory solid tumors. These trials demonstrated that TRX518 treatment is safe and with acceptable side effects, and further investigation is warranted. MK-4166 is another IgG1 agonist anti-GITR mAb with high-affinity interaction with GITR that promotes TCR and the proliferation of TILs, decreasing in vitro the suppressive functions and proliferation of Tregs (Sukumar et al. 2017). There are several phase I studies in combination or not with pembrolizumab (NCT02132754 and NCT02553499). BMS-986156 is an anti-GITR antibody investigated in phase I trials, single or combined with nivolumab in solid cancers (NCT02598960).

4-1BB and 4-1BBL

4-1BB (CD137) is a surface glycoprotein from the tumor necrosis factor receptor family activated by binding on its ligand, 4-1BBL (CD137L), with a co-stimulatory function on various immune cells (Tregs, NK cells, NK T cells). 4-1BBL is expressed on APCs, including B cells, dendritic cells (DC), and macrophages.

The 4-1BB/4-1BBL pathway activation produces co-stimulation signals by JNK, NF-κB, and p38 MAPK pathways and has the consequence of CD4+ and CD8+ T cell activation and proliferation followed by IFN-γ and IL-2 production. Also, 4-1BB promote the cytotoxicity of CD8+ T cells and is involved in IL-15- and IL-21-driven NK cell proliferation (Vidard et al. 2019). Hypoxia-induced factor-1α (HIF-1α) can mediate the high expression of 4-IBB on the surface of TILs, and blocking 4-IBB is followed by a depletion of CD8, CD4, B cells, and NK cells, being considered a possible therapeutic target for cancer treatment (Chester et al. 2018).

Urelumab was the first anti-4-IBB fully human IgG4 monoclonal antibody that demonstrated a cancer treatment potential. In phase I-Ib trials, in combination with nivolumab in melanoma patients, ORR was promising, but it suggested that it was correlated with nivolumab activity, although ORR also included PD-L1 negative cases (NCT01471210 and NCT02253992) (Segal et al. 2017).

Utomilumab is an IgG2 monoclonal antibody that triggers 4-1BB and seems to be safe in phase I clinical trials, without dose-limiting toxicity reported. The same safety profile was described in combination with pembrolizumab, and also some CR and PR confirmed (Tolcher et al. 2017). Other ongoing trials are combined utomilumab with other molecules in various solid or hematologic cancers: avelumab, rituximab, and ibrutinib in lymphomas (NCT03440567); trastuzumab and chemotherapy in breast cancer (NCT03414658); cetuximab and chemotherapy in colorectal cancer (NCT03290937); or anti-OX40 antibody in triple negative breast cancer (NCT03971409).

OX40 and OX40L

Other members of the TNF receptor superfamily is OX40 (CD134) and its ligand OX40L (CD252), usually found on APCs (Willoughby et al. 2017). OX40 is responsible for the promotion of the survival and proliferation of CD4 and CD8 T cells. Other functions of OX40 are leading to the genesis of Th1 and Th2 cells and regulating IL-17 production. OX40L is the ligand of OX40 and is expressed on B cells, endothelial cells, epithelial cells, APCs, NK cells, and other activated T cells (Fu et al. 2020). OX40/OX40L interaction results in promoting of T cells and also decreases the suppressive capacity of Tregs (Polesso et al. 2019). OX40 is also involved in reactivations in memory T cells, having demonstrated an increased number of memory cells after OX40 agonist supplementation. Based on the decreased tumor growth, improved antitumor responses, and prolonged survival in different cancer models after treatment with OX40 as monotherapy or combined with other treatments, multiple clinical studies were initiated, still ongoing, at different stages to evaluate the safety and efficacy of OX40/OX40L agonists (Fu et al. 2020).

5.2.2.2.2 Inhibitory Targets

LAG-3 (Lymphocyte Activation Gene 3)

LAG-3 (CD 223) is a molecule located nearby CD4. Similar to CD4, LAG-3 is binding to MHC-II on APCs (Workman et al. 2002) and is expressed on CD8+ and

CD4+ effector T cells, Tr1 cells, CD4+Treg, pDCs, B cells, and some NK cells. LAG-3 is an MHC-II ligand and suppresses CD4+ T cell proliferation and slows down the cytokine response (Huard et al. 1995). The enhanced level of LAG-3 is stimulated by IL-12, IL-7, and IL-2 on human-activated CD4+ T cells. LAG-3 enhances the proliferation of LAG-3-positive T cells and NK cells. LAG-3 and PD-1 are both expressed and upregulated on TILs in the tumor microenvironment, and also LAG-3 has a high expression in regulatory IL-10. It was demonstrated that LAG-3 suppresses the effector T cell responses (Long et al. 2018). IMP321 is a form of LAG-3 that can increase IL-12 production. In a phase I clinical trial of IMP321 combined with paclitaxel in metastatic breast cancer patients was obtained an 50% objective response rate. This optimistic result has determined the initiation of a phase 2b clinical trial, which is ongoing (NCT02614833).

VISTA (B7-H5)

VISTA (PD-1H, Gi24, Dies1, SISP1) is expressed on myeloid cells, macrophages, DCs, and neutrophils. VISTA is highest expressed also in initiated T cells, including memory CD4+ T cells and Tregs. There is no expression of VISTA in B cells and low expression in NK cells and CD8+ T cells (Xu et al. 2018).

Based on its expression, VISTA exerts both receptor and ligand functions. VISTA actions as a ligand but also as a receptor to slow down T cell activity. VISTA can be enhanced on myeloid-derived suppressor cells (MDSCs) from acute myeloid leukemia (AML) patients, decreasing the inhibition of CD8+ T cell activation (Wang et al. 2018). Decreased induction of Tregs can be a consequence of VISTA blockade, reducing the functions of natural Tregs. Can be concluded that VISTA may slow down Tregs and promote naïve T cell resistance to Tregs' suppression. It has been found that tumor regression has been reported when administered an anti-VISTA monotherapy in some preclinical studies in melanoma models (Le Mercier et al. 2014). Some molecules are tested actually on early-phase clinical trials: JNJ-61610588 (an anti-VISTA monoclonal antibody) and CA-170 (a small-molecule antagonist that selectively targets VISTA and PD-L1) (Xu et al. 2018; Lee et al. 2017).

TIM-3

Tim-3 (T cell immunoglobulin and mucin domain 3) is an Ig superfamily protein and has ligands represented by galectin-9 (Gal-9), HMGB1, CEACAM-1, and PtdSer (Banerjee and Kane 2018). Tim-3 has different interactions with its ligands, resulting in different results ranging from inhibition of innate immune responses to promotion of apoptosis of Th1 cells, promotion of cross-presentation by dendritic cells (DCs), and the tolerance of T cell (Du et al. 2017). Tim-3 was reported as being active in advanced head and neck cancers, where it is expressed together with PD-1 on TILs after PD-1 blockade (Das et al. 2017). Several clinical studies, related with the safety and efficacy of anti-Tim-3 antibodies, are still ongoing.

TIGIT

Also known as VSig9, Vstm3, or WUCAM, TIGIT is a member of the CD28 family, with actions similar to LAG-3 as a co-inhibitory receptor (Joller et al. 2011). TIGIT represents part of the ligand/receptor network, in which it binds with high affinity on its ligands, PVR2 and PVR3. These ligands are expressed on APCs and tumor cells and shared with DNAM-1. The TIGIT binds with PVR ligands, resulting in inhibition of IFN-γ production followed by downregulation of NK cells. The expression of TIGIT is correlated with IL-10 level and CTLA-4 and PD-1 in Tregs (Stamm et al. 2018).

It was described as a dynamic axis TIGIT/DNAM-1/PVR/CD96, where the signals from TIGIT and CD96 are opposing to stimulatory signals from DNAM-1 (Blake et al. 2016). An in vitro synergistic effect on the proliferation of immune cell, followed by tumor removal and stimulation of protective memory responses due to the inhibition of TIGIT and TIM-3 or PD-1, was demonstrated. Also, it seems that the functions of TIGIT in TME can be correlated with the microbiome. An anti-TIGIT candidate drug, OMP-313M32, was found, which realized a reduction of tumor volume in humanized NSG mice. Another TIGIT antibody, OMP-313R12, was demonstrated to promote tumor growth suppression in a murine colorectal cancer model. The combination of OMP-313R12 with an anti-PD-L1 improved the overall survival in the mice model. Phase 1/2 clinical trials are initiated with anti-TIGIT antibodies, BMS-986207 combined with nivolumab and MTIG7192A combined with atezolizumab (Xu 2020).

5.2.3 Vaccines

Cancer vaccines are designed for the patients to elicit the immune response to fight cancer. Cancer vaccines can be classified into several classes: neoantigen, nucleic acid, dendritic cell, and whole tumor cell vaccines. Neoantigens are proteins with individual specificity, which are generated by mutations in the tumor cell genome antigens that have originated from somatic DNA alterations. Due to their strong immunogenicity and lack of expression in normal tissues, neoantigen vaccines are designed to have specific immunogenicity and tumor properties that can virtually eliminate the risk of off-target side effects while hardening the immune response to destroy cancer cells (Li et al. 2017a, b).

Neoantigens are different from the traditional tumor-associated antigen (TAA). TAA is present in normal tissues but is not unique in tumor tissue, especially in proliferating tumor cells expressing MART-1, HER2, MAGE, and MUC1. Neoantigens express stronger immunogenicity than TAA and higher affinity toward MHC, not being able to be affected by central immunological tolerance. For neoantigen recognition from tumor cells and normal cells, high-throughput sequencing techniques, such as whole-exome sequencing technology, are used. Different types of software applications for the identification of neoantigens were described (Yadav et al. 2014; Ott et al. 2017; Sahin et al. 2017). As foreign antigens, neoantigens can not only promote the antitumor immune response but also decrease

the risk of autoimmunity. Neoantigen-activated T cells can generate highly active T cells, which receptors have a powerful affinity toward MHC-neoantigen-peptide complexes, avoiding the clearance by central immune tolerance (Stone et al. 2015). Due to the fast development of sequencing technology and the highlight of bioinformatics algorithms, it is now possible to accurately find tumor neoantigens and also to predict their immunogenicity and MHC affinity. Also, nucleic acid vaccines that contain mRNA or DNA encoding neoantigens were developed. The delivery to APCs can be intracellular (mRNA) or intranuclear (DNA), producing an antigen expression (Kreiter et al. 2010). These antigens are presented to T lymphocytes, which destroy tumor cells that express antigens with the same epitope. RNA vaccines can bypass the integration into the host cell genome. Various clinical trials of DNA and RNA vaccines have failed actually to demonstrate the efficacy due to the delivery barriers and immunogenicity (Hilf et al. 2019).

Tumor cell lysate-derived vaccines represent other cancer therapy and are classified into two classes: autologous cancer vaccines and allogeneic cancer vaccines. Tumor cell lysates derived from patients (autologous cancer vaccination) or another member of the same species (allogeneic cancer vaccination) are presented by MHC (major histocompatibility complex) molecules to trigger immune responses (Robbins et al. 2011). DC-based vaccines contain engineered DCs derived from patients. These vaccines express TAAs (tumor-associated antigens) that stimulate the antitumor activity of T lymphocytes. An FDA-approved DC-based vaccine is sipuleucel-T (Provenge®) that was developed using autologous peripheral blood mononuclear cells and activation with PAP-GM-CSF (Yang et al. 2019).

5.2.4 Cellular Adoptive Immunotherapy

Cellular immunotherapy represents collection, activation, expansion, modification, and administration of tumor-infiltrating lymphocytes, engineered natural killer cells, T cells, or chimeric antigen receptor (CAR) T cells. There are three major types of cellular therapy described, including TILs, T cell receptor-modified cells (TCRs), and CAR T cells. The fourth, NK cell therapy is developing (Barrett et al. 2015). Engineered T lymphocytes that express chimeric antigen receptors have shown promising antitumoral effects in hematologic cancers, such as chronic lymphocytic leukemia and non-Hodgkin lymphoma and relapsed or refractory acute lymphoblastic leukemia.

The success of CAR T cell therapy in solid tumors has been limited due to heterogeneity of antigen expression, presence in the tumor microenvironment of networks involved in immunosuppression, limiting CAR T cell function, and mobility (Mirzaei et al. 2017). In solid tumors, typical tumor-associated antigens, such as CEA, GD2, MSLN, HER2, EGFR, and many other tumor antigens like MUC1, PSMA, PSCA, FAP, and IL-13Rα2, have different expression on the surface of different cancer cells (Townsend et al. 2018).

Actually, four generations of CAR-Ts were developed, and the difference among each generation is generally related with the construction of the intracellular domain.

The first-generation CAR consists of CD3-ζ or Fc receptor γ (FcRγ) in an intracellular motif, which triggers a temporary T cell activation (Brocker and Karjalainen 1995). The second and third generations of CARs were made following the principle of the first generation, where one or more co-stimulatory molecules (OX40, CD28, 4-1BB) were added. These signaling domains enhance the proliferation of T cells and promote the cytokine secretion, increasing the antitumoral effect (Finney et al. 2004). The fourth generation of CAR T cells involves "armored T cells" and is designed by adding an inducible cytokine-producing cassette that includes cytokines (IL-12, IL-15, IL-18, IL-21) and ligands of receptors on other immune cells or tumor cells (CD40L) (Van Schandevyl et al. 2020). These cytokines can trigger TILs, CAR T cells, and other cells, such as macrophages and NK cells (Avanzi et al. 2018).

5.2.5 Mechanisms of Resistance to Immune Checkpoint Blockades in Cancer

Unprecedent results in clinical trials of cancer immunotherapy were obtained by ICIs, which target CTLA-4 and the PD-1/PD-L1 axis. But an overwhelming obstacle to durable responses to therapy or nonresponses is represented by innate or acquired drug resistance. It was demonstrated that resistance could appear in every stage of the tumor. Molecular mechanisms for the resistance of immune checkpoint blockades (ICB) can be classified into the following: 1. tumor-derived mechanism, 2. T cell-based mechanism, and 3. TME-determined resistance.

5.2.5.1 Tumor-Derived Resistance

Tumor cells develop epigenetic and genetic alterations to avoid the immune cell recognition and destruction and enhance immune evasion, recurrence, growth, and metastasis during the ICI treatments. The following strategies are generated by tumor cells:

5.2.5.1.1 The Lack of Antigenic Proteins on the Tumor Cell Surface

The most direct factor of avoiding recognition by immune cells is the lack of antigenic proteins, such as viral antigens (VAs), cancer-testis antigens (CTAs), tumor-specific antigens (TSAs), and tumor-associated antigens (TAAs). The acquired resistance to immune checkpoint therapy can be generated by the lack of cell surface antigens, such as genetic deletion and genetic and epigenetic modification of T cells. Intrinsic resistance was reported to be generated by low mutational burden and overlapping surface proteins (Gubin et al. 2014).

5.2.5.1.2 Modulations and Mutations in the Oncogenic Signaling Pathway

Some oncogenic signaling pathways can undergo mutations that lead to resistance of tumor cells. For example, Wnt/β-catenin pathway can suppress the dendritic cell-recruiting cytokine CCL4, preventing T cell infiltration (Spranger et al. 2015). The expression of CD47 and PD-L1 can be upregulated by MYC and STAT3 oncogenes, by directly binding to their promoters to intrude the antitumor immunity (Atsaves

et al. 2017). Also, the activation of MAPK pathway can be suppressed by proteins, like VEGF and IL-8, followed by the inhibition of the recruit and improve the T cells (Liu et al. 2013). Mutated or suppressed molecules from IFN-γ pathway could promote tumor cells' escape from its destroying effect (Dunn et al. 2005).

5.2.5.1.3 PD-L1 Expression

The main important element involved in therapeutic resistance to ICI treatment is represented by the immunosuppressive cell surface ligand PD-L1, which makes tumor cells block the activated T cells to find the tumoral neoantigens. Several molecules and signaling pathways were found to be correlated to PD-L1 expression, including mutation of EGFR, MYC overexpression, suppression of PARP, aberrant IFN-γ pathway, CDK5 disruption, amplification of PDJ, loss of PTEN, and PI3K/AKT mutations (Akbay et al. 2013). These disorders are influencing the antitumor T cell responses. New data reveals that there are some variants of PD-L1 generated by tumor cells that can be "decoys" of PD-L1 targeted antibody and may promote resistance to PD-L1 blockade in NSCLC (Gong et al. 2019).

5.2.5.2 Innate PD-1 Resistance (IPRES)

A cluster of genes correlated to mesenchymal transition (WNT5A, TWIST2, AXL, FAP, and TAGLN), immunosuppression (VEGFA, VEGFC, IL-10), and monocyte and macrophage chemotaxis (CCL7, CCL8, CCL2) that were overexpressed in nonresponding tumors was reported (Hugo et al. 2016).

5.2.5.2.1 Epigenetic Modifications

The abnormal epigenetic modification is the main cause of disturbing gene expression. It was proved that histone deacetylase inhibitors promote the expression of MHC and tumor-associated antigens and, in consequence, are improving the antitumor effect of ICI treatment. It was supposed that histone deacetylase inhibitors are involved in the promotion of immune treatment resistance (Vo et al. 2009).

5.2.5.2.2 Absence of Antigen Presentation

Impaired cell surface expression of MHC class I can be produced by the loss of B2M expression and is followed by a damage of antigen presentation to cytotoxic T cells. It was demonstrated in a study of 4512 tumors from 11 types of cancer that deletions and harmful alterations in B2M and HLA class I alleles are correlated with a gene expression signature of cytotoxic immune cells, followed by evasion of cytotoxic T cells' antigen-specific response by tumor cells. In another study of ICI-resistant lung cancer, loss of B2M and MHC-I expression was reported in 75 patients with colorectal carcinoma. The identified B2M mutations and protein loss were proposed as causes of resistance to ICI treatment (Middha et al. 2019).

5.2.5.2.3 T Cell-Based Resistance

T cells can recognize portions of specific antigens on tumor cells, which are presented by dendritic cells with their MHC. The activation of T cell receptors and

signaling pathways are controlling this function of T cells, activating the recognition and killing of cancer cells.

5.2.5.2.4 Absence of T Cells

Loss or absence of T cell function or cells is followed by nonresponsiveness/resistance to the immune checkpoint blockade. The lack of T cells in the regional TME can be the reason of the failure of tumor infiltration and abnormal distribution of functional T cells. It was suggested that the β-catenin pathway suppresses CD8+ T cell proliferation in colorectal cancer (Xue et al. 2019).

5.2.5.2.5 Inhibitory Immune Checkpoints

Alternative inhibitor checkpoints, like LAG-3, TIGIT, TIM-3, CD73, CD38, B7-H3, and A2A receptors, were also involved in ICI treatment resistance. LAG-3, VISTA, and TIGIT checkpoint inhibitors are expressed on the surface of T cells function as compensatory inhibitors of T cell function (Topalian et al. 2015).

5.2.5.2.6 Impaired Formation of T Cell Memory

Some subtypes of effector T cells turn into effector memory T cells being assisted by the DCs and helper CD4+ T cells for obtaining long-term immune memory. In this context, the impairment of the genesis of T cell memory due to epigenetic modifications could be followed by the failure of ICI therapy. In patients with burden tumors, it demonstrated limited reacquisition of memory T cell response with a shorter time of persistence of the information (Pauken et al. 2016).

5.2.5.3 Tumor Microenvironment-Determined Resistance

TME represents a separate pool containing first-value modulators of immune activities against tumors, separate from tumor cells and T cells. These modulators are represented by immunosuppressive cells, cytokines, chemokines, and other molecules.

5.2.5.3.1 Immunosuppressive Cells

Tregs, TAMs, MDSCs, and CAFs are crucial non-tumor cellular components of the tumor-extrinsic mechanisms of primary and adaptive resistance to ICI treatments. MDSCs stimulate tumoral functions, such as invasion, metastasis, and angiogenesis, and they suppress the responses of T cell through reactive oxygen production, local nutrient depletion, and nitrosylation of local chemokines. Normally, MDSCs are positive for CD33 and CD11b, and it was observed that granulocytic MDSCs are CD15+ and monocytic MDSCs are CD14+ (Gabrilovich et al. 2012; Yang et al. 2008). The presence of MDSCs in TME negatively influences the survival in patients with breast and colorectal cancer. A small proportion of intratumoral MDSCs in the TME may enhance the responses to ICI treatments (Solito et al. 2011; Meyer et al. 2014). Tregs are a category of CD4+ T cells existing in TME, which reduce the proliferation and function of local effector CD8 T cells (Teffs). This function is fulfilled through cell contact or by secreting cytokines, such as TGF-β, IL-35, and IL-10.

The depletion of Tregs in TME recovers antitumor immunity (Sakaguchi et al. 2008; Viehl et al. 2006). A poor response to ICI treatments has been correlated with an increased Teff/Treg ratio. The majority of human tumors are infiltrated by Tregs, and the presence of Tregs in TME is related to a weak immunologic antitumor response. In a retrospective study, it was reported that cancer patients have better outcomes of CTLA-4 therapy when demonstrated with the existence of a high amount of FoxP3+ Tregs (Hamid et al. 2011). Tumor-associated macrophages (TAMs) are also involved in the response to immunotherapy. TAMs may exist as classically activated macrophages (M1 macrophage) or alternatively activated macrophages (M2 macrophage) within different microenvironments. M1 macrophages promote an antitumor response by the immune system, while M2 macrophages enhance tumorigenic activities (Chanmee et al. 2014). It was observed that TAMs can slow down T cell responses through B7-H4 in ovarian carcinoma and PD-L1 in hepatocellular.

Also, a low frequency of TAMs enhances IFNs and suppressed tumor development (Kryczek et al. 2006; Kuang et al. 2009). Cancer-associated fibroblasts (CAFs) positive for fibroblast activation protein-α (FAP) enhance the ICB resistance by influencing the distribution of T cells in the tumors. CAFs are involved in extracellular matrix production that physically separates T cells and tumor cells. Also, FAP + CAFs recruit MDSCs into TME by secreting CXCL12, followed by the suppression of T cells (Feig et al. 2013; Yang et al. 2016).

5.2.5.3.2 Immunosuppressive Molecules

The cytokines released in TME by macrophages can develop the local suppression of immune responses. TGF-β was described as a potent negative regulator of effector T cells (Teff) (Park et al. 2018; Lin and Zhao 2015). In a clinical trial in bladder cancer patients, where the disease was resistant to PD-L1 blockade, was found that TGF-β can be upregulated by CAFs and collagen-rich extracellular matrices, which suppress the recruitment of CD8+ T cells into TME (Mariathasan et al. 2018).

Another immunosuppressive molecule is indolaimine-2, 3-deoxygenase (IDO), which might be promoted by IFN-γ and slow down the functions of T effector cells (Teff). IDO inhibitors express antitumor effects when are combined with ICBs (Spranger et al. 2014). Furthermore, other molecules involved were reported potentially competent in ICB resistance: CD73, CEACAM1, adenosine, TIM-3, and CDKs (Gray-Owen and Blumberg 2006; Koyama et al. 2016). Some chemokines may recruit MDSCs and Tregs in TME. For example, CCR4 is expressed in TME by Tregs, and CCR4 inhibitors can suppress the recruitment of Tregs. CCR4 and other molecules, such as CXCL12, CCL5, CCL7, and CXCL8, promote ADCC, decreasing the level of Tregs (Chang et al. 2012; Gil et al. 2014).

5.2.5.3.3 Aberrant Regulation of Signaling Pathways

The PI3K/AKT/mTOR pathway is implicated in the modulation of various cellular functions, including survival, proliferation, and motility. The dysfunctions of PI3K/AKT/mTOR pathway are correlated with the innate resistance to PD-1/PD-L1 blockade (Bai et al. 2017). Also, it was found that the loss of PTEN in melanoma

patients may be followed by the overexpression of cytokines, thus modulating the resistance to ICBs. The suppression of the PI3Kβ could enhance the efficiency of PD-1/PD-L1 blockades. Other studies demonstrate that the activation of Wnt/β-catenin axis may promote T cell exclusion from TME and induce resistance to PD-1/PD-L1 activities (Peng et al. 2016; Spranger et al. 2015).

ICB resistance also includes other cellular signaling pathways like JAK/STAT/IFN-γ and ERK/Erk MAPK pathways (Shin et al. 2017; Hugo et al. 2015).

5.2.5.4 Microbiome Modulation

The gut microbiota is represented by a large number of microorganisms, consisting of essential and opportunistic microorganisms, generally hosted in the gastrointestinal tract as viruses, bacteria, fungi, protozoa, phages, and also archaea. Gut microbiota composition might be useful to explain the various effects of treatment, and manipulating gut microbiota in the appropriate future could be a hopeful adjuvant treatment for cancer immunotherapy. The regulatory effect of gut microbiota on the gut mucosal immune system is currently accepted. It has been demonstrated that *Bacteroides fragilis* promote the CD4+ naive T cell to be transformed into Treg and induce the production of cytokines (Round and Mazmanian 2010). It was found that most Treg cells from the colon belonged to Tregs derived from the thymus, which recognized the antigens from bacteria, such as *Clostridiales*, *Lactobacillus*, and *Bacteroides*, that could favor the tolerance to these bacteria.

It was showed that antibiotics which are decreasing mainly the members of the Clostridium family in gut microbiota composition, produce a decreased number of colonic Tregs (Cebula et al. 2013). Some commensals such as *Escherichia coli* can enhance the pro-inflammatory gut immunity in a "love-hate" relationship (Ivanov et al. 2009; Tomkovich and Jobin 2016).

The regulatory effect of gut microbiota can enhance a regulatory effect on the localized mucosal immune system and also on host systemic immunity via lymphocyte homing, cytokine secretion, cross-reactivation, and recirculation. The detection of immune cells demonstrated that increased gut *Faecalibacterium* is related to an elevated number of CD4+ or CD8+ T cells (Gopalakrishnan et al. 2018). The influence of gut microbiota on the efficacy of anti-PD-1 treatment in metastatic melanoma patients was also noticed. Some bacteria, such as *B. longum*, *Bifidobacterium adolescentis*, *Enterococcus faecium*, *Klebsiella pneumoniae*, *Parabacteroides merdae*, and *Lactobacillus* species, were found significantly elevated in responders at anti-PD-1 treatment, while *Roseburia intestinalis* and *Ruminococcus obeum* were enriched in nonresponders. The influence of gut microbiota was widely studied, and some evidences demonstrate these interferences. A group of bacteria, including *B. adolescentis* and *B. longum*, is enhancing PD-1 by elevating the secretion of IFN-γ and increasing CD8+ tumor-infiltrating T cells (Sivan et al. 2015; Matson et al. 2018).

Bifidobacterium are enhancing the function of DC, upregulating tumor-specific CD8+ T, increasing pro-inflammatory cytokine, and enhancing PD-1 blockade. Other enhancers of PD-1 blockade are *Faecalibacterium*, which increase CD8+

and CD4+ T in circulation and tumor, and *Akkermansia muciniphila*, which promote CXCR3 + CCR9 + CD4+ T cell and the ability of DC with the production of IL-12 (Routy et al. 2018). *Ruminococcus obeum* and *Roseburia intestinalis* were found elevated in patients resistant to anti-PD-1 treatment. The promotion of CTLA-4 blockade by *Bacteroides fragilis* and *Faecalibacterium* was also demonstrated (Vétizou et al. 2015).

5.3 Nanotechnologies in Cancer Immunotherapy

5.3.1 The Value of EPR Effect in Nano-Immunotherapy

The enhanced permeability and retention (EPR) effect was first reported during the studies of inflammation related to bacterial infection (Matsumoto et al. 1984). The EPR effect represents a unique phenomenon of solid tumors that is correlated to anatomical and pathophysiological characteristics. These features can be the inadequate architecture of the vessels; large gaps between endothelial cells in blood vessels; vascular mediators in excess, such as bradykinin, carbon monoxide, nitric oxide, and vascular endothelial growth factor; and defective lymphatic recovery, leading to significant extravasation of plasma components and nanomedicines. EPR effect provided an accelerate development of macromolecular antitumoral drugs, called nanomedicines (Maeda 2010). Different EPR effects were observed in different tumors or different areas of the same tumor, especially in large tumors. Also, EPR effect is a dynamic phenomenon involving pathophysiological factors, biological events inside the body, tumoral growth, and inflammatory processes.

EPR effect represents the basic concept of tumor targeting with nanomedicines and is related with the size, biocompatibility, and conformation of macromolecules. The surface of charge and halftime in circulation are other critical points for the tumor-targeting nanomedicines (Zhao et al. 2005; Campbell et al. 2002). The concept of EPR-based tumor targeting was investigated in recent studies, and the potential of exploring transcytosis for tumor targeting by nanomedicines, especially in highly stromal solid tumors such as pancreatic cancer with low EPR effect, was described (Liu et al. 2019). The EPR effect and nanomedicine effectiveness can be improved by pharmacological and physical treatments employed for the remodeling of the tumor microenvironment. The improvement of the EPR effect can be obtained by incorporating more strategies, such as physical alteration, additional molecular targeting, or physiological modulating of the tumor microenvironment (Park et al. 2019).

5.3.2 Nanoparticles Designed for Modeling Cancer Immunotherapy

Nanotechnology is emerging as a multidisciplinary area that is changing the treatment of many diseases including cancer in the twenty-first century (Farokhzad and

Langer 2009). Also, in the last decade, immunotherapy represents a turning point in cancer treatment. Actually, an interaction between immunotherapy and cancer nanomedicine be described, whixh is demonstrated in various preclinical studies. In the last years, there was an expansion of applications of nanotechnology in the field of medicine, which have also developed novel design concepts to cancer immunotherapy, the so-called nano-immunotherapy (Peer et al. 2007). Various types of materials have been experimented for biomedical applications, including metals, carbon structures, lipids, polymers, and inorganic materials, that can be used for NP production. The delivery of the nanoparticles (NPs) containing bioactive molecules has the goals to increase therapeutic efficacy and reduce side effects of these molecules with improving pharmacokinetics and biodistribution.

There are various applications for which NPs can be used for enhancing immunotherapy in cancer. Some examples are the delivery of antigens and adjuvants as vaccines and the delivery of molecules, antibodies, and viruses, targeting specific cells, such as APCs or dendritic cells that interact for modifying the tumor microenvironment.

Generally, NPs are classified in different categories related with their physical and chemical properties, such as material size, shape, type, charge, and surface chemical modifications elasticity, and now it is generally accepted that all these properties influence their kinetics, bio-distribution, cellular uptake, immunogenicity, and loading efficiency (Grimaldi et al. 2017; Goldberg 2015; Fan and Moon 2015; Kesharwani et al. 2020). Certain properties must be taken into account in choosing the appropriate nanoparticles. The particles of approximately 100 nm, a surface zeta potential between + and - 10 mV, and a PEG surface layer are typically preferred for their optimal pharmacokinetics and biodistribution. Not only the functions of NPs can be drug delivery but also the target of the immune system. The main advantage of nanomaterials is derived from their size, facilitating the intake of immune cells. Various types of NPs that direct target the immune system to generate cytokines and induce humoral and cellular immunity are described (Fontana et al. 2017; Mahjub et al. 2018).

The advantages related to the administration of nanocarriers are the following: their size, facilitating the intake of immune cells; the delivery of therapeutic compounds to a specific target; and in addition, the improvement of immunostimulatory compounds safety profile, allowing an increase in the dosage. Also, the nanocarriers themselves may work as an adjuvant, reducing the need for the coadministration of adjuvants and antigens. Nanoparticles can action as a protective delivery vehicle for many types of cargo related with stability, solubility, and period of half-life. (Qiu et al. 2017).

5.3.2.1 Classification of Nanotechnologies for Cancer Immunotherapy

At present, nanomaterials used in cancer immunotherapy can be classified into polymeric NPs, lipid nanocarriers, metal NPs, mesoporous silica NPs (MSNs), exosome, carbon nanotubes (CNTs), and virus-like particles (VLPs) (Rosalia et al. 2015; Hassan et al. 2016; Wong et al. 2006, Gupta et al. 2014).

5.3.2.1.1 Polymeric Nanoparticles

Polymeric NPs, such as PLGA, dendrimers, and micelles, have been used in several drug delivery and targeting vehicles. These polymeric nanoparticles have some advantages such as versatility in morphology, size, and surface functionalization. Also, they can display high loading of the therapeutics, form hydrogels, or self-assembly into micelles. The disadvantages are the following: proinflammatory molecules' production upon degradation of the polymer and the uncertain degradation and inactivation of the therapeutic payload in the preparation process.

5.3.2.1.2 PLGA

PLGA (poly lactic-co-glycolic acid) is an FDA-approved frequently used polymer, which is biocompatible and biodegradable and can encapsulate many biologically active compounds with low toxicity. PLGA microspheres can target the pathways for MHC class I and II molecules and enhance the maturation of dendritic cells (Waeckerle-Men and Groettrup 2005). PLGA nanoparticles interact with DCs without any recognition of this specific character. PLGA for siRNAs, cytokine agonists, or CpG-coated tumor antigen transportation were designed to enhance the DC uptake of antigens and activation of immune responses of both CTL (CD8+) and Th (CD4+) (Kim et al. 2018; Kokate et al. 2016).

Another nanomolecule, PEGylated IL-10 (pegilodecakin), releases high concentrations of IL-10, increasing the infiltration of TME and cytotoxic activity of CD8+ T cells (Mumm et al. 2011). IL-10 is acting on TILs and determines the upregulation of MHC molecules in the TME followed by tumoral rejection. Antitumor activity was reported as monotherapy in renal cell carcinoma, in uveal melanoma (Naing et al. 2016), and also combined with a PD-1 inhibitor in NSCLC and with FOLFOX in pancreatic tumors (Hecht et al. 2018). It was demonstrated that pegilodecakin promotes a sustained elevation in serum of Th1 and Th2 cytokines and simultaneously leads to a reduction of the cytokines Th17 and TGF-β, responsible for tumor-associated inflammation. As a consequence, pegilodecakin stimulates the expansion of CD8+ T cells. This mechanism associated with the induction of immunologic memory is responsible for the prolonging tumor responses, without severe adverse events. Clinical trials assessing the safety and activity of pegilodecakin combined with anti-PD-1 antibody inhibitors in patients with solid tumors are ongoing (Naing et al. 2019).

DCs are taken up by the PLGA NPs without any recognition of this specific character. The development of an efficient delivery system that incorporates trastuzumab and doxorubicin into poly(lactic-*co*-glycolic) acid nanoparticles, capable of inhibiting the regulatory pathways of cancer cells and stimulate the ADCC, was reported (Colzani et al. 2018) [258]. In vitro results showed that PLGA nanoparticles are more suitable to target DCs than PLGA microparticles are, with a 100-fold higher efficiency in the delivery of hD1 for nanoparticles (Cruz et al. 2010).

NP can be designed with a modification of the surface with more densities of monoclonal antibody (mAb) to target the cluster −205 (DEC-205) receptor, followed by DC immune stimulation with a higher interleukin-10 (IL-10) production

and an enhanced antitumor response and prolonged survival. In this manner, PLGA NP containing antigenic peptides can target DCs for vaccine delivery, followed by the cytotoxic T cell response and blocking the immune escape mechanism of tumor cells (Bandyopadhyay and Fine 2011). Tumor cells develop genetic and epigenetic alterations to prevent being recognized and destroyed by immune cells and promote immune evasion (Luo et al. 2018).

5.3.2.1.3 Dendrimers

Dendrimers are extensively branched macromolecules, composed of a core and cavities to entrap drugs. Dendrimers are suitable for modified drug delivery due to their well-defined chemical structure, with water solubility (Nanjwade et al. 2009). A direct interaction of dendrimers and immune cells was described. Poly (phosphorhydrazone) dendrimers have developed a preliminary activation of monocytes followed by a selective proliferation of NK cells with anticancer activity (Perise-Barrios et al. 2015). Tumor reduction by chemo-immunotherapy, using dendrimers as carriers, was reported. CpG oligonucleotide as an immune-stimulating agent interacted with doxorubicin, and this complex was targeted by prostate-specific membrane RNA aptamer (Lee et al. 2011).

5.3.2.1.4 Lipid Nanocarriers

Lipid nanocarriers are represented by liposomes, solid-lipid NPs, and phospholipids micelles. Liposomes are characterized by high biocompatibility and are vesicles compounded of one or more bilayers of natural or synthetic phospholipids. The structure of liposomes has many similarities with a cell membrane: hydrophobic tails of phospholipids cluster together while hydrophilic heads. The existence of a hydrophobic and hydrophilic compartment causes the liposomes to have the ability to cargo different kinds of compounds and release them safely, without affecting their metabolism (Torchilin 2005). Liposomes are spherical vesicles with one or more lipid layers containing a watery core so that they can transport both hydrophilic and lipophilic agents (Bulbake et al. 2017; Bozzuto and Molinari 2015).

Ovalbumin (OVA) can deliver IFN-encoding pDNA to the DCs via liposomes. There is a combining efficacy of OVA and the action of IFN-encoding pDNA in mice tumors that enhance the antitumor effect through the CTL activation (Yuba et al. 2015). Other pH-sensitive liposomes are *curdlan* and *mannan* used as bioactive polysaccharides, which deliver antigenic proteins into the cytosol of dendritic cells (Yuba et al. 2017). 2030-cyclic guanosine monophosphate-adenosine monophosphate (cGAMP) was included in liposomes and its delivery facilitates the improving the activity of STING agonists results in immunological memory that stimulate the tumor cells rechallenging (Koshy et al. 2017).

Gene delivery for enhancing immunotherapy is represented by the delivery of RNA lipoplex to DC cells that trigger the immune mechanisms of IFN-α, resulting in DC maturation. PEGylation is frequently used for the delivery of siRNA. An example is pH-sensitive cationic lipid named YSK05 that was developed as a multifunctional envelope-type nanodevice (MEND). PEGylated YSK05-MEND promotes gene silencing when administrated intratumorally (Sato et al. 2012).

5.3.2.1.5 Micelles

Micelles are vesicular particles with many opportunities as carriers for imaging or cancer therapeutics. The synthesis of micelles is relative easy compared to other nanocarriers, being also biodegradable and nontoxic and with the facility to deliver intracytoplasmatic (Peng et al. 2018; Volovat et al. 2020). They are used to carry ovalbumin (OVA) or regulating metabolism-related enzymes such as IR780, resulting in the slowing down of IDO followed by the activation of T lymphocytes and consequently inhibition of distal tumor growth (abscopal effect) (Li et al. 2017a, b). Micelles containing zinc and protoporphyrin IX target TAMs and stimulate the immune system, resulting in the promotion of ROS and inhibition of STAT3 expression. The stimulation of T lymphocytes by the repolarization of TAMs is followed by tumor regression (Liu et al. 2018).

5.3.2.1.6 Metal NPs

Gold Nanoparticles (AuNPs)

These nanoparticle systems can be carriers for antigens and gene or oligonucleotide to specific sites. The surface of Au NPs can create covalent and non-covalent interactions with various biomolecules, such as DNA, peptides, and antibodies (Kong et al. 2017).

There is an impact of AuNPs on some subcellular organelles as the mitochondria and the nucleus with its subcompartments of cancer cells, these organelles being related to cancer cell growth, survival, proliferation and death. The combination of AuNPs with photothermal ablation is a promising concept and is searched in different trials (Kodiha et al. 2015). Gold NPs are used in delivering CgP oligonucleotides that promote the infiltration of macrophages and dendritic cells followed by the inhibition of tumor growth (Lin et al. 2013).

Delivery of adjuvants such as OVA or CpG for immunotherapy is made by different sizes and shaped of gold nanoparticles, such as nanoshells, nanostars and nanorods, and it was remarked that the sizes of 15 nm have the best efficacy for the immunotherapeutic delivery of antigens (Dykman et al. 2018).

Iron Oxide Nanoparticles

These nanoparticles are powerful carriers for vaccine delivery. They can have a direct effect by polarization of immune cells, such macrophages and DCs, increasing immune response, or can be used as a delivery system of OVA with a function of immune potentiator (Zhao et al. 2018). FDA has approved supplementation with ferumoxytol in mammary cancer, due to an intrinsic therapeutic effect. In vitro, it was demonstrated that adenocarcinoma cells incubated together with ferumoxytol and macrophages can increase the activity of caspase-3. Also, macrophages exposed to ferumoxytol can induce pro-inflammatory Th1-type responses in macrophages (Zanganeh et al. 2016).

5.3.2.1.7 Inorganic Nonmetallic NPs

Mesoporous Silica NPs (MSNs)

MSNs are solid materials with a porous honeycomb-like structure containing hundreds of empty mesopores capable of absorbing large quantities of bioactive molecules (Slowing et al. 2008). Mesoporous silica materials can trigger various interactions with biosystems being related to physical and chemical properties. These properties are particle size, porosity, shape, and surface functionality of the materials and contribute to biodegradation, biodistribution, and, more importantly, their interaction with immune cells (Nguyen et al. 2019).

Mesoporous silica materials are degradable in physiological conditions via hydrolysis in the silica matrix, being related to the profile of guest molecules, particle size, surface functionality, concentration, porosity, and morphology.

Also, mesoporous silica can be released to body tissues and have a renal excretion. Mesoporous silica materials are nontoxic because of its compound, the silicic acid (Croissant et al. 2017).

Vallhov et al. demonstrated that the smaller particles and lower concentrations of mesoporous silica affected human monocyte-derived dendritic cells MDDC to a minor degree compared to the larger particles and higher concentrations, being suggested the hypothesis of use in vaccines therapy terms of viability, uptake, and immune regulatory markers, as a component of cancer vaccines (Vallhov et al. 2007). A mesoporous silica (XLMSNs+OVA+CpG-ODN) platform was developed and successfully induced dendritic cells (DC) maturation with high levels of CD86 expression, and elevated secretion of cytokines (Kwon et al. 2017). MSNs were found useful for the transportation of drugs and siRNAs, which induce the secretion of cytokines (Guo et al. 2012).

Carbon Nanotubes (CNTs)

CNTs represent cylindrical models composed of carbon and showed multiple potentials as tumor antigen nanocarriers. CNTs are multiwalled carbon nanotubes (MWNTs) and were successfully used to deliver OVA and cytosine-phosphate-guanine oligodeoxynucleotide (CpG) to antigen-presenting cells (APCs) (Hassan et al. 2016). Also, photothermal ablation of primary tumors with single-walled carbon nanotubes was demonstrated to stimulate immune responses in combination with anti-CTLA-4 therapy, to prevent the process of metastasis (Wang et al. 2014a, b).

5.3.2.1.8 Exosomes (EXOs)

Exosomes are extracellular vesicles with the size of 30–100 nm released by the majority of cells, with functions of communicators among cells to cargo lipids, proteins, and nucleic acids among cells and organs, being involved in the progression of cancer. The biogenesis of exosomes takes place via the invagination of plasmatic membrane to form endosomes. The maturation process of exosomes happens during the intracellular moving of endosomes from the PM to the center of the cell, with the participation of lipids and tetraspanins, with the formation of

multivesicular bodies (MVBs) that carry mRNA, noncoding RNA, and DNA. Exosomes are vesicles involved in various physiological and pathological processes in the immune system; their roles are as activators, mediators, and modulators. The secretion of exosomes is a characteristic of both lymphoid and myeloid lineages and also of many types of cells involving TME and cancer cells, the so-called tumor-derived exosomes containing growth factors and microRNAs (e.g., miR-423-5p and miR-675) (Raposo et al. 1996; Yang et al. 2018). A combined use of GM-CSF treatment and exosomes derived from ascites demonstrated the potency of combined therapy in inducing tumor-specific cytotoxic T cell, which determined the responses in a phase I trial. Another trial has demonstrated the safety and therapeutic efficacy of DC-derived exosomes carrying tumor MAGE peptides, resulting in the improvement of antitumor immunity and responses in patients with advanced non-small cell lung cancer (Morse et al. 2005).

Exosomes loaded with IFN and MHC class I and II administrated in patients with advanced lung cancer improved the NK cell-mediated antitumor response, with prolonged overall survival of the patients in a phase II clinical trial (Besse et al. 2015). Chimeric antigen (CAR) T cell-derived exosomes also showed benefits in controlling the immune-related adverse event such as cytokine storm syndrome and in improving the clinical responses (Tang et al. 2015; Chen et al. 2019a, b, c).

5.3.2.1.9 Engineered Viruses

Virus-like Particles (VLPs)

VLPs have a 20–100 nm in size and are artificial nanostructures containing viruses without the ability to replicate. VLPs can stimulate immune responses, being immunogenic, and can target immune cells as an engineered vaccine. A VPL-based vaccine using cowpea mosaic virus used as a delivery vehicle and also as an immunotherapeutic agent was reported. VPLs specifically target TME cells and tumor cells and can be used as a nanocarrier for tumor antigens and drugs (Smith et al. 2013; Lizotte et al. 2016).

Oncolytic Viruses

Oncolytic viruses seem to be "the new wave" in designing nanotechnologies. These viruses selectively infect tumor cells and determine cell lysis. The targets of these treatments are to initiate a local change in TME and, also, to have a systemic effect on tumor cells with minimum side effects (Guo et al. 2014).

The first-in-class FDA-approved oncolytic virus is talimogene laherparepvec, used in advanced melanoma. Many clinical trials are underway with various modified viruses, including adenovirus, poliovirus, reovirus, vaccinia virus, Seneca Valley virus, parvovirus, coxsackie virus, measles, Newcastle disease virus, and vesicular stomatitis virus (Fountzilas et al. 2017).

5.4 A Possible Mathematical Model for Cellular Communications Mechanisms

Intercellular communication has been closely scrutinized, because it enhances the swapping of data between cells, either through direct contact or by employing diverse secreted molecules. The secretion of extracellular vesicles (EVs) is a regularly occurring process, because they have been detected in assorted biological fluids, including blood. These are cell-derived constructions that make possible the swapping of nucleic acids, lipids, and proteins between cells, having a function in cell signaling (Colombo et al., 2014).

Cell communication and the microenvironment have a vital role in cancer development and tumor growth (Kahlert and Kalluri, 2013). Exosomes are a category of EVs secreted by most types of cells, playing a role in cell communication with other nearby or distant cells, immune response, cancer developing, and organ-specific metastasis (Liu et al., 2016). They are constructed of a phospholipid double layer and their dimensions revolve around 50–100 nm in diameter (Wang et al., 2014a, b). They embed all molecular constituents of a cell, such as DNA, miRNA, mRNA, or proteins. Although exosomes have been initially defined 50 years ago (Wolf, 1967), their fledgling role in cancer development as well as a potential biomarker has been thoroughly studied for the past 10 years.

Regularly used models are most of the time based on the rather unreasonable assumption that variables depicting the dynamics pertaining to any cellular complex system are differentiable (Mitchell, 2009). As such, the successful employment of the previously mentioned models should be viewed as sequential—that is, on domains in which differentiability and integrability still hold true. The differentiable and integrable mathematical processes are defective when the dynamics of any cellular complex system encompass nonlinearity, as well as chaoticity. Nevertheless, in order to explain such dynamics—but still being able to make use of differential mathematical procedures—it is imperative to specifically insert the concept of scale resolution into the description of physical variables and, furthermore, into the expression of fundamental equations which control these dynamics. This implies that that any variable controlled by space and time coordinates, in a classical sense, will rely on both space and time coordinates as well as on scale resolutions in this new mathematical sense (which is one of the non-differentiability and non-integrability). To put it differently, instead of working with a variable explained by means of a non-differentiable function, approximations of said mathematical function will be employed, acquired by its mediation at diverse scale resolutions. Subsequently, any variable purported to depict the dynamics of any cellular complex system will operate as the boundary of a collection of mathematical functions, this being non-differentiable in the case of zero scale resolution and differentiable for the case of nonzero scale resolutions (Nottale, 2011; Mandelbrot, 1983).

This procedure of portraying the dynamics of any cellular complex system involves the elaboration of new geometrical constructs and of new mathematical models. For the said developments, the motion laws, which are invariant to spatial and temporal transformations, are integrated with scale laws, which are invariant to

the transformations of spatial and temporal scales. In the author's view, the sought-after geometrical construct can be established on the notion of a "multifractal," and the corresponding mathematical model can be established with the help of the Fractal Theory of Motion, in a random but constant fractal dimension. In the case of biological complex systems, the analysis of dynamics is comparable to the one explained in Merches and Agop (2016).

The basic hypothesis of the considered model states that the dynamics of structural units of any cellular complex system can be described by means of continuous but non-differentiable motion curves (multifractal motion curves). Said multifractal motion curves display the trait of self-similarity in every one of their points, which can be equated into a characteristic of holography (every part mirrors the whole). Basically, the discussion revolves around "holographic applications of structural unit dynamics pertaining to any cellular complex system" by means of multifractal "regimes" of Riccati-type equations (i.e., depicting the dynamics of structural units belonging to any cellular complex system by means of Riccati-type equations at diverse scale resolutions).

As such, the Fractal Theory of Motion in the shape of scale relativity for describing the communication mechanisms becomes operational through the scale covariant derivative (Agop and Paun, 2017; Agop and Merches, 2019):

$$\frac{\widehat{d}}{dt} = \partial_t + \widehat{V}^l \partial_l + \frac{1}{4}(dt)^{\left(\frac{2}{D_f}\right)-1} D^{lp} \partial_l \partial_p, \tag{5.1}$$

where

$$\begin{aligned} \widehat{V}^l &= V_D^l - V_F^l \\ D^{lp} &= d^{lp} - i\widehat{d}^{lp} \\ d^{lp} &= \lambda_+^l \lambda_+^p - \lambda_-^l \lambda_-^p \\ \widehat{d}^{lp} &= \lambda_+^l \lambda_+^p + \lambda_-^l \lambda_-^p \end{aligned} \tag{5.2}$$

$$\partial_t = \frac{\partial}{\partial t}, \partial_l = \frac{\partial}{\partial x^l}, \partial_l \partial_p = \frac{\partial}{\partial x^l} \frac{\partial}{\partial x^p}, i = \sqrt{-1}, l, p = 1, 2, 3$$

In the previous equations, x^l represents the fractal spatial coordinate; t represents the non-fractal time, with the function of an affine parameter of the motion curves; $\widehat{V}^l$ represents the complex velocity; V_D^l represents the differential velocity nondependent of the scale resolution dt; V_F^l represents the non-differentiable velocity dependent on the scale resolution; D_F represents the fractal dimension of the motion curve; D^{lp} represents the constant tensor connected to the differentiable-non-differentiable transition, $\lambda_+^l(\lambda_+^p)$ is the constant vector connected to the backward differentiable-non-differentiable physical processes; and $\lambda_-^l\left(\lambda_-^p\right)$ is the constant vector connected

to the forward differentiable-non-differentiable physical processes. A variety of modes exists, and along with them, a diverse collection of definitions of fractal dimensions: more specifically, the fractal dimension in the expression of Kolmogorov, the fractal dimension in the expression of Hausdorff-Besicovitch, etc. (Mandelbrot, 1983). By choosing one of said definitions and working with it in the context of the cellular complex system dynamics, the value of the fractal dimension has to be constant and random for the totality of the dynamical analysis: as an example, it can be frequently found that $D_f < 2$ for correlative processes, $D_f > 2$ for non-correlative processes, etc. (Mandelbrot, 1983).

Furthermore, acquiescing the functionality of the principle of scale covariance (i.e., employing the operator (1) to the cell mass M, in the lack of any external restraint), the dynamics can be described though the differentiable equation:

$$\frac{\widehat{d}M}{dt} = \partial_t M + \widehat{V}^l \partial_l M + \frac{1}{4}(dt)^{\left(\frac{2}{D_f}\right)-1} D^{lp} \partial_l \partial_p M = 0 \tag{5.3}$$

This means that the temporal variation of the cell mass ($\partial_t M$), the "fractal convection" of the cell mas ($\widehat{V}^l \partial_l M$), and the "fractal dissipation" of the cell mass ($\frac{1}{4}(dt)^{\left(\frac{2}{D_f}\right)-1} D^{lp} \partial_l \partial_p M$) achieve their equilibrium in any point belonging to the fractal curve. Particularly, if the dynamics are expressed by stochastic processes of Markov type (Mandelbrot, 1983), then:

$$\lambda_+^l \lambda_+^p = \lambda_-^l \lambda_-^p = 2\lambda \delta^{lp} \tag{5.4}$$

where λ is a coefficient linked to the differentiable-non-differentiable shift and $\delta^{lp}$is the Kronecker's pseudo tensor:

$$\delta^{lp} = \begin{cases} 1 & i = l \\ 0 & i \neq l \end{cases} \tag{5.5}$$

In these circumstances, the differential Eq. (5.3) is simply expressed:

$$\frac{\widehat{d}M}{dt} = \partial_t M + \widehat{V}^l \partial_l M - i\lambda (dt)^{\left(\frac{2}{D_f}\right)-1} \partial^l \partial_l M = 0 \tag{5.6}$$

or by separating the cell dynamics on scale resolutions:

$$\partial_t M + \widehat{V}^l \partial_l M = 0 \tag{5.7}$$

at differentiable scale resolution and

$$-V_F^l \partial_l M - \lambda (dt)^{\left(\frac{2}{D_f}\right)-1} \partial^l \partial_l M = 0 \tag{5.8}$$

at non-differentiable scale resolution. Since the cell dynamics implies complex dynamics (self-structuring, Fickian and non-Fickian-type diffusion, etc.) at a differentiable-non-differentiable scale (i.e., at a mesoscopic scale), from (7) and (8) by adding them, the fractal diffusion equations are obtained:

$$\partial_t M + \left(V_D^l - V_F^l\right) \partial_l M = \lambda (dt)^{\left(\frac{2}{D_f}\right)-1} \partial^l \partial_l M \tag{5.9}$$

Moreover, if the $V_D^l \equiv V_F^l$ condition is employed, which specifies the synchronization of the cell kinetics at the two scale resolutions (differentiable and non-differentiable), (5.9) can be simplified as follows:

$$\partial_t M = \lambda (dt)^{\left(\frac{2}{D_f}\right)-1} \partial^l \partial_l M \tag{5.10}$$

In the one-dimensional stationary case, (5.10) becomes:

$$\frac{d^2 M}{dx^2} + k_0^2 M = 0 \tag{5.11}$$

with

$$k_0^2 = \frac{\lambda}{2m_0 \lambda^2 (dt)^{\left(\frac{4}{D_f}\right)-2}} \tag{5.12}$$

In (5.12), λ is a variable separation constant and m_0 is the residual mass of the cellular structure unit. The solution of (5.11) may be expressed as:

$$M(x) = he^{i(k_0\, x+\theta)} + \overline{h} e^{-i(k_0\, x+\theta)} \tag{5.13}$$

where h is the complex amplitude, $\overline{h}$ is the complex conjugate of h, and θ is a phase. As such, h, $\overline{h}$, and θ mark each structural unit contained in a possible cellular system, which exhibits, as a "fundamental property," the same k_0.

Equation (5.11) has a "hidden" symmetry by means of a homographic group of fractal type. Assuredly, the ratio ε of two independent and linear solutions of (5.11) is a solution of Schwartz's differential equation of fractal type—for the classical case, see (Cartan, 1951):

$$\{\epsilon, x\} = \frac{d}{dx}\left(\frac{\ddot{\epsilon}}{\dot{\epsilon}}\right) - \frac{1}{2}\left(\frac{\ddot{\epsilon}}{\dot{\epsilon}}\right)^2 = 2k_0^2 \tag{5.14}$$

$$\dot{\epsilon} = \frac{d\epsilon}{dx}, \ddot{\epsilon} = \frac{d^2\epsilon}{dx^2} \tag{5.15}$$

The left part of (5.14) is invariant in relation to the homographic transformations of fractal type:

$$\epsilon \leftrightarrow \epsilon' = \frac{a\epsilon + b}{c\epsilon + d} \tag{5.16}$$

with a, b, c, and d real parameters (of fractal type). The relation (5.16), which is corresponding to all probable values of said parameters, outlines the group SL (2R) of fractal type.

As such, all the cellular structural units containing the same k_0 are in biunivocal correspondence with the transformations of the group SL(2R) of the fractal type. This enables the construction of a "personal" parameter of fractal type ε for each cellular structural unit, separately. Thus, as a "guide," it is selected in the general form of the solution of (5.14), which is expressed as

$$\epsilon' = l + m \tan(k_0 x + \theta) \tag{5.17}$$

Indeed, by means of l, m, and θ, it is possible to depict any cellular structural unit. In such a context, recognizing the phase from (5.17) together with the one from (5.13), the "personal" parameter of multifractal type is expressed as:

$$\epsilon' = \frac{h + \overline{h}\epsilon}{1 + h}, h = l + im, \overline{h} = l - im, \epsilon \equiv e^{2i(k_0 x + \theta)} \tag{5.18}$$

The fact that (5.17) is also a solution of (5.14) implies, by expliciting (5.16), the group of SL(2R) fractal type:

$$h' = \frac{ah + b}{ch + d}, \overline{h}' = \frac{a\overline{h} + b}{c\overline{h} + d}, k' = \frac{c\overline{h} + b}{ch + d}\, k \tag{5.19}$$

In group (5.19), the phase of k is moved, taking into account the amplitude of the cellular structural units at the transition between the different structural units of any cellular. This shows the fact that this group functions as "synchronization modes," a process in which both amplitudes and phases participate. Delaying amplitudes and phases represents the usual "synchronization," but in this model, it must be a particular case.

The structure of group (5.19) is typical of SL(2R) one, which can be taken in the standard form

$$[A_1, A_2] = A_1, [A_2, A_3] = A_3, [A_3, A_1] = -2A_1 \tag{5.20}$$

where A_k, $k = 1, 2, 3$ are the infinitesimal generators of the group. Due to the fact that the group is simple transitive, these generators can be easily calculated as the components of the Cartan coframe of multifractal type from the relation

$$d(f)=\sum\frac{\partial f}{\partial x^k}dx^k=\left\{\begin{array}{c}\omega^1\left[h^2\frac{\partial}{\partial h}+\overline{h}^2\frac{\partial}{\partial\overline{h}}+(h-\overline{h})k\frac{\partial}{\partial k}\right]+\\+2\omega^2\left(h\frac{\partial}{\partial h}+\overline{h}\frac{\partial}{\partial\overline{h}}\right)+\omega^2\left(\frac{\partial}{\partial h}+\frac{\partial}{\partial\overline{h}}\right)\end{array}\right\} \tag{5.21}$$

where ω^k are the components of the Cartan coframe of fractal type, which can be calculated from the system:

$$\begin{aligned}dh&=\omega^1h^2+2\omega^2h+\omega^3\\d\overline{h}&=\omega^1\overline{h}^2+2\omega^2\overline{h}+\omega^3\\dk&=\omega^1(h-\overline{h})\end{aligned} \tag{5.22}$$

As a consequence, both the infinitesimal generators and the coframe of the fractal types can be calculated, by identifying the right-hand side of (5.21) with the standard dot product of SL(2R) algebra of fractal type:

$$\omega^1A_3+\omega^3A_1-2\omega^2A_2 \tag{5.23}$$

so that

$$A_1=\frac{\partial}{\partial h}+\frac{\partial}{\partial\overline{h}},A_2=h\frac{\partial}{\partial h}+\overline{h}\frac{\partial}{\partial\overline{h}},A_3=h^2\frac{\partial}{\partial h}+\overline{h}^2\frac{\partial}{\partial\overline{h}}+(h-\overline{h})k\frac{\partial}{\partial k} \tag{5.24}$$

and

$$\omega^1=\frac{dk}{(h-\overline{h})k},2\omega^2=\frac{dh-d\overline{h}}{h-\overline{h}}-\frac{h+\overline{h}}{h-\overline{h}}\frac{dk}{k},\omega^3=\frac{hdh-hd\overline{h}}{h-\overline{h}}+\frac{h\overline{h}dk}{(h-\overline{h})k} \tag{5.25}$$

It should be taken into consideration that, in (Agop and Merches, 2019), the process does not work with the previous differential forms but with the absolute invariant differentials:

$$\omega^1=\frac{dk}{(h-\overline{h})k},\omega^2=i\left(\frac{dk}{k}-\frac{dh+d\overline{h}}{h-\overline{h}}\right),\omega^3=\frac{kd\overline{h}}{h-\overline{h}} \tag{5.26}$$

The advantage of this depiction is that it highlights the connection with the Poincaré representation of the Lobachevsky plane. Indeed, the metric here is as follows:

$$\frac{ds^2}{g}=\left(\omega^2\right)^2-4\omega^1\omega^2=\left(\frac{dk}{k}-\frac{dh+d\overline{h}}{h-\overline{h}}\right)^2+4\frac{kd\overline{h}}{h-\overline{h}} \tag{5.27}$$

where g is a constant.

These metrics minimize that of Poincaré in case when $\omega^2 = 0$, which describe the variable θ as the "angle of parallelism" (in Levi-Civita sense) of the hyperbolic plane of fractal type—the connection of the fractal type (Agop and Merches, 2019). Now, it is a favorable moment to return to homographic transformation of fractal type (5.16). Taking into consideration the previously presented implications of this transformation, each structural unit of any cellular system can be located with the help of four homogeneous coordinates (a, b, c, d) or three nonhomogeneous coordinates, when a parallelism of direction in Levi-Civita sense becomes functional on the manifold induced by SL(2R) group of fractal type. Now, the simultaneity condition of the free structural units of any cellular system can be distinguished using different methods, from a Riccati equation of fractal type in pure differentials of multifractal type (this can be named Riccati gauge of fractal type):

$$d\frac{a\varepsilon + b}{c\varepsilon + d} = 0 \tag{5.28}$$

which implies

$$d\varepsilon = \omega^1\varepsilon^2 + \omega^2\varepsilon + \omega^3 \tag{5.29}$$

where ω^1, ω^2, and ω^3 are the components of the Cartan coframe of fractal type given through the relations (5.25). Therefore, in order to describe the dynamics of any cellular system as a succession of states of an ensemble of simultaneous structural units, as it were, it is more than enough to have three differentiable 1-forms, representing a coframe of SL(2R) algebra of multifractal type. Therefore, a state of a cellular system in a given dynamics can be arranged in a systematic way as a metric plane space, i.e., a Riemannian three-dimensional space of multifractal type. According to this last idea, the geodesics of Riemannian space of fractal type are given by particular conservations of equations of fractal type:

$$\omega^1 = a^1 d\tau, \quad \omega^2 = a^2 d\tau, \quad \omega^3 = a^3 d\tau \tag{5.30}$$

where a^1, a^2, and a^3 are constant and τ is the affine parameter of the geodesics, so that, along these geodesics of differential Eq. (5.29), it represents an ordinary differential of Riccati type:

$$\frac{d\varepsilon}{d\tau} = a^1\varepsilon^2 + 2\omega^2\varepsilon + a^3 \tag{5.31}$$

Let us take into account the following form of the previous equation:

$$A\frac{d\varepsilon}{d\tau} - \varepsilon + 2B\varepsilon + AC = 0 \tag{5.32}$$

where

$$A = \frac{1}{a^1}, B = -2\frac{a^2}{a^1}, AC = -\frac{a^3}{a^1} \tag{5.33}$$

As long as the roots of the polynomial

$$P(\varepsilon) = \varepsilon^2 + 2B\varepsilon - AC \tag{5.34}$$

can be written in the form

$$\varepsilon_1 = B + iA\Omega, \varepsilon_2 = B - iA\Omega, \Omega^2 = \frac{C}{A} - \left(\frac{B}{A}\right)^2 \tag{5.35}$$

the change of variable

$$z = \frac{\varepsilon - \varepsilon_1}{\varepsilon - \varepsilon_2} \tag{5.36}$$

converts (5.32) in

$$\dot{z} = 2i\Omega z \tag{5.37}$$

of solution

$$z(\tau) = z(0)e^{2i\Omega\tau} \tag{5.38}$$

As such, if the initial condition $z(0)$ is easily expressed, then it is possible to establish the general solution of (5.31), by writing the transformation (5.36) as follows:

$$\varepsilon = \frac{\varepsilon_1 + re^{2i\Omega(\tau-\tau_0)}}{1 + re^{2i\Omega(\tau-\tau_0)}} \tag{5.39}$$

where r and τ_0 are two integration constants. Using (5.35), we can write this result in real terms:

$$z = B + A\Omega \cdot \left(\frac{2r\ sin\left[2\Omega(t-t_0)\right]}{1 + r^2 + 2r\ cos\left[2\Omega(t-t_0)\right]} + i\frac{1 - r^2}{1 + r^2 + 2r\ cos\left[2\Omega(t-t_0)\right]}\right) \tag{5.40}$$

In Fig. 5.1a,b, we can notice a periodic release of specific information to the cell through a controllable period of time. In Fig. 5.1c,d, transitions of these dynamics into more complex behaviors can be distinguished. It is possible to detect a damped periodical scenario useful to control the high doses of the specific cell. The intermittence communication of cells is based on a controllable modulating frequency (which is selected as a control parameter). By controlling the concentration and

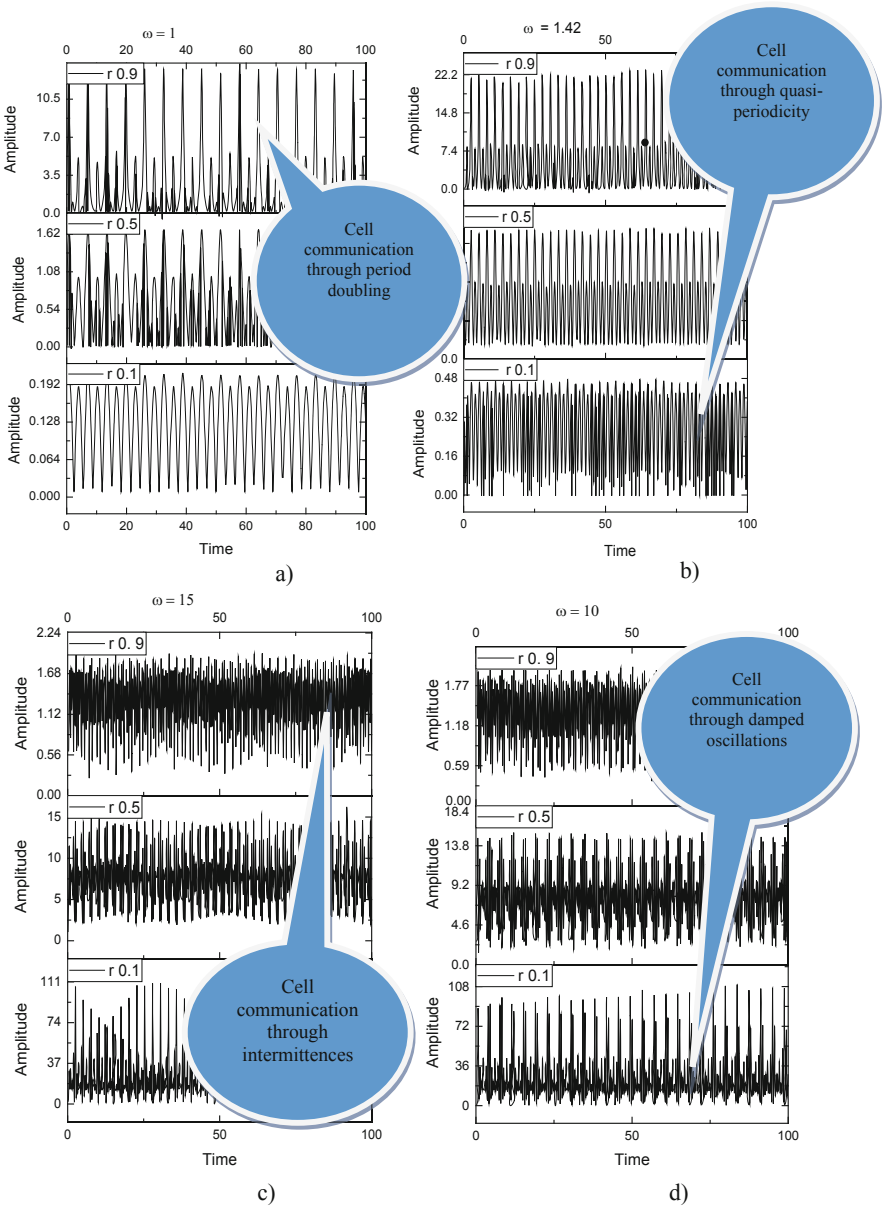

Fig. 5.1 Dependences with time of *Rez* for different values of the ω and r: a) $\omega = 1$, $r = 0.1; 0.5; 0.9$; b) $\omega = 1.42$, $r = 0.1; 0.5; 0.9$; c) $\omega = 10$, $r = 0.1; 0.5; 0.9$; d) $\omega = 15$, $r = 0.1; 0.5; 0.9$. Cell dynamics through multifractal self-modulation at a non-differentiable scale in the form of period doubling, quasi-periodicity, damped oscillations and intermittency (Ailincai et al., 2020)

the frequency of information release, a modulated response to the system is finally reached. In the fractal paradigm, various scenarios for the cell communication mechanism have been acquired. These support in understanding the behavior of cells that may benefit from such complex scenarios.

The 3D and 2D (contour plot) dependences of Rez on Ω and t, for a constant value of r, are shown in Fig. 5.2. In a situation like this, the communication dynamics involve multifractal self-modulation (at different non-differentiable scale resolutions appointed by the maximum values of ω) of the network dynamics. The comprehensive evolution of the system is clearly presented in Fig. 5.2. There, the damped cell communication scenario for various concentrations of information (controlled in the model by ω) can be observed. For various non-differentiable scale resolutions, different scenarios such as modulated information noticed in Fig. 5.2b were obtained, while an intermittent communication can be observed in Fig. 5.2c. The latter scenarios reveal complex communication scenarios and depend strongly on the physical properties of the cell matrix and the biological communication conditions. Finally, the model anticipates the presence of unwanted regimes with a quasi-chaotic communication. It is worth paying attention to the fact that the complete chaotic dynamic is never reached; instead, the adjustments made through the control parameter ω will force the system in doubling period state. Thus, even when the dynamic can apparently be chaotic, it can be very well rectified toward a more controllable state.

5.5 Nanomedicine to Enhance Immunotherapy

The aims of nanomedicine in cancer are to improve the direct destroy of cancer cells by enhancing the delivery of therapeutic drugs to tumors and metastases. Nanomedicine formulations improve anticancer immunity and to synergize with clinically established immunotherapeutics, existing some principal directions to be explored: to target cancer cells, to target the tumor immune microenvironment (TIME) and to target the peripheral immune system. Current cancer immunotherapies are often based on the use of ACT, therapeutic cancer vaccines, and monoclonal antibodies.

5.5.1 Cancer Cell Targeting

Nanomedicines can be used to enhance the induction of immunogenic cell death (ICD). ICD represents a specific mode of cell death, associated with the releasing of tumor antigens and danger-associated molecular model. This model is an important trigger and enhancer of anticancer immunity. ICD can be induced by chemotherapy (e.g., oxaliplatin, doxorubicin, cyclophosphamide) or radiotherapy, magnetic fluid hyperthermia, photodynamic therapy, or other stimuli (Duan et al. 2019). The nanoparticles designed for ICD provide a new way to enhance immunotherapy to

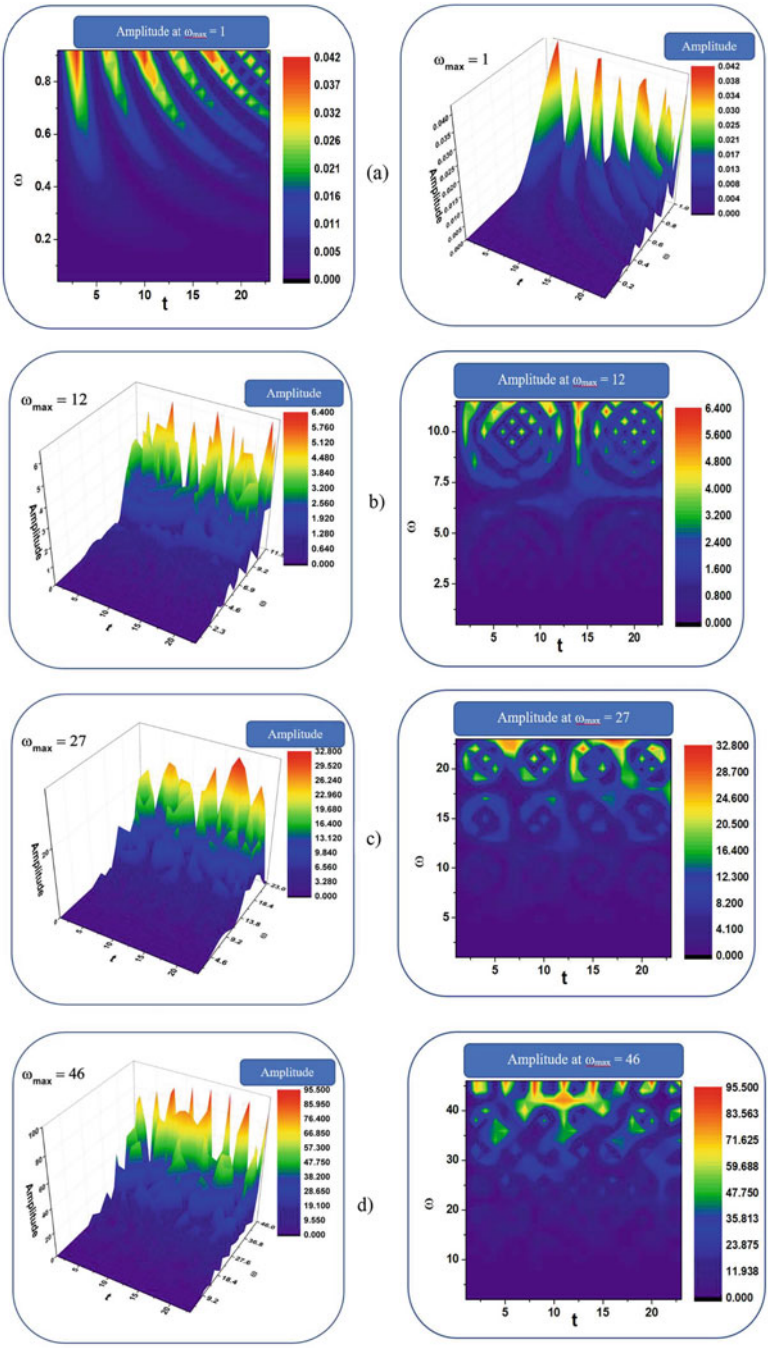

Fig. 5.2 3D and 2D dependences of *Rez* for different values of ω and t at a constant value of: a) $\omega = 1$, $r = 0.5$; b) $\omega = 12$, $r = 0.5$; c) $\omega = 27$, $r = 0.5$; d) $\omega = 46$, $r = 0.5$. (Ailincai et al., 2020)

be more efficient, by combining with ICD-inducing modalities, such as radio-, photo-, and chemotherapy.

This immunogenic cell death (ICD) is characterized by the release of TAAs and DMAPs, such as pro-inflammatory cytokines, which enhance the presentation of TAAs to immune cells. ICD can promote immune-stimulatory or annihilate immune suppressive effects for the activation, proliferation and tumor infiltration of T cells to synergize with current immunotherapies. ICD is characterized by the translocation of calreticulin (CRT) to the cell surface and releasing ATP and the high mobility group box 1 protein (HMGB1) into the extracellular environment. These modifications alert the immune system, resulting in the processing of tumor antigens by APCs and generation of cytotoxic T cells, which eradicate tumors and metastases. Doxorubicin-loaded liposomes (Caelyx/Doxil) can increase the efficacy of immunotherapy when combined (Rios-Doria et al. 2015). Doxil was combined with anti-PD-1, anti-PD-L1, and anti-CTLA4 antibodies and tumor necrosis factor receptor-α agonists. Doxil administration enhances CD80 expression on mature dendritic cells in the tumor. Also, in monocytic and granulocytic myeloid cells, the CD80 expression was increased, suggesting that Doxil may induce these tumor-infiltrating cells, activating an antitumor T cell response. It is supposed that Doxil through ICD promotes the proliferation of DC and CD8+ T cells. It was demonstrated that the immunopotentiation is higher for Doxil compared with doxorubicin administered in the same dose. Similar results were reported with oxaliplatin-loaded PLGA nanoparticles that induce ICD and are more efficient to activate the immune system than free oxaliplatin (Zhao et al. 2016).

For tumor-targeted delivery, immunotherapeutic agents combined with photodynamic therapy-radiotherapy were also used. It was demonstrated that pyrolipidic-loaded inorganic nanoparticles enhance the immunoactivation and ICD induction via photodynamic therapy when combined with anti-PD-L1. This ICD induction is promoting the serum levels of cytokines as TNF-α, IL-6, and IFN-γ and is also improving the tumor infiltration of CD8+ and CD4+ cells, eradicating primary tumor, and preventing lung metastasis through the abscopal effect (Duan et al. 2016).

Currently, it is confirmed that the abscopal effect represents a phenomenon, whereby local radiotherapy induces a systematic immune response and the regression of metastatic lesions (Min et al. 2017) [316]. Blocking TGF-β activity during radiation therapy was observed to generate CD8+ T cell responses to endogenous tumor antigens in poorly immunogenic mouse carcinomas. It was supposed that TGF-β activity is a major obstacle for radiotherapy to generate an in situ tumor vaccine. The addition of anti-PD-1 and/or anti-CD137 antibodies extended survival achieved with radiation and TGF-β blockade (Rodríguez-Ruiz et al. 2019). An example of nano-radio-immunotherapy is the administration of lipid nanoparticles loaded with rhenium-188 in combination with radiotherapy in gliomas at rats, being found that the nanoparticles increased the levels of circulating cytokines and tumor-infiltrating immune cells (Yang et al. 2017). Nanomedicines are inducing ICD followed by improving the immunity by inhibiting the systemic lymphocyte toxicity, which also potentiates the immunotherapy outcomes (Mathios et al. 2016). Also,

nanoparticles can be locally injected or locally activated and can induce systemic immunity via abscopal effect (Mulder and Gnjatic 2017). The European Medicines Agency approved the intratumoral administration of NBTXR3 hafnium oxide nanoparticles induced abscopal effect induced by radiotherapy for patients with locally advanced soft-tissue sarcomas (Bonvalot et al. 2019).

5.5.2 Tumor Immune Microenvironment (TIME) Targeting

Immunosuppressive pathways and mediators are currently upregulated in the TIME, by increasing infiltration of immunosuppressive cells, such as tumor-associated macrophages (TAM) and MDSC, into tumors and by enhancing the levels of soluble inhibitors, such as indoleamine 2,3-dioxygenase (IDO) and transforming growth factor beta (TGF-β).

5.5.2.1 Targeting Antigen-Presenting Cells: Dendritic Cells (DCs)

Dendritic cells (DCs) are the most efficient APC in the body, being multifunctional regulators of immunity. It is well recognized that antigen endocytosed by DCs is presented by MHC class II molecules and restricted to CD4+ T cell presentation (Naing et al. 2016). The antigen is released from the endosome into the cytosol during the cross-presentation process and, through alternative pathways, presented on MHC class I molecules to CD8+ cytotoxic T cells (Sallusto and Lanzavecchia 1994). Therapeutic cancer vaccines consist of tumor-associated antigens and adjuvants that target dendritic cells (DCs) and tumor-specific T cells for enhancing antitumor immunity.

Positive therapeutic outcomes in preclinical and clinical investigations by immunotherapy with exogenously manipulated DCs were reported (Kandalaft et al. 2013). This was demonstrated by the first DC-based vaccine for cancer immunotherapy, sipuleucel-T, approved by the FDA in patients with castration-resistant prostate cancer.

Promising strategies in the targeted delivery of immunomodulatory factors to DCs in vivo are the nano- and microparticles of various shapes, sizes, and compositions (e.g., lipids, polymers, metals), which have been extensively studied as vehicles for in vivo drug delivery (Moon et al. 2012). There were developed strategies for APC targeting that bind surface moieties to increase retention and uptake, to minimize off-target drug interactions, and to produce more potent immune responses due to selectively taken up by APCs. Specific formulations with surface moieties include α-CD40, α-DEC205, and α-CD11c antibodies (Cruz et al. 2014). Microparticles modified with antibodies against CD11c or DEC-205, highly expressed on DCs, or functionalized with peptides P-D2 or RGD, targeting intercellular adhesion molecule-4 and surface integrins, respectively, were demonstrated to be efficient in targeting DCs (Lewis et al. 2012). Targeting ligands have been also studied to improve uptake by DCs. It showed that covalent coupling of α-CD40 to PLGA nanoparticles containing antigens, Pam3CSK4 and poly(I:C), a TLR2 and

TLR3 agonist, respectively, enhance selective DC uptake and activated DCs in vivo (Rosalia et al. 2015).

RNA modulation was also involved in DCs targeting by nanomedicines. In a study on specific delivery systems, SiRNA carried by liposomes was targeted to DCs for silencing CD40 expression in vitro. Consequently, this co-stimulatory protein has a low expression on the surface of DCs, leading to the generation of Treg cells involved in immunosuppression (Zheng et al. 2010).

Also, in clinical testing for vaccines, low-immunogenicity lipid-based RNA nanoparticles, designed for delivering mRNA into DCs, were developed. These widely used cationic lipid materials (DOTMA, DOTAP, and DOPE) and anionic mRNA form RNA-lipoplexes to ensure an efficient and precise DC-targeting mRNA delivery without the need for molecular ligands, such as antibodies (Kranz et al. 2016).

Nanoparticle-mediated hyperthermia enhances various proinflammatory cytokines within treated tumors and activated DCs. Combination therapy of magnetic nanoparticle-induced hyperthermia, radiotherapy, and a virus-like particle adjuvant was found effective in dogs with spontaneously arising oral melanoma (Hoopes et al. 2018).

5.5.2.2 Targeting Tumor-Associated Macrophages (TAM)

Nanomedicines are first collected in tumors through passive/active targeting mechanisms and then are involved in local tumor immunosuppression mediated by MDSC, TAM, and/or soluble inhibitors, reducing the immunosuppression in the TIME with the increasing of the infiltration, maturation, proliferation, survival, and/or activity of effector immune cells. TAM represents a major population of immune cells with an M2-like phenotype in tumors, which have pro-tumoral functions, reducing the infiltration of effector T cells (Prendergast et al. 2017). It was demonstrated that ferumoxytol, a superparamagnetic iron oxide nanoparticle formulation FDA-approved for the treatment of iron deficiency anemia, changes M2-like TAM into M1-like TAM and inhibits the growth of primary and metastatic tumors in the liver and lungs. In another study, it was found that cyclodextrin nanoparticles are targeting a small-molecule toll-like receptor 7/8 agonist to macrophages in the TIME, producing an induction of M2 to M1 polarization, enhancing the efficacy of checkpoint-inhibiting immunotherapy (Rodell et al. 2018).

The increasing and macrophages M1 phenotype is followed by improving the outcome of checkpoint blockade therapy were obtain with CaCO3 nanoparticles functionalized with anti-CD47 antibodies. These nanoparticles were locally administrated as an in situ forming hydrogel during tumor surgery, and it was observed that CaCO3 reacted with protons in the TIME. The anti-CD47 antibodies were incorporated also to block the "don't eat me" signal on tumor cells (Chen et al. 2019a, b, c). Two immunosuppressive molecules from TIME, IDO, and TGF-β were also targeted by nanoparticles.

5.5.2.3 Targeting Indoleamine 2,3-Dioxygenase (IDO)

IDO enhances the conversion of tryptophan to kynurenine, a T cell suppressing metabolite (Prendergast et al. 2017). Small-molecule IDO inhibitors, incorporated in nanomedicine formulations, were tested in preclinical and clinical trials, and synergy between IDO inhibitor-loaded nanomedicines and photodynamic therapy and radiotherapy was shown (Lu et al. 2016). An IDO inhibitor was combined with the ICD inducer oxaliplatin in lipid-coated mesoporous silica nanoparticles, followed by tumor reduction in a mouse model of pancreatic ductal adenocarcinoma (Lu et al. 2017). Another IDO inhibitor was used together with a peptide that blocked PD-L1 in peptide-based nanoparticles. This inhibitor effectively inhibited melanoma growth in mice by stimulating anticancer immunity (Cheng et al. 2018).

5.5.2.4 Targeting TGF-β

TGF-β was found to be an important immunosuppressive factor in tumors that slow down the efficacy of checkpoint-inhibiting immunotherapy (Tauriello et al. 2018). A small-molecule TGF-β inhibitor encapsulated in PEGylated immune-liposomes was demonstrated to increase the expression of T cells triggering receptors CD90 and CD45 (Zheng et al. 2017). TGF-β siRNA-containing nanoparticles, which slow down TGF-β expression in tumors and synergized with cancer vaccination, were developed (Xu et al. 2014).

5.5.3 Peripheral Immune System Targeting

Immune compartments located outside of tumors, represented by the peripheral immune system, are of increased interest in the last years for nanomedicines. The peripheral immune system mainly composed of secondary lymphoid organs, such as the lymph nodes and the spleen, is the place where antigen presentation and cytotoxic T cell generation happen. These compartments are frequently impaired in cancer occurrence and progression. Restoring the functions of the peripheral immune system can be conducted by potentiation of antigen presentation and by engineering T cells. Subcutaneous or intradermal administration of antigen-containing nanoparticles drains in lymph nodes and is more efficiently processed by APCs (Swartz et al. 2012). CpG conjugated in nanoparticles or loaded together with peptide antigens in nanodiscs was administered in local injections targeting lymph nodes for promoting anticancer immunity (Kuai et al. 2017). Also, molecule toll-like receptor 7/8 agonist imidazoquinoline entrapped in nanogels or CpG bounded with albumin was injected locally or systemically to reach the lymph nodes. Such vaccines demonstrate the tolerability of the adjuvants (Nuhn et al. 2016).

Another antitumor vaccine was developed using PLGA nanoparticles containing antigens, which were administrated targeting the lymph nodes to deliver the antigens to DCs. A significant improvement of immunotherapy and the abscopal effect ex vivo in tumor-bearing mice receiving αPD-1 immunotherapy treatment were demonstrated (Molino et al. 2017; Min et al. 2017). Was reported a nanovaccine,

including a mixture of an antigen and a synthetic polymeric nanoparticle, PC7A NP which after administration deliver the antigens to antigen-presenting cells from lymph nodes activating type I interferon via stimulator of interferon genes (STING). This vaccine in combination with anti-PD-1 antibody demonstrated great synergy, with a 100% survival over 60 days in a TC-1 tumor model (Luo et al. 2017). Another strategy is generating cytotoxic T cells to replace APCs. Synthetic APCs were designed based on polypeptide modified with anti-CD3 antibodies included in the polymer chain, which enhanced the expression of CD69 and promoted the production of IFN-γ (Hammink et al. 2017). Were also prepared magnetosomes as versatile artificial APCs based on clusters of iron nanoparticles with a coat of leukocyte membranes including peptide-loaded MHC complex class I and anti-CD28 antibodies as co-stimulatory ligands. These synthetic APCs produce activation of cytotoxic T cells, and they promote tumor inhibition when administered together with T cells in tumor-bearing mice (Zhang et al. 2017). Liposomes, loaded with the cytokines IL-15 superagonist and IL-21 or cytokine-based nanogels modulating the release of IL-15, were also studied (Stephan et al. 2010). Antigen-encoding mRNAs, within a lipoplex, have already been studied in clinical trials, in monotherapy or combined with immunotherapeutics (Kranz et al. 2016).

Talimogene laherparepvec (T-VEC) is a type I herpes simplex virus (HSV-1), which preferentially replicates in tumor cells and is inducing a systemic antitumor immunity capable of eradicating tumor at distance. Locally, T-VEC influences the immunosuppressive tumor microenvironment, followed by the local release of interferons, chemokines, pathogen-associated molecular pattern (PAMP), and danger-associated molecular pattern (DAMP) factors. It enhanced the migration and maturation of dendritic cells that migrate to regional lymph nodes and present the antigens to CD4 and CD8 cytotoxic T cells. Also, it influenced the evolution of distant metastasis, lower than in injected tumor, this being the reason for combinatory T-VEC-checkpoint inhibitors (Kohlhapp and Kaufman 2016).

In clinical studies, OPTiM trial in stage IIIB-IVM1a melanoma patients demonstrated a 4.4-month longer median overall survival (OS) in patients receiving T-VEC compared with GM-CSF, and an estimated 5-year survival for the T-VEC arm was 33.4% (Andtbacka et al. 2019). A phase III trial of T-VEC/placebo plus pembrolizumab in unresectable stage IIIB-IVM1c melanoma is ongoing (MASTERKEY 265; NCT02263508) (Long et al. 2015). Another phase II randomized trial in resectable stage IIIB/C or IV melanoma treated with T-VEC in neoadjuvant demonstrated a higher percentage of recurrence free (33.5%) and a higher overall survival at 1 year (95.9%) (NCT02211131) (Dummer et al. 2019).

New viruses used in oncolytic vaccines are the following: coxsackieviruses, Cavatak®; adenovirus, Telomelysin (OBP-301); reovirus: Reolysin®; and Newcastle disease virus (Trager et al. 2020). Oncolytic virus therapy remains a promising treatment option for locally advanced melanoma and also for metastatic disease in combination with checkpoint inhibitors.

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6 Advancements in the Field of Oral, Intravenous, and Inhaled Immunomodulators Using Nanotechnology

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Abstract

Despite a great progress in the field of conventional delivery of immunomodulators, development of newer techniques and drugs is greatly required due to intrinsic instability of immunomodulators in vivo, related toxicity, and the required multiple administrations. Nanotechnology has emerged as an effective platform for overcoming these problems associated with conventional delivery of immunomodulators. Oral, intravenous, or inhalation route is used for the administration of immunomodulators during lung diseases or cancer for the release of different types of peptides, nucleic acids, as well as drugs to the site of action, and this efficacy is further enhanced by implementation of nanotechnology. Nanosized drug delivery systems create an occasion to enhance the cellular and humoral immune responses. Nanoscale size particles also facilitate uptake by the mucosa as well as gut-associated lymphoid tissue and the phagocytic cells that efficiently recognized and present an antigen. A number of studies on various types of lung diseases have shown advantages of inhaled and intravenous nanomedicines by direct local deliverance of immunomodulators specifically to the diseased cell. Other advantages of using nanoparticles include greater surface-volume ratio and variable surfaces for specific delivering of the

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immunomodulators to specific cells. The focus of this chapter is on summarizing the recent condition and developing way in such nanotechnology-based oral, intravenous, and inhaled immunotherapy as well as the function of nanosize particles as a carrier of immunomodulators, and depots for sustained immunostimulation along with associated advantages and limitations.

Keywords

Immunomodulators · Nanotechnology · Immunostimulation · Nanovaccines · Autoimmune diseases · Inhaled nanomedicines

6.1 Introduction

Immune system is ordinarily a complicated protective system in birds, fish, amphibians, reptiles, and mammals (vertebrates) that protects them from disease-causing or harmful agents. It is capable of producing different types of molecules and cells that are able in recognizing and eliminating different types of unknown or foreign cells (Manu and Kuttan 2009). Immune system modulation denotes some alteration in the immune reaction which may participate in the involvement of expression, induction, inhibition, or amplification of any kind of part or phase of the immune response. Therefore, immunomodulator is a kind of substance that is used for its effect on the immune system (Manu and Kuttan 2009). In targeted populations, macrophages, dendritic cells (DCs), and monocytes are various sites of immunomodulation. The first innate protective lines which are circulating or staying in tissues and apoptotic cells and reimbursing pathogens are monocytes and macrophages that are capable of changing over the stimuli of immune system and discharging antigens and cytokines (Manu and Kuttan 2009).

Immunopharmacology is a division of pharmacology having intention to find out new thing for immuno-modulator. On the basis of effects, the immunomodulators are classified into two different types of classes that are immunostimulators and immunosuppressants. The agent that prevents or suppresses response of immune system is known as immunosuppressants, e.g., interferons, mycophenolic acid, fingolimod, TNF-binding proteins, prolonged use of opioids, etc. These immunosuppressants are used in transplantation of organ during that they prevent rejection of transplanted organ and also used for treating autoimmune disorders including psoriasis, rheumatoid arthritis, and crohn's disease. Several cancer treatments work as immunosuppressants. On the ther hand, those substance that encourage or stimulate the response of immune systems by increasing or inducing response of its constituents such as the recombinant cytokines, monoclonal antibodies, monoclonal antibody cytokine antagonists, and granulocyte- macrophage colony-stimulating factors are known as immunostimulators (Roshan and Savitri 2013)

A lot of immunostimulatory and immunomodulatory substrates like chemokines, cytokines, and antibodies acting on specific sites are recognized for their vital

function in response to the enhanced immunosuppressive cancer micromilieu. Interleukin-2 is a type of cytokine that helps in growth of effector potential of cytotoxic T lymphocyte (CTL) and has exposed scientific effectiveness in malignant tumor. Renal carcinoma interleukin-2 has revealed improved effectiveness of some other immunotherapies. Interleukin-21 and interleukin-18 are some kind of cytokines which alter adaptive and innate immune responses via stimulation of natural killer (NK) cells, $CD4^+/CD8^+$ T cells, and B cells although as suppressing T_{reg} cells (Shukla and Steinmetz 2016). In biomedical research, specific targeted treatments have been established to be a major challenge owing to the multifaceted regulatory setup of the immune processes. In such sense, nanotechnologies propose the opportunity for the precise release of the antigen or drug to the preferred cell population and the co-discharge of the specific drugs along with plenty of immunomodulatory molecules. Moreover, nanotechnologies also decrease the chance of degradation of drug and enhanced the half-life of drugs (Irvine et al. 2015; Shukla and Steinmetz 2016).

Switching of immune system acts as the base of novel and promising treatment for several severe diseases which are mainly prevalent of our time like HIV, diabetes, and cancers. The contribution of nanotechnology is exponentially growing, and also the modulation field is expanded due to development of treatments (Smith et al. 2013; Irvine et al. 2015). Physicochemical property and composition of nanocarriers play a major role in influencing their interaction with immune cells. Incorporation of key molecule in nanoform mimics the size of microorganisms that have involvement in immune processes like cytokines, TLR agonists, etc., and thus the immune cells take up these nanocarriers which further alter the responses of immune cells. Also, the nanocarriers are capable of targeting the specific sites and can support the favored entry of key molecules to precise population of immune cell (Getts et al. 2015; Gutjahr et al. 2016; Gause et al. 2017). Prominently, because of same kind of nature, the nanotechnology proposes the chance to strengthen the preferred facet of immunomodulation, which maybe (i) the initiation of immunotolerance against immunoactive drugs and antigens, or (ii) the commencement of immune system to produce an immune reaction in opposition to a definite disease-causing agent. The first choice progresses the chances of scheming communicable diseases which don't react very well to conventional vaccines like HIV and tuberculosis, along with others (Delany et al. 2014). The subsequent or second, and less explored, choice focuses mainly on improving the vaccines for autoimmune diseases and developing the targeted delivery of immunomodulators. The ability of nanotechnology to obtain various response outcomes from its versatility, added through the definite mixture and particular option of its molecular constituents and commencing physicochemical characteristics of nanosystems (Serra and Santamaria 2015). A number of immunomodulatory and immunostimulatory molecules such as cytokines, chemokines and targeted antibodies have been identified for their important roles in countering the extremely immunosuppressive tumor microenvironment.

6.2 Role of Nanotechnology in Delivery of Immunomodulators

Immunomodulatory molecules loaded nanoformulations enhanced bioavailability because of significantly extended movement of transporter carriers, in vivo stability of against enzyme degradation and serum deactivation (Christian and Hunter 2012). For example, *i/v* administration of liposomal formulation having cytokine like IFN-α, IFN-g, TNF-α, or IL-2, increases the plasma residence time (Veen et al. 1998; Petros and DeSimone 2010; Kedar et al. 2000). Moreover, intramuscular, intraperitoneal, intranasal, or subcutaneous administration of liposomal formulation with cytokine and polymeric nanoparticles produces local depot and raises transit time of their payloads at target site (Anderson et al. 1991).

Particularly, nanoparticles-based drug deliverance encourages their superior retention and accumulation in tumor owing to enhanced permeability and retention (EPR) effect, whereas diminishing off-target complete toxicity, thus enhancing prospective for clinical transformation of these treatments (Maeda et al. 2013). Building on enhanced permeability and retention effect-mediated nanoparticle habitat and nanotechnology is experiencing progress in specific target and transformation of immunosuppressive tumor micromilieu to accomplish efficiency of immunotherapies. On the basis of passive tumor homing characteristics, lipid-coated calcium phosphate nanoparticles (LCP-NPs) are utilized for immunomodulation by transporting TGF-β siRNA and thus losing regulation level of immunosuppressive TGF-β inside tumor. These nanoparticles are also utilized to release a wide spectrum of anti-inflammatory triterpenoid-methyl-2-cyano-3, 12-dioxooleana-1, 9(11)-dien-28-oate that considerably decreased T_{reg} and MDSC's populations. The deliverance of immunostimulatory candidates by these are combined with vaccination strategy by means of LCP vaccine releasing Trp 2 peptide (tumor antigen) and CpG oligonucleotide (appurtenant) to DCs. This combinational therapy showed superior efficiency over vaccine-only uses for treatments (Xu et al. 2014).

In the same way, EPR-mediated accretion of liposomes' encapsulated polymer nanogels is used for intra-tumoral release of IL-2 and TGF-β receptor-I inhibitor-SB505124, resulting into inhibition of TGF-β receptors I and successive development of NK cells and T cells (Park et al. 2012). Also, by transporting PD-L1 siRNA via polyethyleneimine (PEI) liposomes, PD-L1 levels have been taped down foremost to immunosuppressive to immunostimulatory phenotype mutation in mouse and human ovarian tumor-allied DCs with following augmentation in $CD8^+$ T cell counts and enhanced survival of mice (Cubillos-Ruiz et al. 2009). Deliverance of liposomal IL-2 also indicated improved therapeutic potential with decreased toxicity in a mixture of other tumors as well as lung and liver cancers foremost considerable diminution in tumor growth (Neville et al. 2001).

Immunostimulatory liposomes conjugated with interleukin-2 and anti-CD137 antibodies, targeted to activate T cells that direct to increase dosing of IL-2 inside tumor when they were administered directly through systemic injections vs. intratumorally. The intratumoral administration outcome showed elevated ratios of tumor-infiltrating CD8þ T cells over regulatory T cells in conventional melanomas (Kwong et al. 2013). Similarly, PEGylated liposomal preparation

has been employed to inject agonistic anti-CD40 antibodies and TLR agonist CpG compounds by means of intratumoral administration resulting in considerable inhibition of tumor although appropriating sufficient amount to targeted sites and decreasing systemic leakage, consequently diminishing off-target inflammatory activity (Kwong et al. 2011). Likewise, intratumoral injection of CpG payloads on gold nanoparticles has revealed to stimulate considerable macrophage and DC infiltration in tumors, extensively influenced growth of tumor due to accumulation of CpG oligonucleotides, and decreased enhanced dose necessities of *i/v* injections (Lin et al. 2013).

Tumor-associated macrophages (TAMs) efficiently captured CpG oligonucleotide and anti-IL-10 and anti-IL-10 receptor antisense oligonucleotides by their encapsulation in nanocomplexes and changed macrophage phenotypes that resulted in significant antitumor effect (Huang et al. 2012). To improve this, mannose-modified polymeric micelles that contained acid-sensitive PEG modifications are formulated for improving partition in TAMs over macrophages associated with the mononuclear phagocyte system (Zhu et al. 2013). RGD-targeted single-walled carbon nanotubes have revealed improved accumulation of tumor via administering Ly6Chi monocytes in systemic flow (Smith et al. 2014). Mouse vascular endothelial growth factor (VEGF)-siRNA with TAM-targeting M2 peptide were loaded into gold nanoparticles that highlighted effectiveness of nanoparticles for targeting of phagocytic cells for activation of immune, tissue, and cell specificity (Conde et al. 2015; Kumar et al. 2015). Self-assembled nanoparticles obtained from viruses produce powerful immune response against weakly immunogenic tumors by rising generation of inflammatory cytokines within activated leukocytes (Lizotte et al. 2016).

Particular cytokines, growth factors, or a mixture of immune stimulants for making better immune cell functions can be delivered by nanoparticles. With recent advancements in genome editing, nanoparticles can be employed for deliverance of nucleic acids like siRNA to repair particular syndrome related genes in vivo (Smith et al. 2017).

Biological interactions among antigen-presenting cells and T cells can be mimicked by liposomes or polymeric nanoparticles because these are designed to directly mimic functions of immune cell (Gao et al. 2015). Modification of polydimethylsiloxane (PDMS) particles with activation of antibodies to CD3 and CD28 is valuable in improving growth of CD^{4+} and CD^{8+} T cells in vitro. Polymeric nanoparticles of multivalent synthetic dendritic cells can increase the efficiency for activation of T cells (Lambert et al. 2017).

The identification or engineering of nano-constructs for modulation of specific steps along the immune activation cascade is a new area in this field of nanomedicine such as ferumoxytol, a nanoparticle of iron oxide approved by USFDA for cure of anemia. Its systemic injection drastically declined the growth of tumor (Zanganeh et al. 2016). Fucoidan-dextran-loaded magnetic nanoparticles were modified with T cell activators and PD-L1 inhibitors to develop a multifunctional complex (termedIO@FuDex3). Its magnetic core helps in vivo magnetic map-reading for improving tumor targeting and minimizing off-target effect. Their surface was

modified with polyethylene glycol and folic acid, for improving intracellular uptake and capability to specific cell targeting (Chiang et al. 2018).

Nanodiscs loaded with immunogenic cell death (ICD)-inducing agent enhanced their pharmacokinetic profiles and growth of cancer (Kuai et al. 2018). Polyacrylamide-loaded NPs prepared by Birrenbach and Speiser are considered as the primary proof for possibility of nanotechnology in vaccination. This vaccine increased the response of immune cells against antigen IgG and tetanus toxoid when administered subcutaneously to guinea pigs (Birrenbach and Speiser 1976). The idea of "single-dose vaccines" was proposed by Preis and Langer which was based on chance for controlling discharge of proteins through polymeric beads which lead to the base for designing of nanovaccines and controlled antigen delivery systems (Preis and Langer 1979).

Almeida et al. developed microspheres of tetanus toxoid with PLA for nasal route with particle sizes of 500 and 800 nm. These nanosystems resulted in more and long-acting anti-tetanus Ig titers because of their capability to cross the nasal epithelium. They observed that tetanus toxoid-loaded chitosan nanoparticles resulted in an increased mucosal and humoral response through intranasal route in contrast to the administration of free antigens or the antigen associated with alum (Almeida et al. 1993). Various researches are carried out for development of nanotechnology-based vaccines for a variety of diseases. Some of them are discussed below.

The recombinant hepatitis B surface antigen (rHBsAg) was developed for intramuscular route as a nanoformulation by its association with chitosan NPs. It resulted in greater IgG immunogenic response than the control alum formulation (Prego et al. 2010). This antigen was also developed as chitosan-based nanocapsules for intramuscular administration (Vicente et al. 2013). Recent approach on layer-by-layer strategy was employed for encapsulation of this antigen with protamine or polyarginine (cationic polymer). After intranasal and intramuscular administration, this formulation revealed greater balanced Th1/Th2 ratio (Correia-Pinto et al. 2015). Currently, the most promising vaccine for development of an HIV vaccine is under clinical trial that is based on the combination of antigen (Gag) and protease (Pro), HIV gp120 envelope recombinant gp adsorbed onto alum (Rerks-Ngarm et al. 2009). Kasturi et al. developed PLGA-based NPs by using adjuvants (TLR 4/7/8 ligands) as with SIVsmE660 for intravaginal administration. This formulation showed improved shield of nonhuman primates for 12th low-dose challenge (Kasturi et al. 2017).

Carbon nanotube-polymer nanoparticle complexes were formulated as an APC mimic proposal. In this, scientists attached on carbon nanotubes pMHC and anti-CD28. These were associated with interlukein-2 and magnetite coloaded PLGA nanoparticles. The developed complex facilitated magnetic separation subsequent to incubation with T cells (Fadel et al. 2014; Zhu et al. 2017a, 2017b). A variety of nanovaccines employed for cancer treatment are enlisted in Table 6.1.

Table 6.1 Nanovaccines for cancer treatment

Formulation	Remark	Mechanism	Reference
Functionalized PLGA-PEG nanoparticles, PLGA nanoparticle, DOTAP-coated PLGA nanoparticles	Tumor-derived protein antigens liberate in radiotherapy	Increases abscopal effect, associated with anti-PD-1	Min et al. (2017)
Nanodisc	Neoantigen peptide, CpG	Mimics high-density lipoprotein, associated with anti-PD-1 and anti-CTLA-4	Kuai et al. (2017)
Nanoparticles of PEG-*b*-PC7A copolymers	Antigen peptide B16F10	Associated with anti-PD-1	Luo et al. (2017)
Microrods of PEI-absorbed mesoporous silica	Neoantigen peptides, CpG	Combined with anti-CTLA-4	Li et al. (2018)
Nanocapsules of self-assembled intertwining CpG-stat3 shRNA and PPT-*g*-PEG copolymers	Neoantigen peptide, CpG, stat3shRNA		Zhu et al. (2017a, 2017b)
Nanoparticles of B16F10 cell membrane-coated PLGA	Tumor cell membrane, CpG	Associated with anti-PD-1 and anti-CTLA-4	Kroll et al. (2017)
Liposomes of dying cancerous cells modified with hyaluronic acid	Dying cancerous cells, CpG	Associated with anti-PD-1	Fan et al. (2017)
Nanoparticles of superparamagnetic FeO, Fucoidan, and aldehyde-functionalized dextran	Anti-PD-L1, anti-CD3, anti-CD28		Chiang et al. (2018)
Nanoparticles of hydrogel of hexapod such as CpG-gold	CpG	Combined with photothermal therapy	Yata et al. (2017)

6.3 Advantages of Nanotechnology over Conventional Drug Delivery for Immunomodulators

Nanoformulations of immunomodulators illustrated various benefits in increasing therapeutic window. Encouragingly, nanotechnology helps in achieving the desired therapeutic effect by solving the existing issues. Various investigations have demonstrated that nanoplatforms' several beneficial evident features are as follows (Feng et al. 2019):

- Co-administration of adjuvants and antigens to same antigen-presenting cells (APCs) or intracellular compartments and extended t1/2 of bioactive cargo molecules by prevention of enzymatic degradation in systemic circulation.
- Size-dependent EPR effect consequences in higher growth in tumor tissues.
- Target to specific tissues/cells via surface modifications.
- Safe trafficking and smart drug discharge via stimuli-sensitive behavior.

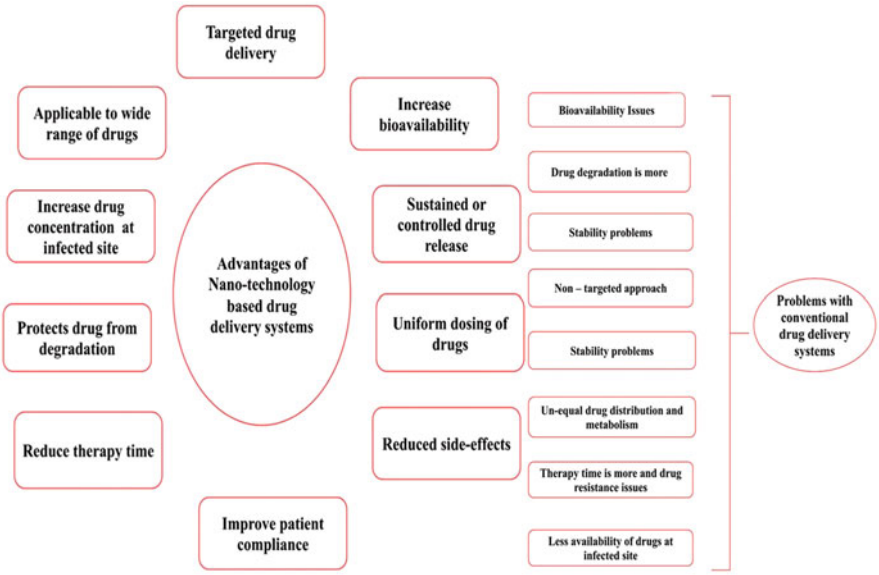

Fig. 6.1 Advantages of nanotechnology-based drug delivery systems

- Lessening of accumulation at off-target organs and tissues results in higher tolerant doses of drugs.
- Activation of potent T cell and surface coupling of both antigens and costimulatory molecules can be done by engineering artificial APCs (aAPCs).
- Microneedle patches can be employed for diversified drug delivery routes like intranasal or subcutaneous.
- Engineered nanoparticles can act as intrinsic immunomodulator.

Opsonization is avoided by PEGylation of nanoparticles. These nanoparticles are filtered through mononuclear phagocytic system and can improve deposition of drug within tumor through active and passive targeting. EPR effect occurs for accumulation and absorption of these infiltrating nanoparticles inside tumors. These nanoparticles actively target the cancer cells through binding of surface moieties and a ligand/receptor manner. Various researches based on nanoformulations have revealed considerable progress in condition of the patients, indicating a drawback of the EPR effect. A valuable benefit of these formulations is controlling over their transport kinetic that helps in deliverance of antigens to specific/target site (Petros and DeSimone 2010). The various benefits of nanotechnology-based drug delivery are summarized in Fig. 6.1. Descriptions of different diseases in which immunomodulators are used are listed in Table 6.2.

Mechanism—The first one is the development of nanocarriers that can act either by passive or active targeting of immune cells. The second one is use of immunomodulators. These molecules transform the response produced for a specific

Table 6.2 Treatment options for selected autoimmune diseases (Dacoba et al. 2017)

Syndrome	Treatment	Route	Mechanism of action (MOA)
Multiple sclerosis	IFN-β	*IM/SC*	In the brain, it balances expression of pro-inflammatory as well as anti-inflammatory agents and declines the number of inflammatory cells crossing the BBB
	Glatiramer acetate	*SC*	Strong binding to major histocompatibility complex molecules that act competitively for antigens of myelin
	Natalizumab	*IV*	Inhibition of immune cell extravasation and inhibition of α-4 integrin
	Immunosuppressants	Oral/*IV*	Inhibition of immune responses at various levels
Type 1 diabetes	Insulin injection	*SC*	Decline in glucose level
Rheumatoid arthritis	NSAIDs	Oral	Prevent the production of prostaglandins and thromboxane
	Corticosteroid	Oral/ intra-articular	Suppression of immune response and regulation of inflammation related genes
	TNFα antagonists	*SC/IV*	Inhibition of TNFα or its receptor
	Disease-modifying antirheumatic drug	Oral/ *SC/IV*	Slowed down progression of diseases by various processes of biomechanics
Inflammatory bowel disease	Amino salicylate	Oral	Expression of gene changeover and inhibition of COX and NF-κβ and its downstream signals
	Corticosteroid	Oral	Inflammation and immune response suppression related genes are regulated
		SC/IV	Suppression of immune responses at various levels
	TNFα antagonists	*SC/IV*	Inhibition of TNFα or its receptors
	Antibiotic	Oral	Bacterial concentration decline in the gut lumen, intestinal microbiota composition alteration
Systemic lupus erythematosus	NSAIDs	Oral	Prevent the production of prostaglandins and thromboxane
	Antimalarial drugs	Oral	Alter lysosome stability, suppressing antigen presentation, prevent synthesis of PGs and cytokine, affect toll-like receptor signaling
	Corticosteroids	Oral	Inflammation and immune response suppression related genes are regulated
	Immunosuppressive agent	*SC/IV*	Inhibition of immune responses at various levels

IM Intramuscular, *IV*, Intravenous, *SC* Subcutaneous

immune cell by activating various receptors that result in cellular or humoral immune responses (Dacoba et al. 2017).

The immune system contains circulating cells (dendritic cells, macrophages, and monocytes) and more static cells (B and T lymphocytes). These cells target the desired type of response by using specific immunomodulator.

Nanotechnology is beneficial for improving the pharmacological potential of cancer immunotherapy chiefly by three aspects (Feng et al. 2019):

- When nucleic acid is used, antigens and adjuvants are protected.
- Proficient deliverance to APCs and beginning of antigen-specific immune responses.
- Reprogramming of TME.

6.4 Different Routes of Administration

6.4.1 Mucosal Administration

This route is best for vaccination and inducing mucosal and systemic immune responses. M cells are associated with mucosa-associated lymphoid tissues (MALT). Activated T and B cells from mucosal are very important for vaccination via mucosal (Brandtzaeg 2007). Moreover, tolerance production can be accessed via administration of nanocarriers for this route (Kim et al. 2002).

6.4.2 Parenteral Administration

The main routes of vaccination are *i/m*, *s/c*, and *i/v*. Formulations of NPs can be administered directly to the nearby lymph node relying upon physical and chemical features and composition of nanoparticles. From literature review, it is summarized that formulations of particle size up to 100 nm can be easily drained to the nearby lymph node. However, particles of diameter less than 10 can be directly transferred to blood capillaries (Kourtis et al. 2013). Several researchers have demonstrated that the drainage of NPs with negative charge drains to LN is aided by their repulsive behavior with extracellular matrix (negative charge) that helps in their lymphatic transportation (Rao et al. 2010). However, after parenteral administration of cationic nanosystems, formation of a depot occurs (Vicente et al. 2014).

Generation of a tolerogenic effect by antigen-loaded nanocarriers occurs through intravenous (IV) administration (Hunter et al. 2014).

6.4.3 Oral Spray Immunization

Globally, development of noninvasive needle-free vaccines is an enormous task done by health division formulators and scientists. Avtushenko et al. (1996)

developed a vaccine for a non-replicating viral vector (tonsils of rhesus macaques) by spraying, and they named this method as tonsillar immunization. This results in the cellular and humoral immune responses that are stimulated by simian immunodeficiency virus (SIV) antigens (Avtushenko et al. 1996). It was found that the reduced level and protection level of viral RNA are nearly equivalent after systemic immunization of the vaccine. For systemic and respiratory tract infections, oral immunization with viral vectors is a novel strategy to consider to evade the issues related with syringes and needles. Furthermore, these viral vectors can be employed for deliverance of vaccines to mucosal lymphatic tissues, removal of epithelial barrier, and prevention of induction tolerance. In the same way, Ankara vaccines for a subtopical delivery into palatine tonsils of rhesus macaques via needle-free injections apparatus induced a significant response and inhibition of viral burden post challenges of SHIV89.6P (Amorij et al. 2007).

6.4.4 Nasal Delivery of Vaccines

The nanocarriers have found considerable advantages for mucosal vaccine delivery in the nanotechnology sector. Indeed, the majority of vaccines containing protein antigens and DNA vaccines are highly unstable in biological milieu. They require protection against degradation and designing of suitable antigen carriers to tackle their poor crossing capability for biological barriers (Hobson et al. 2003). For nasal delivery of vaccines, polymeric nanocarriers are the best alternative/solution for these types of issues. Very weak immune response is induced via nasal delivery of naked plasmid-DNA vaccines because of their efficient and considerable physical and chemical defensive barrier (Zhao et al. 2014). Chitosan-based NPs are employed for enhancing immunogenicity by mucosal deliverance of DNA vaccines. Recently, chitosan NPs were employed for pneumococcal DNA vaccine carrier. Xu et al. (2011) evaluated efficiency of chitosan-encapsulated psaA-NPs that immunized the BALB/c mice via intranasal route. They observed that the cellular, systemic, and mucosal immune responses were improved with chitosan-encapsulated psaA-NPs but decreased the nasopharyngeal carriage in the immunized mice. These findings suggest utility of nasal delivery for deliverance of DNA vaccines against pneumococcal infections (Xu et al. 2011). Moreover, there are various nanovaccine delivery systems that are in clinical phase as shown in Table 6.3. At present, autoimmune disorders are major areas of focus due to their fatal impact on the threat of life. Various nanoformulations approaches have been developed for these disorders as enlisted in Table 6.4.

Table 6.3 Nanovaccine delivery systems that are in clinical trial phase

Name	Inventor company	Application	Nanocarrier	Route of administration	Phase	Reference
SELA-070	Selecta Biosciences	To prevent relapse and smoking cessation	Synthetic vaccine particles	Subcutaneous	I	Zhang et al. (2014)
MAS-1	Nova Immunotherapeutics Limited	For seasonal influenza	Nanoparticle emulsion-based adjuvants	Parenteral	I	http://www.mucosis.com/mimopath.php
FluGem®	Mucosis B.V.	Influenza	Bacterium-like particles	Intranasal	I	http://www.mucosis.com/flugem.php
SynGem®	Mucosis B.V.	Respiratory syncytial virus	Bacterium-like particles	Intranasal	I	http://www.mucosis.com/press_releases_07-11-16.php
VCL-HB01	Vical Inc	Herpes simplex virus 2	Vaxfectin® adjuvants: Cationic lipid-based liposome	Intramuscular	II	http://www.vical.com/technology/vaxfectin/default.asp
ASP0113	Vical Inc	Cytomegalovirus in hematopoietic cell transplant patients	Poloxamer CRL1005+ DNA	Intramuscular	III	http://www.vical.com/technology/dna-technology/poloxamer/default.aspx
HBV003	Vaxine Pty Ltd	Hepatitis B	D-inulin microparticles	Intramuscular	I/II	Hayashi et al. (2017)
		H5N1 avian influenza		Intramuscular	I	https://clinicaltrials.gov/ct2/show/NCT02335164
R21 + Matrix M1	University of Oxford and Novavax	Malaria	Antigen+Matrix M™: saponin-based particles (saponins, synthetic cholesterol and phospholipids)	Intramuscular	I/II	http://novavax.com/page/10/matrix-m-adjuvant-technology

RSV F Vaccine	Novavax	Respiratory syncytial virus	Recombinant F-proteins from respiratory syncytial virus that self-assemble to form NPs	Intramuscular	III	http://novavax.com/page/8/vaccine-technology
RSV F Vaccine + Matrix M	Novavax	Respiratory syncytial virus	Respiratory syncytial virus-F Vaccine + Matrix M™	Intramuscular	II, III	https://clinicaltrials.gov/ct2/show/NCT03026348
LV305	Immune Design	NSLC, melanoma, and sarcoma	Antigen-specific ZVex® vector	Intradermal	I	http://www.immunedesign.com/platforms/
CMB305	Immune Design	NSLC, melanoma, and sarcoma	LV305 + G305 (GLA adjuvant system)	Intramuscular and intradermal	I	https://clinicaltrials.gov/ct2/show/NCT02387125?term=NCT02387125&rank=1
JVRS-100	Juvaris BioTherapeutics Inc	Leukemia	Cationic lipids/non-coding DNA plasmid complexes	Intravenous	I	http://www.juvaris.com/technology/overview.html
1790GAHB	GlaxoSmithKline	Dysentery	Generalized modules of membrane antigen	Intramuscular	I	Gerke et al. (2015)
CTH522-CAF01	Statens Serum Institut	*Chlamydia trachomatis*	Cationic adjuvant system containing dimethyl dioctadecyl ammonium and trehalose 6,6′-dibehenate	Intramuscular	I	https://clinicaltrials.gov/ct2/show/NCT02787109?term=NCT02787109&rank=1

GLA Glucopyranosyl lipid A, *ID* Intradermal, *IM* Intramuscular, *SC* Subcutaneous

Table 6.4 Various nanoformulation approaches for treatment of autoimmune disorders based on generation of antigen-specific tolerance

Formulation	Therapeutic agent	Mode of administration	Model	Remarks	Ref.
PLGA NPs	Antigen of IL-10/MOG	Subcutaneous	EAE	Inhibit development of disease, no delay in disease onset	Cappellano et al. (2014)
	Antigen of PLP	Subcutaneous and intravenous	R-EAE	Disease onset delayed, inhibit relapse episodes	Maldonado et al. (2015)
	Antigen of PLP	Intravenous	R-EAE	Prevent onset of disease, inhibit relapse episodes	McCarthy et al. (2017)
	Type II collagen	Oral	Collagen-induced arthritis	Accumulation of Peyer's patches for longer time, type II collagen antibody level declined, incidence of arthritis reduced	Kim et al. (2002)
PLGA MPs	Rapamycin and MOG antigen	Intra-nodal	EAE	Reduced disease onset permanently	Tostanoski et al. (2016)
	Vitamin D3 and insulin B or TGF-β1 and GM-CSF	SC	Nonobese diabetic	Prevented 40% of disease onset, enhance survival time from 19 to 24 weeks in treated mice	Lewis et al. (2015)
Liposomes	PS and insulin peptides	IP	Nonobese diabetic	Delayed disease onset, declined T1D incidence	Pujol-Autonell et al. (2015)
	PS and MOG peptides	IP	EAE	Delayed disease onset, reduced clinical score	Pujol-Autonell et al. (2017)
	Methylated BSA and NF-κβ inhibitors	SC	Adjuvant-induced arthritis	Reduced joint swelling severity scores	Capini et al. (2009)

Nanocomplexes	pDNA encoding for B and T lymphocyte attenuator and MOG antigen	IP injection of pre-treated dendritic cells	EAE	Postponement in disease onset, lessening of syndrome harshness	Yuan et al. (2014)
	GpG and arginine-modified MOG antigen	SC	EAE	Reduction of clinical score, induced asymptomatic in mice for 24 days	Hess et al. (2016)

BSA Bovine serum albumin, *MOG* Myelin oligodendrocyte protein, *PEG* Pegylated, *PLP* Proteolipid protein, *PS* Phosphatidylserine, *R-EAE* Relapsing-EAE

6.5 Overcoming Antidrug Antibodies: The Next Challenge in Immunomodulation

Biodrugs or biotherapeutics are biological products. These are the recent trending vaccines in this era. The recombinant DNA technology was introduced in the 1970s that launched recombinant insulin in the market which was the base for the utility of biomolecules as therapeutic agents. Biomolecule-based therapies are already in use and also in trials clinically for a number of diseases that show the great possibility of their biotherapeutics. The undesired immunogenicity during preclinical and clinical trial is the major safety concern for their development. Prolonged immunosuppressive treatment is the most frequent therapy for "Pompe disease," a lysosomal storage disorder. However, this treatment can increase the chances of infections and other complications because of systemic immunosuppression (Petros and DeSimone 2010).

6.6 Pros and Cons of Nanotechnology-Based Immunotherapies

T-VEC (immunostimulatory) is a nanotechnology-based platform that has been clinically approved. It is an attenuated version of herpes simplex virus. It particularly divides in tumor cells and shows anticancer potential by stimulating cytokine GM-CSF. This product is available in injectable dosage form that has been accepted for treatment of melanoma patients (Zhang 2015). Formulation of nanoparticles results in undesirable immunotoxicity (due to production of IL-6 and TNF-a and inflammasome response), adverse interactions, and deposition at targeted sites after long-term use. For example, nanoparticles of mesoporous hollow silica and carbon nanotubes cause immunotoxicity, harm the liver, and activate Kupffer cells (Liu et al. 2012). In the same way, nanosize TiO_2 particles result in oxidative stress, activation of neutrophil, and inflammation in lungs (Shvedova et al. 2005; Moon et al. 2010).

6.7 Conclusion

During the last two decades, nanotechnology has exposed vital prospective in the immunotherapeutic field. It can be employed for the modulation of various immune processes that will show good results in vitro and in vivo. In this chapter, a variety of nanotechnology-based formulations have been discussed for activating the immune system and generating tolerance. In such cases, physicochemical properties and composition of formulations play a crucial importance for desired immune response. Use of a particular subset of immune cells can increase specific target by using ligands for specific cell receptors. For the polarization of immune response, use of immunomodulators is a valuable strategy.

Some of the nanoformulations (nanotechnology-based approaches) are currently available in the market. And a variety of nanoformulations for deliverance of vaccines and tolerance generations are being tested in the clinical trial phases. Nanoformulations are being developed for the treatment of some of the most threatening illnesses. There is rapid development in development method and designing strategies in this field during the last decade.

Conflict of Interest None.

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Phytochemicals as the Source of Natural Immunomodulator and Their Role in Cancer Chemoprevention

7

Charu Gupta and Dhan Prakash

Abstract

A well-functioning immune system of the host body plays critical role in the upkeep of regular biological and immunological functions as well as inner milieu. Stable immunity augments protection mechanism against infection, diseases and undesirable pathogens to elude hypersensitivity reactions and immune-related diseases. Immunomodulation is a very wide word which denotes to any alterations in the immune response and may comprise induction, manifestation, magnification or inhibition of any part or stage in the immune response. Recently, the curiosity towards the immune system increased as important objective of injuriousness due to revelation of compounds, medications and environmental toxins. Phytochemicals are naturally occurring compounds with bioactive potential to modulate the immune system, such as alkaloids, polysaccharides, lectins, glycosides, phenolic compounds, flavonoids, anthocyanins, tannins, saponins, terpenoids and sterols. The precise mechanism by which phytochemicals implement anticancer roles is still a matter of investigation. This chapter would thus focus on the various phytochemicals as a source of natural immunomodulators and their role in cancer chemoprevention.

Keywords

Phytochemicals · Immunomodulators · Anticancer · Reactive oxygen species (ROS)

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7.1 Introduction

Conferring to the National Center of Health Statistics, cancer was the perpetrator of approximately 600,000 deaths in 2016 in the USA. It is by far one of the most diverse illnesses to treat. So far, the cancer problem and the disappointment of conservative chemotherapy to attain a decrease in the death rates for collective epithelial malignancies, such as cancer of the lung, colon, breast, prostate and pancreas, shows an acute need for fresh methodologies to govern cancer development (Takuji et al. 2012). One of these tactics is chemoprevention, which is a pharmacological methodology to involvement with the objective of arresting or retreating the course of multistep carcinogenesis. The carcinogenic process may be compelled by mutation(s), and trailed by preceding modifications in phenotypic, epigenetic and genetic events. Pharmacologic inflection of these governing pathways, comprising the actual use of drugs, micronutrients and non-nutrients that hunk mutational injury of DNA, thus deals great prospective for cancer hindrance.

There is a strong relation between dietary intake or dietary habits and cancer growth in man (Russo 2007). Nutritive risk reasons have graded higher than smoking and much higher than pollution or industrial exposures in their relationship with loss due to cancer. However, a quantity of combinations naturally befalling in foods, predominantly anti-oxidative compounds in plants, have shown ability as prospective chemopreventive agents (Peng et al. 2011). These phytonutrients include the yellow, orange and red carotenoid pigments that have just been explored.

In latest years, natural compounds called "phytochemicals", which are existent in fruits, vegetables and plants, have established distinct consideration due to their probability to inhibit with tumour formation and enlargement. Several of these phytochemicals are being used in chemoprevention approaches.

Phytochemicals are actually the non-nutritive plant chemicals' bioactive ingredients that endure or endorse health and befall at the intersection of food and pharmaceutical productions. They have either self-protective or illness defending properties. They are supplementary nutrients and primarily created by plants to provide them defence. Such constituents may array from secluded nutrients, dietary supplements and exact regimens to genetically engineered designer foods, herbal products, processed foods and beverages.

Phytochemicals also unveil strong biological actions such as antioxidant, anti-inflammatory and immunomodulatory when they are directed by the persons. The prospective activities exerted by these agents upkeep the controlling of assured long-term diseases (cancer, cardiovascular diseases) (Koche et al. 2018; Xiao and Bai 2019). In the last two spans, phytochemicals have been normally considered by using few facts of in vivo and in vitro mock-ups, which provided the favourable effects of these compounds in improving the numerous ailments and improving the quality of lifespan.

Phytochemicals are largely defined as polyphenols, flavonoids, isoflavonoids, anthocyanidins, phytoestrogens, terpenoids, carotenoids, limonoids, phytosterols, glucosinolates and fibres. They have remarkable effect on the healthcare scheme

and may offer therapeutic health aids including the preclusion and/or management of ailments and biological maladies. The use or ingestion of carotenoids, such as lycopene, alpha-carotene and beta-carotene, leads to decrease in the threat of cancer, such as breast and prostate tumours. For breast cancer, beta-carotene even lessens the threat of relapse. The usage or ingestion of soybean isoflavones has managed a decline in the threat of lung, prostate, colon (in women only) and breast cancers, although this has been governed by menopausal and oestrogen receptor prominence. The usage or intake of isothiocyanates and indole-3-carbinol also seems to decrease the risk of cancer, such as breast, stomach, colorectal, or prostate tumours. The acceptance of a diet rich in phytochemicals is related with an alteration of cancer threat (Ruiz and Hernández 2016).

Popular foods, such as whole grains, beans, fruits, vegetables and herbs, comprise these phytochemicals. Midst these, fruits and vegetables add to the substantial sources of phytochemicals. These phytochemicals, whichever alone and/or in mixture, have major beneficial prospective in curing numerous conditions. The pertinent health profits are based on science and principles, for fitness rights, functional foods and occurrence of certain phytochemicals. They play affirmative pharmacological properties in human well-being as antioxidants, antibacterial, antifungal, anti-inflammatory, anti-allergic, antispasmodic, chemopreventive, hepato-protective, neuroprotective, hypo-lipidemic, hypotensive, diuretic, CNS stimulant, immunomodulator, carminative and analgesic; prevent aging, diabetes, osteoporosis, DNA damage, cancer and heart diseases; induce apoptosis, and guard from UVB-induced carcinogenesis (Singh et al. 2015). Cancer chemoprevention comprises the usage of different natural or biologic agents to impede or inverse tumour progress. Initial studies have shown that these compounds are able to disturb cell production and cell cycle regulation, and usually contribute in multiple signalling paths which are often disturbed in tumour instigation, production and spread (Howes and Simmonds 2014).

Epidemiological and pre-clinical data endorse that numerous probable phytochemicals and dietary compounds embrace chemopreventive properties, and in vitro and animal studies back that these compounds may control signalling paths involved in cell multiplying and apoptosis in malformed cells, augment the host immune system and alert malignant cells to cytotoxic agents. Regardless of encouraging results from investigational studies, only a restricted number of these compounds have been confirmed in clinical trials and have shown inconstant results (Kotecha et al. 2016).

7.2 Phytochemicals as Immunomodulator

A stimulus of the body's defence is anticipated for certain people such as immunocompromised patients including cancer patients, whereas dominance of the immune response is looked-for others such as transplant receivers or patients with autoimmune or inflammatory diseases (Venkatalakshmi et al. 2016). Clinically,

immune-modulants are distributed in three key classes: immuno-stimulant, immunosuppressant and immunoadjuvant.

Immuno-stimulants are used to help the immune responses against numerous contagions (autoimmunity, allergy, cancer) by quickly triggering both innate and adaptive immune systems. Apparently, these agents display prophylactic action in the healthy persons by exciting the primary immune response and act as an immunotherapeutic agent in patients having primary and secondary immunodeficiency (Clement et al. 2010; Naik and Hule 2009).

Immunosuppressants are used to subdue the pathological immune reactions in autoimmune diseases, hypersensitivity, graft-versus-host-rejection, graft rejection and other immune-mediated ailments. They have the power to lessening the potential of human body which reject the transferred organs, for example, the kidney, liver and heart; hence, these molecules can also be termed as antirejection agents (Yatim and Lakkis 2015)

Immunoadjuvants are used to smooth the immune system by cumulative magnitudeand extent and stimulation of antigen-specific immune response while they do not contain any unusual antigenic activity. These molecules are mainly directed in amalgamation with definite vaccine antigen. In the lack of vaccine, these constituents do not show any antigen activity (Petrovsky 2006)

Inflection of the immune system can be spoken through a range of specific and non-specific tactics. Many agents of synthetic and natural origin have stimulatory, suppressive and controlling activities. Plants are the biosynthetic workroom of phytochemicals.

Natural compounds with probable immune-stimulating activity can be categorized as high- and low-molecular compounds. Terpenoids, phenolic compounds and alkaloids lead among low-molecular immunomodulatory compounds, while polysaccharides lead among high-molecular weight compounds (Venkatalakshmi et al. 2016).

The natural foods, spices and medicinal plants are good sources of antioxidants. Compounds with strong antioxidant activity are also worthy immunomodulators. The immune-modulating activity of numerous phytochemicals such as alkaloids, polysaccharides, lectins, glycosides, phenolic compounds, flavonoids, anthocyanins, tannins, saponins, terpenoids and sterols also explicates the role of antioxidants as immunomodulators and in cancer chemoprevention.

7.2.1 Alky3esaloids as Immunomodulator

- Piperine (*Piper longum*) increases total WBC count, bone marrow cellularity, total antibody making.
- Berberine (*Hydrastis canadensis*) substantially decreases plasma TNF-α, IFN-γ, and NO levels.
- Tetrandrine (*Stephania tetrandra*) destroys cytokine production and impedes NF-κB facilitated release of inflammatory factors.
- Sinomenine (*Sinomenium acutum*) grafts persistence.

7.2.2 Glycosides as Immunomodulators

Glycosides are the plant secondary metabolites comprising a sugar moiety that is attached with non-sugar portions. These metabolites perform numerous beneficial activities in animals and humans; however, many plants store these substances in inactive form which can be stimulated by the help of enzymes existent in the body (Brito-Arias 2007). Pharmacologically active glycosides contain anthocyanin and anthracene. These compounds are primarily involved in the stimulus of central nervous system, cardiac system and immune system. Furthermore, glycosides also show strong antimicrobial action (Nenaah 2013).

Pandey and co-workers studied the immunosuppressive action of eupalitin-3-*O*-β-D-galactopyranoside (Bd-I) obtained from *Boerhaavia diffusa* root. Peripheral blood mononuclear cells (PBMCs) were treated with or without phytohaemagglutinin (PHA) and Bd-I. Treatment of Bd-I displayed a noteworthy inhibition of TNF-α and IL-2 and production of human PBMCs (Pandey et al. 2005). Chiang and colleagues analysed the immunomodulatory effects of aucubin derived from *Plantago major* on PBMCs. ELISA assay was done to analyse the expression of interferon-gamma (IF-γ). Data revealed that management of aucubin enhanced the proliferation of lymphocytes and formation of IL-γ. Thus, aucubin can be a strong immune-stimulatory agent (Chiang et al. 2003).

Examples of some glycosides as immunomodulators are as follows:

- Isorhamnetin-3-O-glucoside (*Urtica dioica*) in vitro immunomodulatory prospective.
- Eupalitin-3-O-β-D-galactopyranoside (*Boerhaavia diffusa*) subdued PHA-stimulated production of peripheral blood mononuclear cells IL-2 and TNF-α.
- Aucubin (*Plantago major*) augments lymphocyte proliferation and excretion of IFN-γ.
- Mangiferin (*Mangifera indica*) boosts the production of IgG1 and IgG2b.

7.2.3 Phenolic Compounds as Immunomodulators

Polyphenols are the naturally derived ancillary metabolites which are extensively found in fruits, vegetables, beverages and cereals. Dependent upon their antioxidant, antimicrobial and anti-inflammatory activities, polyphenols have proved notable effects in many prolonged sicknesses such as neurodegenerative diseases, diabetes and cardiovascular diseases (Mohamed 2014). Presently, an extensive diversity of these molecules are presenting immunomodulatory activity by changing development of nitric oxide and eicosanoids and constraining pro-inflammatory cytokines and gene expressions (Keservani et al. 2010; Chuang and McIntosh 2011).

Kalsum et al. stated the immunomodulatory efficiency of *Propolis trigona* extract by using Sprague-Dawley rats' model. Treatment of ethanolic extract of propolis amended the formation of nitric oxide, phagocytic index and IgG antibodies,

accordingly improving the immune responses (Kalsum 2017). *Plantago* species have been broadly used in the therapy of inflammation, infection and cancer. Chiang et al. assessed the immunomodulatory activity of five phenolic compounds extracted from *Plantago major* on human PBMCs. ELISA and BrdU immunoassay were done to analyse the action of phenolic chemicals on cytokine expressions. Data discovered that treatment of aucubin, ferulic acid, vanillic acid, p-coumaric acid and chlorogenic acids considerably increased the activity of lymphocyte proliferation and making of IFN-γ. Ellagic acid derivative from *Punica granatum* presented both anti-apoptotic and antiproliferative activities against the HT-29 and HCT116 colon cancer cell lines when treated with a dose of 100 μg/mL (Seeram et al. 2005).

Examples of some polyphenols with immunomodulatory activity are as follows:

- Gallic acid propagates B-cell and inhibits mast cell degranulation.
- Ellagic acid (*Punica granatum*) has anti-proliferative and antioxidant activities.
- Chlorogenic acid (*Plantago major*) increases lymphocyte proliferation and excretion of IFN.
- Ferulic acid (*Plantago major*) boosts lymphocyte proliferation and excretion of IFN.
- P-coumaric acid (*Plantago major*) improves lymphocyte proliferation and excretion of IFN.
- Vanillic acid (*Plantago major*) augments lymphocyte production and excretion of IFN.
- Curcumin (*Curcuma longa*) improves bone marrow cellularity, α esterase positive cells and phagocytic activity hinders IL-2 expression and NF-κB.

7.2.4 Flavonoids as Immunomodulators

Flavonoids are considerably dispersed polyphenols found in plant-based foods or in beverages. Above 8000 flavonoid compounds have been recognized which are mostly present in grapes, berries, cranberries, cherries and plums. They are found together in free-state as well as in glycoside form (Marzocchella et al. 2011). Kaempferol, quercetin, myricetin, hesperetin, naringenin, epicatechin gallate and anthocyanin are the chief flavonoid compounds that have definite influence on human healthiness dependent upon their natural activities. Flavonoids have likely protective activity against oxidative cell destruction and also anticancer action. Also, they have also capacity to chunk all the courses of induction, stimulation and progression of tumour. Flavonoids have been used for the regulation of various chronic diseases like atherosclerosis, diabetes and Alzheimer's disease (Marzocchella et al. 2011). Presently, investigators are discovering their influence on immune system activity to make as the strong immunomodulators.

Chang et al. reported the immunomodulatory efficacy of centaurein flavonoid obtained from *Bidens pilosa* by regulating the expression of IFN-γ in Jurkat cells. Additionally, centaurein also controls nuclear factor of activated T-cell activity and NF-κB enhancers which portray it as a strong immunomodulator (Chang et al.

2007). Another study analysed the immunomodulatory strength of *Ziziphus lotus* on oxazolone-induced contact-delayed hypersensitivity (DTH) in mice at a dose of 200 mg/kg. Cytotoxicity assay was made and discovered that methanolic extract of flavonoids remarkably blocks the DTH stirred by oxazolone (Borgi et al. 2008). Additionally, Abd-Alla and colleagues piloted a study to discover the immunomodulatory effects of flavonoid compounds extracted from *Jatropha curcas* on 1-day-old specific pathogen-free (SPF) chicks. A substantial stimulus in both humoral- and cell-mediated immune responses was observed when treated with methanolic extract containing apigenin 7-*o*-β-D-neohesperidoside, orientin, vitexin and apigenin 7-*O*-β-D-galactoside flavonoids (Abd-Alla et al. 2009).

Examples of some flavonoids used as immunomodulators are:

- Centaurein (*Bidens pilosa*)—Increase of IFN-γ promoter activity.
- Apigenin 7-o-β-D-neohesperidoside (*Jatropha curcas*)—Stimulus of humoral- and cell-mediated immune response.
- Apigenin 7-O-β-D-Galactoside (*Jatropha curcas*)—Stimulus of humoral- and cell-mediated immune response.
- Orientin (*Jatropha curcas*)—Stimulus of humoral- and cell-mediated immune response.
- Vitexin (*Jatropha curcas*)—Stimulus of humoral- and cell-mediated immune response.
- Luteolin (*Plantago major*)—Augments lymphocyte proliferation and secretion of IFN.
- Baicalein (*Plantago major*)—Increases lymphocyte proliferation and secretion of IFN.
- Quercetin-3-O-rutinoside (*Urtica dioica*)—Immunomodulation.
- Kaempherol-3-O-rutinoside (*Urtica dioica*)—Immunomodulation.

7.2.5 Immunomodulatory Potentials of Anthocyanins

Anthocyanins, a type of polyphenols, are the water-soluble pigments and can have colour change from red to yellow in several fruits and vegetables. These compounds have revealed encouraging antioxidant and anti-inflammatory activity by controlling several signalling pathways. These favourable effects tend to explore the immunomodulatory activity of anthocyanin (Khoo et al. 2017). A study explored the immunomodulatory and antioxidant effectiveness of anthocyanins resulting from cherries on adjuvant-induced arthritis (AIA) in rats by analysing the expressions of IL-6, prostaglandins E2 (PGE2) and TNF-α. In a study including the administration of anthocyanin extract containing cyanidin, delphinidin, petunidin, malvidin and peonidin with a dose of 75, 150, 300 mg/kg, daily for 28 days, data showed that anthocyanin content unusually suppressed paw swelling and cytokines expressions including IL-6, TNF-α and PGE2 (Behl et al. 2021). An ex vivo human study performed by Rechner and Kroner informed the anti-inflammatory activity of cyanidin-3-glycoside, peonidin, by improving the platelet function (Rechner and

Kroner 2005). Furthermore, Xu et al. showed a comparative study to assess the immunomodulatory activity of anthocyanin fraction on LPS-induced human monocytes mono mac 6. Facts showed that there is no significant difference between the immunomodulatory and antioxidant activity of both anthocyanin fraction and resveratrol (He et al. 2005). Lately, many other studies also testified the antioxidant and immunomodulatory activity of anthocyanins including cyanidins, delphinidin, peonidins and malvidins by reducing levels of IL-6 and TNF-α and refining the insulin sensitivity (Dragano et al. 2013; Karunarathne et al. 2020).

Examples of some anthocyanins with immunomodulatory properties are:

- Cyanidin-3-glycoside (blackberry)—Antioxidant and anti-inflammatory mechanism.
- Peonidin (blackberry)—Antioxidant and anti-inflammatory mechanism.

7.2.6 Immunomodulatory Potentials of Tannins

Tannins are great molecular-weight, water-soluble compounds, frequently existing in plants as a complex with proteins, polysaccharides and alkaloids. Apple, grape, berries, peach and walnuts are the main sources of tannins (Onaolapo and Onaolapo 2019). Many preclinical studies have revealed the immunomodulatory activity of these composites.

Punicalagin (PCG) is an ellagitannin which shows many valuable effects on human body. Lee et al. explored the immunosuppressive activity of PCG resulting from *Punica granatum* reliant upon its action on nuclear factor of activated T-cells (NFAT). Facts showed that treatment of PCG suppressed the expression of IL-2, leukocyte reaction as well as CD3+ T-cell infiltration. Furthermore, PCG also displayed some free radical scavenging activity which advocates that PCG could be a powerful immunosuppressant (Lee et al. 2008). Additional study directed by Reddy and Reddanna described the immunosuppressive activity of chebulagic acid (CA) derived from *Terminalia chebula* on LPS-induced RAW 264.7 cell line. Treatment of CA significantly weakened the expression of IL-2 and TNF-α and ROS production. In addition, a dose-dependent pattern was also realized in inhibition of NF-κB activation, p38, JNK and ERK ½ phosphorylation (Reddy and Reddanna 2009). Additionally, methanolic extract of *Pongamia glabra* (PGE) revealed the immunomodulatory activity in cyclophosphamide-induced myelosuppressed mice when it was treated with a dose of 250 and 500 mg/kg, daily for 13 days. On the 14th day, data discovered that PGE increased the WBC, platelet and DLC counts in a dose-dependent manner (Heroor et al. 2013).

Examples of some tannins with immunomodulatory potential are:

- Chebulagic acid (*Terminalia chebula*)—Downregulation of TNF-α and IL-6.
- Corilagin (*Terminalia chebula*)—Neuroprotection.
- Punicalagin—Free radical scavenging and immunosuppressive action.

7.2.7 Saponins as Immunomodulator

Saponins are the class of naturally arising glycosides which are commonly present in diverse parts including leaves, flowers, shoots, roots, tubers and seeds (Oleszek and Oleszek 2020). Several investigators have confirmed the prospective of plant-derived saponins to prompt immunogenicity of various vaccines, under in vivo and in vitro studies. The usage of saponins as the immunoadjuvants, for their ability in controlling the cell-induced immune system and encouraging the production of antibodies, is one of the most noticeable activities of saponins (Sparg et al. 2004). Various saponin compounds have capability to impede the cancer cells by arresting cell cycle and apoptosis. Ablise et al. assessed the immunotherapeutic activity of glycyrrhizin resulting from *Glycyrrhiza glabra* on rat liver microsomes. Treatment of 1.0 mg/mL glycyrrhizin knowingly impeded the classical complement pathway and boosted the antioxidant activity (Ablise et al. 2004). Another study accomplished by Punturee et al. testified favourable effects of asiaticoside saponin extracted from *Centella asiatica*. Figures showed that treatment of 100 mg/kg asiaticoside considerably phagocytic index and total WBC count when linked to not treated group. Additionally, it also increases the cellular and humoral immune responses (Punturee et al. 2005).

Examples of some saponins with immunomodulatory potential are:

- Asiaticoside (*Centella asiatica*) – Improves phagocytic index and total WBC count.
- Glycyrrhizin (*Glycyrrhiza glabra*) – Hinders classical complement pathway.

7.2.8 Terpenoids as Immunomodulator

Terpenoids, which are secondary metabolites, are the naturally occurring compounds often recognized as isoprenoids because of presence of isoprene units. Carvone, retinol, perillyl alcohol, betulinic acid, β-carotene and α-carotene are the most common examples of terpenoids. In plants, triterpenoids have defensive and microbial protecting activities. These compounds also display many beneficial activities such as antiviral, anti-diabetic, anti-inflammatory, antispasmodic and immunomodulatory activities. The favourable effects of terpenoids on immune system mostly arose due to either making of antibodies or improving T-cell response suppression (Ludwiczuk et al. 2017). Chiou and colleagues assessed the immunomodulatory activity of andrographolide extracted from *Andrographis paniculata*. A dose-dependent activity of andrographolide (1 ± 100 mM) in subduing the making of NO and iNOS was detected (Chiou et al. 2000). Alternative study stated the cytokine modulating activity of boswellic acid resulting from *Boswellia serrata* in paw oedema rat models. Facts showed that the treatment of boswellic acid at a dose of 0.25 mg/paw downregulated the activity of pro-inflammatory mediators and also indicated anti-arthritic activity (Singh et al. 2008). Podder et al. described the immunosuppressive effects of ursolic acid (UA) in macrophage THP-1 cell line.

Treatment of UA considerably dropped the intracellular *Mycobacterium* count by producing NO and ROS. In addition, it also stimulates the phagocytosis process in human monocyte cells and THP-1 cells which proposes the intracellular killing effects of UA in *Mycobacterium tuberculosis* infection (Podder et al. 2015).

Examples of some terpenoids with immunomodulatory potential are as follows:

- Andrographolide (*Andrographis paniculata*) increases the expression of IL-2 Inhibition of NO in endotoxin stimulated macrophages.
- Boswellic acid (*Boswellia serrata*) substantially inhibits mast cell degranulation.
- Ursolic acid stimulates intracellular killing effect of macrophages during *Mycobacterium tuberculosis* infection.

7.2.9 Immunomodulatory Potentials of Sterols

The introduction of glucocorticoids into our armoury of drugs transformed the line for the control of prolonged inflammatory- and immune system-associated ailments. Several in vitro studies have described their immunomodulatory activity by changing the cellular proliferation of T-cells and augmenting the activity of NK-cells in some types of cancer. It has also been anticipated that sterols and sterolins have capability to control the levels of Th1- and Th2-mediated cytokines which further aids in the enhancement of immune responses. Even very little concentrations of phytosterols, β-sitosterol and its glycoside have capability to mend the proliferative reactions of T-cells which make them powerful immunomodulators (Bouic 2002; Patel 2008).

Rasool et al. studied the immunomodulatory activity of withanolide extracted from *Withania somnifera* by using albino Wistar strain rats. Administration of withanolide to the rats considerably curbed the classical complement pathway, hypersensitivity reactions and mitogen-stimulated lymphocyte production. Thus, the study favours the development of withanolide as an effective immunosuppressive agent (Rasool and Varalakshmi 2006). Additionally, β-sitosterol and daucosterol also exhibited the immunomodulatory activity by refining the Th1 and Th2 immune responses against candidiasis spread in mice (Lee et al. 2007). An additional study done by Lee and colleagues testified the immunomodulatory activities of phytosterols extracted from *Clinacanthus nutans* by using murine cells. Mitogen-induced B- and T-cell proliferation and discharge of helper T-cell cytokines were scrutinized to analyse the immunosuppression of phytosterols (stigmasterol, shaftoside, β-sitosterol). Figures showed that usage of phytosterols considerably reduced the T-cell production and enhanced the Th1- and Th2-mediated cytokine expression (Le et al. 2017).

Examples of some sterols with immunomodulatory potential are:

- Withanolide (*Withania somnifera)*—Stimulates murine macrophages, phagocytosis and lysosomal enzyme activity.
- β-Sitosterol—Triggers human peripheral lymphocyte proliferation.

7.3 Carcinogenesis and Phytochemicals

Carcinogenesis is a multi-step progression described by an advancement of distinctive molecular alterations that eventually reprogram and convert a cell to endure uninhibited cellular division (Loeb and Harris 2008). With each disturbance, cells experience alterations primarily characterized by tumour instigation, advancement and evolution (Tokarz and Blasiak 2014).

Tumour beginning is a fast and permanent progression that starts with an exposure to a carcinogenic agent, followed by its spreading and transference to tissues triggering non-lethal mutations in cellular DNA. These "instigated cells" activate to store further irreversible genetic changes which continue with each new cycle of propagation (Barcellos-Hoff et al. 2013). Tumour advancement is a quite extensive and flexible course in which vigorously multiplying preneoplastic cells start to distribute and spread.

More recent data has highlighted the critical part of the tumour microenvironment on the survival and mutation of preneoplastic cells (Quail and Joyce 2013).

Cancer chemoprevention relies on the identification of causes that exactly influence initial phases of cellular transformation (Sapienza and Issa 2016). Naturally occurring phytochemicals have been found to have a wide array of cellular effects. Phytochemicals may check carcinogens from attaining targeted places and upkeep detoxification of very reactive molecules (Royston and Tollefsbol 2015). Some phytochemicals also boost innate immune scrutiny and recover the elimination of transformed cells (Luis Espinoza et al. 2013). Finally, phytochemicals have numerous influences on basic DNA repair mechanisms and may impact tumour suppressors and inhibit cellular propagation pathways (Sapienza and Issa 2016).

7.4 Chemoprevention: Types and Approaches

There are numerous kinds of approaches for cancer chemoprevention.

Primary chemoprevention targets to stop the growth of disease in the overall populace or in specific high-risk peoples. This sort of chemoprevention typically emphasizes on interventions on smoking population (for lung cancer), colorectal cancer and breast cancer.

Secondary chemoprevention emphasizes on persons who have been detected with some kind of tumour or premalignant abrasions that may headway to invasive cancer. This approach objects to limit the progress and advancement of malignant lesions.

Tertiary chemoprevention is directly targeted at checking the relapse or entrance of the new secondary tumours in individuals who have developed a malignance.

7.5 Nutritive Phytochemicals: Part in Malignancy Chemoprevention

Phytochemicals establish a varied set of bioactive compounds classified by chemical structure and comprise polyphenols, alkaloids, carotenoids and nitrogen compounds (Howes and Simmonds 2014). Plants produce phytochemicals to defend themselves against outside pressures and environmental means such as ultraviolet rays and producers of detrimental free radicals. The incorporation of these types of foods into our diet could provide us with the defence that the phytochemicals deliver for the plant (e.g. the ability to nullify free radicals in the body).

The various in vitro and in vivo studies have established added properties of phytochemicals, outside their antioxidant activity. These agents may also impact the production, progression and metastasis of tumours (Vauzour et al. 2010). These and other plant-derived substances may signify natural anticancer drugs. The extent of phytochemicals that we consume is directly associated with the kind of diet that is embraced, so recognizing foods that contain high amounts of phytochemicals might be the beginning for a cherished cancer deterrence approach.

These composites are naturally present in fruits, vegetables, grains and other plant products and are often liable for distinctive plant characteristics such as colour pigment and aroma. Moreover, many are fundamental for host safeguard against viruses, parasites and other outside detrimental agents.

The list of some cancer chemopreventive phytochemicals is mentioned in Table 7.1.

7.6 Phytochemicals: Cancer Chemopreventive

7.6.1 α-Linolenic Acid (ALA)

Alpha-linolenic acid (ALA) is one of the vital omega-3 fatty acids and organic compounds present in seeds (chia and flaxseed), nuts (notably walnuts) and many common vegetable oils. ALA has also been shown to downregulate cell multiplication of prostate, breast and bladder cancer cells (Chamberland and Moon 2015). ALA, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) can differentially obstruct mammary tumour growth by altering the cell membrane fatty acid composition, subduing AA-derived eicosanoid biosynthesis and prompting signalling transcriptional pathways to obstruct cell proliferation and prompt apoptosis.

Animal studies have shown that omega-3 fatty acids may thwart or impede the growth of cancers, signifying that omega-3 fatty acids are vital in cancer physiology. In a study, it was perceived that treatment with 1–5 mM of ALA inhibits cell proliferation, adhesion and invasion in both human and mouse colon cancer cell lines. Remarkably, ALA did not reduce total colony numbers when related to control. By disparity, it was found that size of colony was considerably altered by ALA treatment when matched to control in all colon cancer cell lines (Chamberland and Moon 2015).

Table 7.1 List of some cancer chemopreventive phytochemicals

Phytochemicals	Plant source	Role in cancer chemoprevention
α-Linolenic acid (ALA)	Flax seeds	Cancer protective, lessen danger of coronary heart disease (CHD) (Dave et al. 2020)
Allicin	Garlic, onion	Cancer protective, anti-inflammatory, liver protective (Koh et al. 2020)
Apigenin	Apple, artichoke, basil, celery, Cherry, grapes, nuts, parsley	Chemopreventive, encourages apoptosis and impedes breast and ovarian cancers, anti-inflammatory, antioxidant (Koh et al. 2020)
Carotene	Carrots, leafy greens and red, orange and yellow vegetables, pumpkin	Cancer preventive, increases discharge of immunogenic cytokines IL-1 and TNF-alpha, affords cornea guard against UV light, excites DNA repair enzymes (Koh et al. 2020)
Curcumin	Turmeric	Cancer preventive, anti-inflammatory, antioxidant (Kesharwani et al. 2015; Dave et al. 2020)
Ellagic acid	Cranberry, grapes, pecans, pomegranates, raspberry, strawberry, walnuts	Cancer preventive and antioxidant (Dave et al. 2020)
Ferulic acid	Oats, rice, orange, pineapple, peanut	Cancer protective, bone deterioration (Dave et al. 2020)
Gallic acid	Tea, mango, strawberries, soy	Cytotoxic and anti-oxidative activities, anti-leukemic, antineoplastic, anti-inflammatory (Dave et al. 2020)
Genistein	Alfalfa sprouts, red clover, chickpeas, peanuts	Antioxidant, anticancer agent (Koh et al. 2020)
Lutein	Kale, spinach, red pepper, mango, papaya, kiwi, peaches, squash, honeydew melon, plum, avocado	Protects against colon cancer, absorbs damaging blue light (Dave et al. 2020)
Lycopene	Apricots, papaya, pink guava, tomato, watermelon	Decreases threat of prostate cancer (Koh et al. 2020)
Resveratrol	Blueberry, peanuts, red grapes and red wine	Antioxidant, cancer preventive and aged (Dave et al. 2020)
Silymarin	Milk thistle (*Silybum marianum*)	Guards from UVB-induced carcinogenesis (Dave et al. 2020)
Stigmasterol	Soybean	Cancer preventive
Sulforaphane, glucosinolates	Broccoli sprouts, cabbage, cauliflower, collards, cruciferous vegetables, kale, radish, turnip	Antioxidant, check DNA damage, decrease threat of breast and prostate cancers (Dave et al. 2020)
Ursolic acid	Apple, basil, cranberry, lavender, oregano, rosemary	Anti-inflammatory, antitumor (Koh et al. 2020)
Withaferin, withanolides	*Withania somnifera*	Cancer preventive, immunomodulator (Dave et al. 2020)

The cytotoxic and anti-growth effects of conjugated linolenic acid (CLN) were also detected in other human cancer cell lines, DLD-1 (colorectal adenocarcinoma), HepG2 (well-differentiated hepatocellular carcinoma), A549 (lung alveolar cell carcinoma) and HL-60 (acute promyelocytic leukaemia). A fatty acid mixture rich in CLN (α-elcostcaric acid; 9c,11 t,13 t-18:3) presented dose-dependent growth inhibitory effects via initiation of the apoptotic pathway (Tsuzuki et al. 2004).

The anti-carcinogenic effects of CLN and conjugated linoleic acid (CLA) have been more established by Yasui et al. (2005). In their study, free fatty acids prepared from BGO (BGO-FFA) comprising more than 60% α-eleostearic acid (9c,11 t, l3t-l8:3) exhibited strong tumour growth inhibition and apoptosis induction in three human CRC cell lines, DLD-1, HT-29 and Caco-2, the effects being greater than CLA (9c,11 t-18:2). The study also proved that the inhibitory effects of CLN were related with modulation of peroxisome proliferator-activated receptor gamma (PPAR-γ) expression, which is one of the target molecules for subduing growth of cancer and other chronic diseases (Yasui et al. 2009).

Thus, these fatty acids are undeniably testified to be unique biomolecules having prospective health benefits, but it generally only occurs in very small amounts (<1%) in products of natural origin. The high amount of naturally occurring conjugated linolenic acid present in certain plant seed oils advocates it as much more manageable and easily obtainable for dietary use than formerly supposed. It can be used as a prospective biomolecule and multi-biological function that can be associated with its oxidative stability (Tanaka et al. 2011).

7.6.2 Allicin

Allium intake specifies some associations of *Allium* vegetable consumption with decreased risk of cancer, predominantly cancers of the gastrointestinal tract. Limited intervention studies have been showed to back these links. The bulk of supportive proof on *Allium* vegetables' cancer preventive effects arises from mechanistic studies. These studies highlight probable mechanisms of single sulphur-containing compounds and of many preparations and extracts of these vegetables, including reduced bioactivation of carcinogens, antimicrobial activities and redox alteration. *Allium* vegetables and their components have effects at each stage of carcinogenesis and affect many biological processes that alter cancer risk. The major garlic thiosulfinate produced is allicin (thio-2-propene-1-sulfinic acid S-allyl ester). Allicin and its oil-soluble metabolites are mainly accountable for garlic's odour.

A latest meta-analysis of 19 case-control and 2 cohort studies revealed that eating of great amounts of total *Allium* vegetables decreased risk of gastric cancer when associating the top and bottom feeding groups (odds ratio (OR): 0.54; 95% CI 0.43–0.65) (Zhou et al. 2011). The World Cancer Research Fund/American Institute for Cancer Research (WCRF/AICR) skilled panel also piloted a meta-analysis using 14 case-control studies that studied *Allium* vegetables and stomach cancer and 5 case-control studies that inspected garlic and stomach cancer. Their summary OR was 0.59 (95% CI 0.47–0.74) per 100 g per day for total *Allium* vegetable intake

with high heterogeneity, and 0.41 (95% CI 0.23–0.73) per serving of garlic per day (World Cancer Research Fund 2007). They also conducted meta-analyses of two cohort studies that examined total *Allium* vegetables and cancer, which made a summary effect estimate of 0.55 (95% CI 035–0.87) per 100 g per day with no heterogeneity. They established that due to steady evidence, dose-response relationship and plausible mechanisms, a cancer protective connection between *Allium* vegetables and vegetables was possible (World Cancer Research Fund 2007).

In humans, eating 5 g of garlic per day completely blocked the enhanced urinary excretion of nitrosoproline, an indicator for the synthesis of potentially carcinogenic nitrosamines, that occurred as an outcome of eating supplemental nitrate and proline. More latest data suggests that as little as 1 g of garlic may be sufficient to subdue nitrosoproline formation. Allium allyl sulphur compounds are also effective in blocking DNA alkylation, an early step in nitrosamine carcinogenesis (Nicastro et al. 2015).

7.6.3 Apigenin

Apigenin is a naturally occurring plant flavone (4′',5,7-trihydroxyflavone) plentifully present in common fruits and vegetables including parsley, onions, oranges, tea, chamomile, wheat sprouts and some seasonings. Apigenin has been shown to hold notable anti-inflammatory, antioxidant and anti-carcinogenic properties. In the last few years, substantial progress has been made in studying the biological effects of apigenin at cellular and molecular levels. In recent years, apigenin has been progressively accepted as a cancer chemopreventive agent. Apigenin has been shown to possess anti-mutagenic properties against nitropyrene-induced genotoxicity in Chinese hamster ovary cells. Apigenin has also been shown to inhibit benzo[a]pyrene and 2-aminoanthracene-induced bacterial mutagenesis. Laboratory studies have verified that apigenin supports metal chelation, scavenges free radicals and stimulates phase II detoxification enzymes in cell culture and in in vivo tumour models. Exposure to apigenin prior to a carcinogenic insult has been shown to afford a defending effect in murine skin and colon cancer models. Apigenin is a strong inhibitor of ornithine decarboxylase, an enzyme that plays a main part in tumour advancement. Further, apigenin has been shown to upsurge the intracellular concentration of glutathione, enhancing the endogenous defence against oxidative stress. The anti-carcinogenic effect of apigenin has been validated in a skin carcinogenesis model. Topical application of apigenin inhibited dimethyl benzanthracene-induced skin tumours. Apigenin also lessened UV-induced cancer incidence and improved tumour-free survival in related trials. Other significant targets of apigenin include heat shock proteins, telomerase, fatty acid synthase, matrix metalloproteinases and aryl hydrocarbon receptor activity HER2/neu, all of which have applicability to cancer development and progression (Patel et al. 2007).

The probable health benefits of apigenin have increased owing to its potent antioxidant and anti-inflammatory activities observed in vitro. There is a growing proof from epidemiological and case-control studies that greater consumption of

plant flavonoids decreases the threat of chronic diseases including cancer (Mennen et al. 2004; Xu et al. 2004). In contrast, intake of flavonoid-free diets by healthy human volunteers has been described to lead to a reduction in markers of oxidative stress in blood, viz. plasma antioxidant vitamins, erythrocyte superoxide dismutase (SOD) activity and lymphocyte DNA damage normally related with enhanced disease risk, signifying the beneficial effects of flavonoids on human health (Kim et al. 2003a, 2003b). Apigenin has been shown to have anti-proliferative effects on human breast cancer cell lines with different levels of HER2/neu expression.

The plant species and their parts comprising the highest amounts of apigenin are *Achillea millefolium* (yarrow plant), *Apium graveolens* (celery plant), *Artemisia dracunculus* (tarragon plant), *Camellia sinensis* (tea leaf), *Chamaemelum nobile* (perennial chamomile plant), *Coriandrum sativum* (cilantro fruit), *Digitalis purpurea* (purple foxglove flower), *Echinacea* sp. (coneflower leaf), *Gingko biloba* (biloba leaf), *Glycyrrhiza glabra* (liquorice root), *Linum usitatissimum* (flax plant), *Marrubium vulgare* (horehound plant), *Matricaria recutita* (annual chamomile plant), *Mentha spicata* (spearmint leaf), *Ocimum basilicum* (basil plant) and *Origanum vulgare* (oregano plant) (Patel et al. 2007).

7.6.4 Carotene

Carotenoids covering carotenes and oxy-carotenoids as two main groups are fat-soluble **pigments, broadly dispersed in nature.** The unique arrangement of alternating single and double bonds in the polyene backbone of carotenoids is accountable to quench reactive oxygen species (ROS), while the nature of specific end groups on carotenoids may impact their polarity. The electron-rich character of carotenoids makes them attractive to radicals, thus sparing other cell components (DNA, RNA, carbohydrates, lipids, proteins) from harm. Carotenes along with xanthophylls, astaxanthin, lycopene and lutein seem to offer defence against lung, colorectal, breast, uterine and prostate cancers. They support in prevention of heart disease and supplementation along with vitamin C and E, decrease the danger of developing diabetes and combat Alzheimer's disease. They are generally regarded as safe (GRAS), but increased consumption of carotenoids may cause the skin to turn orange or yellow, known as "carotenodermia". This incidence is completely benign and is unrelated to jaundice that can result from liver disease or other causes. Dietary carotenoids are found in a number of fruits and vegetables, such as green leafy vegetables, spinach, carrots, peaches, apricots and sweet potatoes. Human diet enhanced with carotenoids is useful in decreasing chronic conditions related to coronary heart diseases (CHD), certain cancers and macular degeneration (van het Hof et al. 2002; Prakash and Gupta 2014).

The supplementation with the amalgamation of β-carotene, vitamin E and selenium may impede cancer growth. It has been found that high supplemental intakes of lutein, zeaxanthin, cryptoxanthin, α- and β-carotene, etc. reduced the risk of breast, cervical and lung cancer. Lycopene acts to be particularly effective against cancers of the prostate, digestive tract and lungs and may also protect the body against the

effects of chemotherapy or radiation. They protect against sun damage because of their effect on the immune system, scavenger role towards oxidative substances and shield-like effect on the skin (Prakash and Gupta 2014).

7.6.5 Curcumin

Curcumin is a yellow polyphenol (diferuloylmethane) that is found in the rhizomes of turmeric (*Curcuma longa* Linn). Extensive experimental and clinical works over the earlier span have talked its beneficial effects against numerous ailments including diabetes, cardiovascular disease, arthritis, gastrointestinal ulcers, nephropathy and hepatic disorders. The useful actions of curcumin are linked to its anti-inflammatory, antioxidant and cyto-protective properties (Hosseini and Ghorbani 2015). Furthermore, it has been signified that curcumin has anticancer effects through its manifold activities on mutagenesis, cell cycle regulation, apoptosis, oncogene expression and metastasis. Diverse stages of cancer including origination, advancement and evolution can be affected by curcumin. It was found that this compound enhanced histologic parameters in one out of two patients with resected bladder cancer, one out of six patients with intestinal metaplasia of the stomach and one out of four patients with uterine cervical intraepithelial neoplasm. In a nonrandomized open-label study, 25 patients with pancreatic cancer were registered in an oral curcumin administration. Among them, two patients showed clinical responses: one had stable disease for >18 months and the other had tumour reversion (Dhillon et al. 2008). In a study by Sharma et al. (2001), consumption of curcumin was accompanied by a noteworthy reduction in lymphocytic glutathione S-transferase (GST) activity. The GSTs are a family of phase II detoxification enzymes and have been shown to be involved in the growth of resistance to chemotherapy drugs (Townsend and Tew 2003). The antitumor action of curcumin is facilitated via its anti-proliferative effect in multiple cancers, inhibitory action on transcription factors and downstream gene products, modulatory effect on growth factor receptors and cell adhesion molecules involved in angiogenesis, tumour growth and metastasis (Wilken et al. 2011). Recent data have suggested that curcumin may act by subduing the Sp-1 activation and its downstream genes, including ADEM10, calmodulin, EPHB2, HDAC4 and SEPP1 in a concentration-dependent manner in colorectal cancer cell lines; these results are steady with other studies, which have testified that curcumin could subdue the Sp-1 activity in bladder cancer and could reduce DNA binding activity of Sp-1 in non-small cell lung carcinoma cells. Latest data support that ER stress and autophagy may as well play a role in the apoptosis process, which is encouraged by the curcumin analogue B19 in an epithelial ovarian tumour cell line and that autophagy inhibition could surge curcumin analogue-induced apoptosis by prompting severe ER stress (Vallianou et al. 2015).

7.6.6 Lycopene

Lycopene is a naturally occurring carotenoid found in many fruits and vegetables, mainly with high concentration in tomatoes and tomato-based products. They are of great importance because of their potential activity in decreasing the menaces of tumours such as breast and prostate cancer. The way of action of their anticancer activity is due to their strong antioxidant and anti-proliferative activity and modulation of immune function (Liu 2004; Elliott 2005).

These phytochemicals are found in high amounts in carrot and tomatoes. Lycopene is the most abundant carotenoid in tomatoes (*Lycopersicon esculentum* L.) with concentrations ranging from 0.9 to 4.2 mg/100 g dependent upon the variety. Tomato sauce and ketchup are concentrated sources of lycopene (33–68 mg/100 g) equalled to unprocessed tomatoes (van Breemen and Pajkovic 2008). Other edible sources of lycopene comprise rose hips (Bohm et al. 2003), watermelon, papaya, pink grapefruit and guava (Mangels et al. 1993).

Lycopene was related with a 30–40% decrease of prostate cancer particularly in progressive stage of disease. Positive results were also observed with patients suffering from breast cancer. It was found that women with high plasma levels of carotenoids showed the statistically significant 18–28% decrease in the threat of breast cancer (Eliassen et al. 2012).

Lycopene has been found in a number of epidemiologic studies to be related with a lesser risk of prostate cancer (Thompson 2007). There are various studies backing the intake of lycopene and tomato products as a probable contributor to the lessening of prostate cancer risk (Giovannucci et al. 2002). In a forthcoming, case-controlled clinical trial, Chen et al. and Kim et al. established that regular consumption of commercial spaghetti sauce (30 mg of lycopene in 200 g of sauce) in pasta dishes for 3 weeks prior to radical prostatectomy caused considerably reduced oxidative DNA damage in prostate tissue matched to controls and improved apoptosis of prostate cancer epithelial cells (Chen et al. 2001; Kim et al. 2003a, 2003b; Konijeti et al. 2010).

In yet another preclinical study, it was proved that administration of antioxidants (including lycopene, selenium and vitamin E) in the diet of Lady transgenic mice subdued prostate cancer development and improved disease-free survival (Venkateswaran et al. 2004).

In an alternative study, the result of food processing on lycopene content in processed tomato products was compared with the raw ones. It was found that lycopene is more bioavailable in processed tomato products than in raw tomatoes, since arrangement of cis-isomers of lycopene during food processing and storage may raise its biological activity (Soares et al. 2019).

7.6.7 Resveratrol

Resveratrol (RES, 3,5,4′-trihydroxy-*trans*-stilbene) is a naturally arising polyphenol present in plenty of dietary stuffs, such as grapes, wine, nuts, berries and many other

human foods (Berman et al. 2017). It frequently occurs as a white powder with moderate water solubility (0.03 mg/mL). The molecular skeleton of RES is made by two phenolic rings, one with a *para* hydroxyl group and the other with an *ortho* double hydroxyl groups. The two benzene rings are linked through a double bond that gives isomers with *cis* and *trans* configuration. Generally, the most mentioned RES is the *trans* isomer, which is the most plentiful and biologically active complex. It is described that the total content of RES is around 50–100 μg/g in fresh grape skin, 5.1 μg/g in boiled peanuts, 0.31 μg/g in peanut butter and 0.98–1.80 mg/L in red wine (Cal et al. 2003). Besides, a great amount of RES is also found in Itadori plants and tea, and the commercial grape juice contains about 4 mg/L of RES (Burns et al. 2002).

Various studies have revealed that RES possessed chemo-protective effects, such as cardioprotective activity and neuroprotective activity (Cho et al. 2017; Riba et al. 2017; Sarubbo et al. 2017; Cai et al. 2018). Upon co-administration with chemotherapeutic agents, RES could lessen the associated side effects while boosting the healing efficiency related with cancer chemotherapy. The hostile effects induced by chemotherapeutic agents are all the time the hurdles for their wide-ranging application in clinic. As a naturally occurring multifunctional molecule, RES has been reported to be capable of performing shielding effects to lessen the associated side effects brought by chemotherapeutic drugs.

Resveratrol shows cardioprotective, nephro-protective, hepato-protective and gastrointestinal protective effects in UVR-induced skin cancer and as a synergistic agent in cancer chemotherapy (Xiao et al. 2019).

RES controls numerous pathways involved in cell cycle, apoptosis and inflammation. In addition to the chemopreventive and chemo-protective effects, RES also validates strong anticancer activity (Sarkar et al. 2009). It is known that a solitary treatment often validates weak activity, partial efficiency and drug resistance. The amalgamation therapy newly established through concurrently coalescing more than two drugs often carries improved healing results. As a naturally occurring small molecule, RES has been shown to be capable of facilitating cancer therapy via aiming multiple pathways involving cancer origination, advancement and evolution (Elshaer et al. 2018). Cancer initiation is the primary stage in cancer growth, and a critical biomarker for this event is the raised level of oestrogen-DNA adducts in tissue, which specifies a high risk of cancer, such as in the aetiology of breast cancer and prostate cancer (Pruthi et al. 2012). RES can hinder stimulating enzymes such as CYP19 (aromatase) and CYP1B1 (a kind of cytochrome P450 enzyme), and induce the manifestation of detoxification enzyme of NQO1 (NAD(P)H: quinone oxidoreductase 1), thus delaying the formation of oestrogen-DNA adducts to safeguard against oestrogen-initiated cancer. The synergistic effect of RES-mediated chemotherapy is also moderately accredited to the interfering action to cancer beginning. In addition, RES-induced cell sensitization and the involvement of RES in the modulation of cell cycle, particularly in S-phase, also play important roles for its synergistic effects (Lee et al. 2013).

7.6.8 Silymarin

As a usual product acquired from the fruits and seeds of the milk thistle plant (*Silybum marianum L.*), silymarin is a compound that contains silibinin, isosilybin-b, silydianin and silychristin, which are flavonoids. For numerous years, it has been used as a functional food in liver protection and in dealing with chronic epilepsy (Hackett et al. 2013; Chen et al. 2009).

Latest studies have shown that silymarin modifies the manifestation of proteins correlated to cell cycle regulation and apoptosis and thus controls the equilibrium between cell viability and apoptosis and displays anti-inflammatory, vascularization inhibitory, anti-oxidative and anti-metastasis effects (Surai 2015; Katiyar 2005). It has also been described to unveil anticancer effects in liver (Féher and Lengyel 2012), colorectal (Eo et al. 2016), breast (Hajighasemlou et al. 2014), lung (Wu et al. 2016) and prostate cancer (Deep et al. 2006).

Humanoid scientific trials have examined milk thistle or silymarin mainly in folks with hepatitis or cirrhosis, although minor studies have been recounted about individuals with acute lymphoblastic leukaemia, prostate cancer, breast cancer, head and neck cancer and hepatocellular carcinoma.

In a study, silymarin (100 mg/kg) considerably reduced the AGS tumour volume and improved apoptosis, as measured by the TUNEL assay, approving its tumour inhibitory effect. Immunohistochemical staining showed a preeminent expression of p-JNK and p-p38 as well as reduced manifestation of p-ERK1/2 associated with silymarin treatment. Silymarin was shown to decrease tumour growth through inhibition of p-ERK and stimulation of p-p38 and p-JNK in human gastric cancer cells. These effects showed that silymarin has likely to advance as a cancer therapeutic due to its growth inhibitory effects and generation of apoptosis in human gastric cancer cells (Kim et al. 2019).

Laboratory trials conducted using cancer cell lines have proposed that silibinin improves the efficiency of cisplatin and doxorubicin against ovarian and breast cancer cells (Scambia et al. 1996). Silibinin appears to have straight anticancer effects against prostate, breast and ectocervical tumour cells (Bhatia et al. 1999). Silibinin may also disturb the cell cycle in cancer cells by slowing down cell growth, as confirmed with prostate cancer cell lines (Zi and Agarwal 1999). Laboratory studies using leukaemia cell lines established that silibinin did not encouraged the growth of leukaemia cells (Duthie et al. 1997).

Activated protein-1 (AP-1), a composite containing homo- or heterodimers of the members of jun and fos family of proteins, controls the expression of numerous genes involved in malicious alteration. In particular, AP-1 is known to endorse epithelial to mesenchymal transition of tumour cells that is reflected as a key step in cancer metastasis. Our preceding studies have shown that silibinin subdues UVB-induced AP-1 and NF-kB instigation in mouse skin models (Deep et al. 2006). Newly, a study has testified that silibinin decreases PMA-induced invasion of MCF-7 cells through the specific inhibition of AP-1-dependent MMP-9 gene expression. These outcomes recommend that by overpowering the cancer cell invasion through the precise inhibition of AP-1-dependent MMP-9 gene expression,

silibinin epitomizes a likely anti-metastatic agent. Together, the anti-invasive as well as anti-metastatic prospective of silibinin could be of great importance in the development of a budding cancer therapy (Féher and Lengyel 2012).

7.6.9 Stigmasterol (Phytosterol)

Stigmasterol, a naturally occurring 6–6–6-5 mono-hydroxy phytosterol, possesses anti-inflammatory activities and has been projected as entrant for anticancer agents. The effect of stigmasterol on tumour and endothelial cells in vitro and their anticancer activities in vivo was explored. The outcomes confirmed that stigmasterol inhibited cell viability, migration and morphogenesis of human umbilical vein endothelial cells (HUVECs) but not cholangiocarcinoma (CCA) cells. Expression analyses disclosed that the treatment of both complexes considerably reduced the transcript level of tumour necrosis factor-α (TNF-α), and Western blot analyses additionally showed a decline in downstream effector levels of VEGFR-2 signalling, including phosphorylated forms of Src, Akt, PCL and FAK, which were released by TNF-α treatment. In vivo, stigmasterol dislocated tumour angiogenesis and reduced the growth of CCA tumour xenografts. Immunohistochemical analyses established a decrease in CD31-positive vessel content and macrophage recruitment upon treatment. These findings show that stigmasterol successfully targets tumour endothelial cells and suppresses CCA tumour growth by their anti-inflammatory activities and is an attractive candidate for anticancer treatment of CCA tumours (Kangsamaksin et al. 2017).

In a latest study, the anti-proliferative effects of normally consumed phytosterol "stigmasterol" against human cancerous breast (MCF-7) and liver (HepG2) cells and non-cancerous human embryonic kidney (HEK293) cells were assessed. The cytotoxicity concentration of stigmasterol against the MCF-7 (IC50; 27.38 μM) and HepG-2 (IC50; 25.80 μM) cells were greater than the HEK29 (IC50; 421.74 μM) cells, as determined by MTT assay. The cytotoxicity outcome was also established by the LDH assays ($r > 0.983$). The anti-proliferative potential of stigmasterol was also calculated at the molecular level. The RT-PCR results exhibited high expression levels of pro-apoptotic genes, whereas negative expression of anti-apoptotic genes (bcl-2). Both stigmasterol-treated cancerous cell lines revealed a growth in expression of the gene of caspase-9 and caspase-3. Conferring to the gene expression analysis outcomes, stigmasterol possibly stimulates the apoptosis signalling pathway, and hence genomic DNA fragments were perceived through gel electrophoresis. From the results, it was established that stigmasterol has apoptosis-inducing property and therefore to be assessed as an anticancer therapeutic in the animal model (Al-Fatlawi 2019).

7.6.10 Sulforaphane (SFN)

Sulforaphane is an isothiocyanate (ITC) complex that has been derived from cruciferous vegetables. It was revealed in several studies to be dynamic against numerous cancer types including pancreatic, prostate, breast, lung, cervical and colorectal cancers. Sulforaphane applies its therapeutics action by a range of mechanisms, such as by detoxifying carcinogens and oxidants through obstruction of phase I metabolic enzymes and by arresting cell cycle in the G2/M and G1 phase to hinder cell proliferation. The most prominent observation was the ability of sulforaphane to potentiate the activity of several classes of anticancer agents including paclitaxel, docetaxel and gemcitabine through additive and synergistic effects (Kamal et al. 2020).

SFN can be found in cruciferous vegetables, such as broccoli, cauliflower, Brussels sprouts, cabbage, kale and kohlrabi (Xia et al. 2019). SFN is stated to control cancer cell persistence via inhibition of cell multiplying and spur of apoptosis in a range of cancers (Bernkopf et al. 2018; Kan et al. 2018). It has the prospective to treat breast cancer also. Among the ITC members, SFN has been the most extensively explored regarding its pathological roles and molecular mechanisms both in vivo and in vitro.

Several researchers trust that the anticancer effects of SFN in bladder cancer (BC) are mainly related with caspase- and mitochondria-associated pathways. Nonetheless, there are other cancer-related factors convoluted. For example, SFN can impede DNA damage prompted by chemical carcinogens in BC T24 cells (Ding et al. 2010). Additionally, SFN-induced oxidative stress through ROS has been proposed as a key modulator (Park et al. 2014; Jo et al. 2014). Nuclear factor-erythroid 2-related factor-2 (Nrf2) regulation and endoplasmic reticulum (ER) stress are also related with SFN and carcinogenesis, pathological behaviour and cell persistence in UC. Unusually, these Nrf2 and ER signalling pathways are important features in the answer to oxidative stress and anti-oxidative activities (Leone et al. 2017). A study revealed that an enhanced insulin-like growth-factor-binding protein-3 (IGFBP-3) and curtailed nuclear factor-kappa B (NF-κB) expression by SFN are linked with the anti-proliferative effect of SFN in the BC cell line BIU87. Remarkably, the authors also established that SFN kindles apoptosis and cell cycle arrest at the G2/M phase, resulting from IGFBP-3 and NF-κB regulation (Dang et al. 2014). As IGFBP-3 and NF-κB are recognized to have pro-apoptotic and anti-apoptotic functions, respectively, in numerous malignancies (Patel et al. 2018), this spur of apoptosis by SPN via increased IGFBP-3 and decreased NF-κB levels is in agreement with proven findings. Additional report on the association between SFN-induced anticancer effects and growth factors confirmed that 20 μM SFN leads to a 2.6-, 3.0- or 3.1-fold increase in the G_2/M phase compared with that of controls in three BC cell lines (RT4, J82 and UM-UC-3, respectively) (Abbaoui et al. 2012). In addition, SFN prompts apoptosis in RT4 and UM-UC-3 cells. Thus, these findings show that upregulation of caspase-3/caspase-7 and PARP activity and downregulation of survivin, EGFR and HER2/neu are the fundamental molecular mechanisms (Mastuo et al. 2020).

Studies have revealed SFN hinders cell production, induces apoptosis, rests cell cycle and has antioxidant activities. Growing reactive oxygen species (ROS) yields oxidative stress and stimulates inflammatory transcription factors, and these end in inflammation leading to cancer. Growing antioxidant potential of cells and ascertaining new targets to lessen ROS creation diminishes oxidative stress, and it eventually condenses cancer risks. In short, SFN efficiently disturbs histone deacetylases intricate in chromatin remodelling, gene expression and Nrf2 antioxidant signalling.

7.6.11 Glucosinolates (Isothiocyanates)

Isothiocyanates (ITCs) are natural compounds of high medicinal value that are existing in cruciferous vegetables such as broccoli, watercress, Brussels sprouts, cabbage, cauliflower and Japanese radish. They are existent as conjugates in the genus *Brassica* of cruciferous vegetables (Lai et al. 2010). ITCs are recognized for their chemopreventive activity and facilitate anti-carcinogenic activity by subduing the initiation of carcinogens and growing their detoxification. The high content of glucosinolates, which accumulates ITCs in cruciferous vegetables, confers anti-cancerous effects. ITCs destroy tumour growth by initiation of oxidative stress-facilitated apoptosis, making cell cycle arrest and impeding angiogenesis and metastasis (Wu et al. 2009).

Benzyl isothiocyanate (BITC) is one of the main classes of ITCs that employ potential health aids to humans. It is widely found in *Alliaria petiolata*, pilu oil, watercress, garden cress and papaya seeds (Nakamura et al. 2007). BITC impacts several key signalling pathways which are deliberated to be the symbols of cancer. In addition, BITC alerts tumours to chemotherapy and has significant anticancer effects against several human malignancies like leukaemia (Xu and Thornalley 2000), breast cancer (Sehrawat et al. 2013), prostate cancer (Cho et al. 2016), lung cancer (Wu et al. 2010), pancreatic cancer (Sahu and Srivastava 2009) colon cancer (Lai et al. 2010) and hepatocellular carcinoma (Zhu et al. 2017). A printed study established that BITC prompts DNA damage in human pancreatic cells. It was also revealed that DNA damage originates G_2/M cell cycle arrest and apoptosis (Zhang et al. 2006). Another study proved BITC facilitated inhibition of the migration and invasion of human colon cancer cells. The anti-invasive effect of BITC was through downregulation of MMP-2/MMP-9 and urokinase-type plasminogen activator (uPA) associated with protein kinase C (PKC) and MAPK signalling pathways (Lai et al. 2010). BITC also showed antitumor effects by potentiating p53 signalling in breast cancer cells.

Phenethyl isothiocyanate (PEITC) is an additional isothiocyanate largely existing in cruciferous plants. PEITC is one of the vigorous ingredients of cruciferous vegetables that has been widely studied for its anticancer properties in glioblastoma, prostate cancer, breast cancer and leukaemia (Gupta and Srivastava 2014).

Numerous readings have specified that ingesting of cruciferous vegetables such as broccoli, watercress and garden cress leads to chemoprevention in many rodent models (Wang and Chiao 2010).

7.6.12 Ursolic Acid

Ursolic acid is a plant-derived pentacyclic triterpenoid originating from several medicinal herbs and fruits. An accumulative amount of proof supports the anticancer effect of ursolic acid in several cancer cells. Ursolic acid (UA) is extensively found in fruits and vegetables having the capability to impede breast cancer (BC) spread, angiogenesis and metastasis, halt cell cycle, prompt apoptosis, forage free radicals and control numerous anti-apoptotic and pro-apoptotic proteins.

UA may be a powerful inhibitor of nuclear factor kappa-light-chain-enhancer of activated B-cells (NF-κB), and downregulates the manifestation of apoptosis suppressor proteins, such as B-cell lymphoma-2 (BCL2) and BCL-XL in numerous cancer cell lines, comprising human colorectal carcinoma. UA shows the role of an anticancer agent through numerous signalling paths, plus the STAT3 pathway (Prasad et al. 2012). But whether UA can hinder human colorectal carcinoma cancer-initiating cells has not been testified to our information.

UA has also shown probable anticancer, anti-inflammatory and antioxidant activities in some human breast cancer (BC) cells. It has generated clinical interest owing to its anti-inflammatory, anti-oxidative, anti-apoptotic and anti-carcinogenic effects.

UA has showed chemopreventive and healing effects of cancer mainly through prompting apoptosis, hindering cell proliferation and checking tumour angiogenesis and metastasis. UA nano-formulations could increase the solubility and bioavailability of UA as well as show improved inhibitory effect on tumour growth and metastasis (Zou et al. 2019). Numerous data have also verified that UA inhibited tumorigenesis and cancer cell proliferation, moderated apoptosis and cell cycle development and encouraged autophagy (Kashyap et al. 2016; Jiang et al. 2018).

7.6.13 Withaferin and Withanolides

The anticancer property of *W. somnifera* was first stated by Devi and colleagues (Devi et al. 1992), who presented that intraperitoneal administration of alcoholic root extract of the plant totally retreated the growth of sarcoma-180 cells inoculated in naked mice. Following studies have confirmed that the hydroalcoholic root extract of the plant weakened 20-methylcholathrene (20MC)-induced development of fibrosarcoma in Balb/C mice when directed intraperitoneally or by gavage (Prakash et al. 2001). Davis and Kuttan (2001) have further stated the inhibitory effect of *W. somnifera* extract on chemically induced mouse skin tumour growth. According to a latest study, regular administration of root extract of the plant distinctly abridged methylnitrosourea-induced rat mammary tumorigenesis (Khazal et al. 2013). These

anticancer properties of Ashwagandha are attributable to withanolides, a class of bioactive constituents isolated from *W. somnifera*. Withaferin-A (4β,5β,6β,22*R*)-4,27-dihydroxy-5,6-22,26-diepoxyergosta-2,24-diene-1,26-di one) is the first antitumor withanolide that was extracted from leaves of the plant back in 1967.

Yang et al. first described the mechanism-based anticancer activity of withaferin-A, which abridged the progress of human prostate cancer (PC3) cells' tumour xenograft in naked mice by hindering the tumour angiogenesis and prompting intra-tumoural apoptosis (Lahat et al. 2010).

Withaferin-A has been informed to subdue mouse melanoma (B16F1) tumour growth in vivo. In additional skin cancer xenograft model using 92.1 uveal melanoma cells, about 29% of mice treated with withaferin-A showed an ample clinical response, while 43% of the animals exhibited cancer advance upon termination of treatment. Li and colleagues have lately informed that withaferin-A reduced the tumour diversity, though not the occurrence, of DMBA-initiated and 12-O-tetradecanoylphorbol-13-acetate (TPA) helped mouse skin tumour development partly by obstructive the expression of acetyl-CoA carboxylase-1 (ACC1) and the instigation of activator protein-1 (AP-1) (Li et al. 2016).

7.7 Conclusion

Dealing with metastasized cancers rests a challenge regardless of contemporary diagnostics and treatment procedures. Therefore, substitute methodologies are required. Chemoprevention using dietary phytochemicals such as α-linolenic acid, allicin, apigenin, carotene, curcumin, ellagic acid, ferulic acid, gallic acid, genistein, lutein, lycopene, resveratrol, silymarin, stigmasterol, sulforaphane, glucosinolates, ursolic acid, withaferin and withanolides in the preclusion of start and/or evolution of cancer poses an encouraging alternate approach.

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Part III

Infectious and Autoimmune Diseases

Immunomodulators and Autoimmune Liver Diseases

8

Kaligotla Venkata Subrahmanya Anirudh and Prameela Kandra

Abstract

Autoimmune liver disease (AiLD) is a series of progressive and chronic inflammation of the bile duct and liver cells arising due to impaired coordination between the components of one's immune systems ultimately leading to the destruction of the liver. This disease primarily constitutes autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC) under its wing. Immunomodulatory therapy established itself as a robust approach by providing a platform to treat such diseases. Although tremendous efforts have been put forth for instituting immunomodulatory therapy for PSC, the lack of positive results in a majority of the experimental studies prevailed with intense research is currently underway. This chapter unfolds with a brief perspective on the epidemiological, pathogenetic and clinical studies of AiLDs and dives deep into understanding the intricate dynamics of immune response during the pathogenesis of AIH. This study also highlights the numerous immunomodulators emphasizing its therapeutic potential for treating AiLDs.

Keywords

Hepatic cell inflammation · AiLDs · Immunomodulators · Sclerosing cholangitis · Primary biliary cirrhosis

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8.1 Introduction

The fundamental task of the immune system is to recognize self-cells from non-self-cells. If the immune system fails in discriminating those cells, it may cause autoimmune diseases. Significant damage causing a chronic and progressive inflammation to the hepatocytes and biliary ducts induced by the lack of coordination between the regulatory and effector cells of one's own immune system is a characteristic hallmark of autoimmune liver disease (AiLD). It constitutes primary biliary cirrhosis (PBC), autoimmune hepatitis (AIH) and primary sclerosing cholangitis (PSC) under its umbrella. In 1950, Waldenstrom observed liver cirrhosis in young females as a consequence of increased gamma globulins in the patient's serum, jaundice and amenorrhoea and described it to be autoimmune hepatitis (Lowe and John 2018).

In general, any imbalance of Tregs (regulatory T cells) which commonly play a major role in homeostasis and hindering unwanted autoimmune reactions causes autoimmune liver disease. The foremost requisite for diagnosing autoimmune hepatitis involves eliminating some of the possible causes of chronic hepatitis, for example, drug-induced liver injury (DILI), hepatitis caused by viruses and non-alcoholic steatohepatitis (NASH), as there is a high possibility of these to respond to immunotherapy. Some of the tangible characteristics of autoimmune hepatitis (AIH) involve the presence of a large concentration of ANA (antinuclear antibodies) and specific immunoglobulins (hypergammaglobulinemia) in the patient's serum, lymphoplasmacytic infiltration and death of limiting plates in the liver called piecemeal necrosis. As previously mentioned, hepatic injury is most commonly associated with AIH, whereas intra-hepatic damage to the bile ducts is observed as in primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC).

As early as in the 1950s, it was observed that corticosteroids were successful in causing a therapeutic response added with an early relapse potential. In the coming years, its beneficiary prospects combined with specific immunomodulators might lay the foundation for newer approaches. The main motive behind treating AIH by immunomodulation involving immunomodulators such as prednisolone and tacrolimus under the first line and second line of therapy is to achieve suppression of the patient's immune system. The only reason causing a setback to the current advancements in clinical trials of AIH is the limited incidence and substantial variations found during clinical studies. Apart from these, anti-CD20 monoclonal antibody and antitumour necrosis factor therapies are used for immunomodulation. Immunomodulation by using UDCA (ursodeoxycholic acid) and latest therapies such as obeticholic acid is employed for treating primary biliary cirrhosis. Generally, immunoglobulin infusion treatment is used for treating patients suffering from itching, a symptom of PBC which occurs in people aged 40–60 years. PBC commonly occurs in association with ulcerative colitis and may involve both intra- or extrahepatic injuries in male patients with a high probability of the development of dangerous cancers like bowel and gall bladder cancer (Wang and Zheng 2013; Than and Oo 2015). Further differentiation among the key aspects of the three diseases has been listed out (Table 8.1).

Table 8.1 Major differences between the different forms of AiLDs

Description	ATH	PBC	PSC
Specific disease incidence according to age	At any age	In people between 40 and 60 years of age	Common around 40 years of age
Specific disease predominance according to gender	Females	Females	Males
Elevated Ig levels in serum	Ig G	Ig M	–
Liver test transaminase levels	Fivefold rise in AST, ALT Onefold rise in ALP, GGT	Stable single fold rise in ALT, AST Threefold rise in ALP, GGT	Onefold fluctuated rise in ALT, AST Threefold fluctuated rise in ALP, GGT
Occurrence of granulomas	No	Yes	Has been found to occur very rarely in <10% of total cases
Autoantibodies detected	ANA, SMA, LKM1 SLA/LP (10–30% of AIH patients)	AMA, gp210	P-ANCA
Treatment with first line of therapy (immunosuppression)	Corticosteroids and UDCA	UDCA only	UDCA only
Causes a damage to	Hepatocytes	Intra-hepatic biliary duct	Both intra-hepatic and extrahepatic biliary ducts
Possible disease development	Hypothyroidism Coeliac disease Diabetes Arthritis Vitiligo RA	Hypothyroidism Coeliac disease Diabetes Arthritis	IBD

Some of the factors that could cause autoimmune liver disease has been put forth through the 'multiple hit hypothesis', that explains the possible interplay between genetic components such the X-chromosome and human leucocyte antigen, environmental factors, broadly classified as infectious and non-infectious agents, epigenetic, a person's immune system involving innate, etc. and have been depicted (Liberal and Grant 2016) (Fig. 8.1).

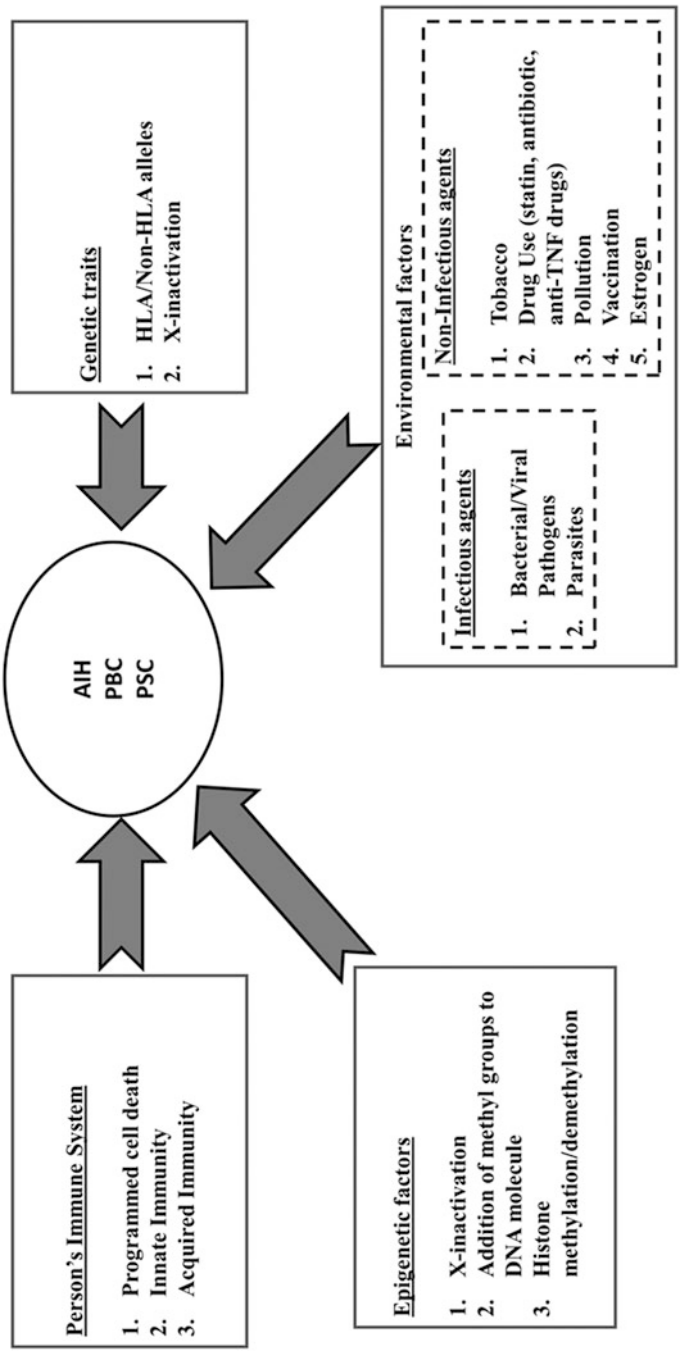

Fig. 8.1 Outline of different factors causing autoimmune diseases (Multiple Hit Hypothesis)

8.2 Autoimmune Hepatitis (AIH)

8.2.1 Epidemiological Characteristics

Autoimmune hepatitis is found to be more prevalent in women with nearly four females getting affected per male and can affect females of any age right from children to senescent. AIH can also be associated with other deadly diseases such as diabetes, arthritis and thyroid disorders. Therefore, before diagnosing potential AIH patients, it is a prerequisite to check the patient's family history with regard to such diseases. As shown through various studies, the incidence of AIH and the necessary clinical course varies depending on the ethnicity. For example, a study conducted showed that the people from North America were affected in higher numbers compared to their Caucasian counterparts where nearly 20 people were affected among one lakh of people. Another study proved black population to be severely affected by this disease resulting in cirrhosis. However, the people from Asia had higher mortality rate and generally contracted the disease at the later stages in their lives. In the United States, the ratio of incidence of this disease when analysed between females and males was 3.5:1, with another study showing women to constitute a total of 76 per cent among the total number of people to get diagnosed with AIH. Heterogenous trends in clinical studies showed the difference in ethnic groups and factors such as genetic traits and environmental factors to have a crucial role in outcome (Werner et al. 2008; Wong et al. 2012; Blachier et al. 2013; Czaja 2013).

8.2.2 Disease Pathogenesis and Mechanism of Immune Response in AIH

Unravelling the true cause and understanding the progression and development of such a disease is a very complex process and is yet to be fully understood. As mentioned before according to multiple hit hypothesis, it is presumed that several genetic and environmental factors such as viral induced infection are directed towards the liver antigens causing considerable inflammation and scarring (Liberal et al. 2011). As described, one may also develop AIH by using drugs such as adalimumab during anti-TNF procedure and antibiotics such as minocycline or by using rosuvastatin (statins). However, in response to either self- or non-self-antigens, adaptive immunity aided by the B lymphocytes and T lymphocytes (specifically, the glycoproteinaceous cluster of differentiation 4 and 8 T cells) plays a critical part in the disease's immunopathogenesis with the NK (natural killer) cells and macrophages mediating the effector responses (Oo et al. 2010a; Makol et al. 2011; Czaja 2011a; Liberal et al. 2013). A vital performer under the umbrella of the CD3 subset making up nearly 2 per cent is the Tregs (regulatory T cells). It is further classified into three distinct subsets, namely, CD4+, 25 and 127, that help in preventing autoimmune diseases and sustaining homeostasis (Wang and Zheng 2013; Muratori and Longhi 2013). Development of the regulatory T cell lineage

takes place at the surface epithelium of the medullary thymus as a consequence of self-antigens being presented. The constitutional characteristics and progression of Treg cells are mediated by a transcription factor, forkhead box P3 (Foxp3). In Autoimmune hepatitis, this Treg function is corrupted (Oo et al. 2010b; Muratori and Longhi 2013).

As discussed earlier, the genetic predisposition of a person, mainly HLA (human leucocyte antigen) haplotypes, determines the presentation of the autoantigenic peptides and recognition of T helper cells, for example, the binding of a peptide to DRB1*0301. The T cells then recognize them within the liver, thus becoming autoreactive. One crucial evidence suggesting the immune response mediated through T cells causing severe inflammation and condition such as fibrosis is the association of autoimmune hepatitis with several heterogenous haplotypes such as HLADR3 or HLA DRB1*0301 (Caucasian population), HLA DRB1*0401 (European population) and HLA DRB1*0405 (Japanese population). Apart from various haplotypes which pose to be a strong genetic predisposition, other vital co-stimulators of T cells, namely, CTLA-4 (cytotoxic lymphocyte antigen-4) and AIRE (autoimmune regulator) gene, which are necessary for imparting tolerance have shown strong associations with AIH (Lowe and John 2018). A detailed outline of the internal dynamics of the immune response that occurs during AIH has been represented in Fig. 8.2.

8.2.3 Clinical Features (Serum Autoantibody Studies)

For diagnosing autoimmune hepatitis, serologic tests are vital as the occurrence of autoantibodies in the patient's serum is what distinguishes this disease from others. In the case of AIH, some of the significant biochemical deformities observed are a substantial rise in transaminase, hyperbilirubinemia quantities along with a heterogenous rise in the levels of immunoglobulin G and alkaline phosphatase in the patient's serum during clinical studies. Some of the serological antibody markers essential for diagnosing AIH are antinuclear antibodies (ANA) which are found to be most useful for diagnosis when expressed along with anti-smooth muscle antibodies (ASMA) with a diagnostic accuracy of nearly 74 per cent. As mentioned, ASMA is important as it reacts against cytoskeletal elements such as F-actin. Another highly accurate antibody for diagnosing AIH is anti-soluble liver antigen (anti-SLA) or liver pancreas (LP) antibody coupled with ELISA for testing and has an accuracy of 99 per cent. But it is present only in 15–20 per cent of the patients in the United States. Apart from type 1 AIH diagnosing antibodies, the anti-LKM1 antibody to liver/kidney microsome type 1 is a marker for type 2 and is mainly found in children and rarely found in adults. It is similar to the antigen of hepatitis C targeting CYP2D6 antigen and is associated with DRB1*0701 allele which is highly prevalent in the southern parts of Europe (Washington 2007). Around 80% of the patients diagnosed with AIH may have ANA or smooth muscle antibodies (SMA) or sometimes both.

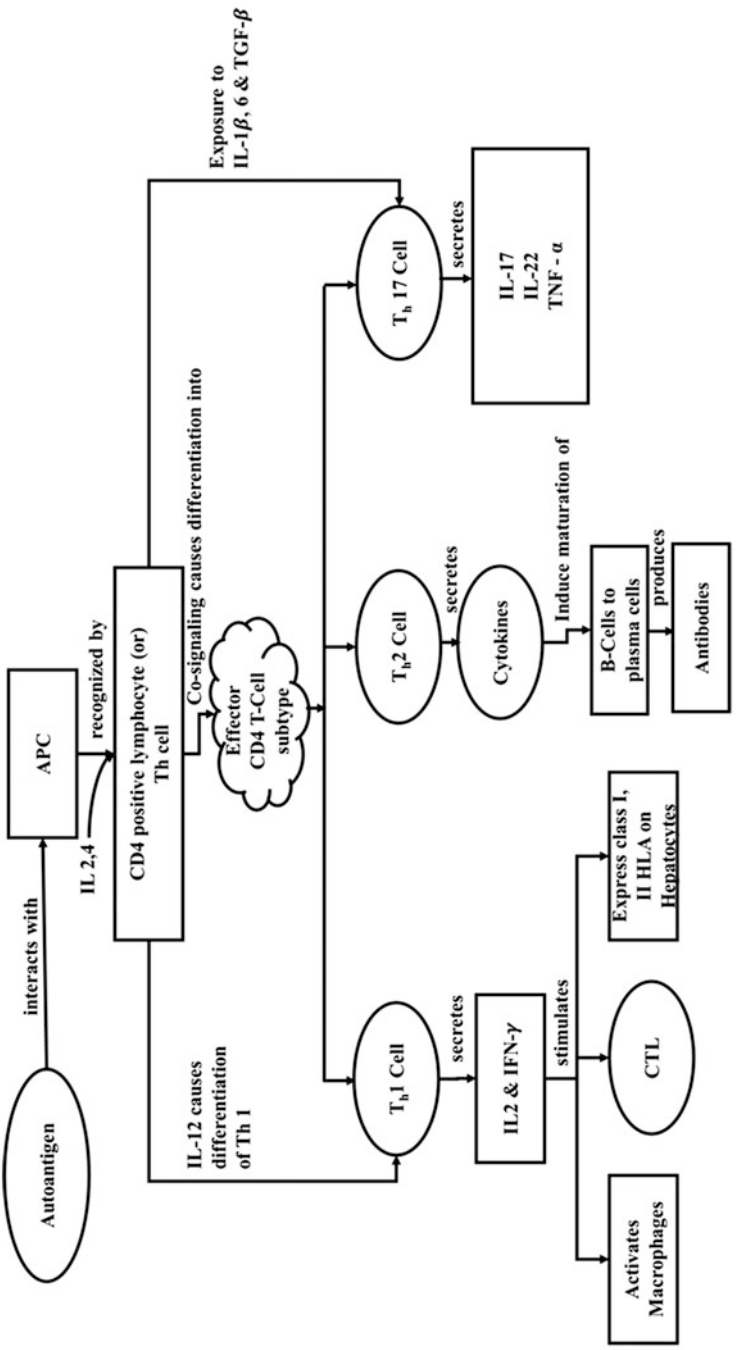

Fig. 8.2 Mechanism of immune response during AIH pathogenesis

ANA mainly reacts with DNA and histone molecules which constitute a major chunk, and such a scenario is also seen in the case of the disease lupus. The accuracy of the diagnostic tests mainly depends on the type of technique used. For instance, solid phase enzyme immunoassay and indirect immunofluorescence assays are highly employed for better results. However, ELISA is not preferred because the recombinant antigens differ from antigens detected in immunofluorescence assay. One advantage solid phase immunofluorescence assay has when compared to indirect immunofluorescence is its antigen specificity and very rapid post-testing results which is found to be lacking in the latter method (Hennes et al. 2008b; Czaja 2011b).

8.2.4 Classification of AIH

Autoimmune hepatitis is mainly classified into three types, namely, Type 1, Type 2 and Type 3, depending on the type of antibody profile. In AIH, antibody profile is of vital importance for distinguishing the distinct groups during pathogenesis but is not much useful in terms of clinical trials. However, Type 1 AIH is distinguished by ANA or SMA or sometimes both. It is more prevalent in the ages between 15 and 20 years or higher at 70 years. It is commonly found in Type 2 AIH patients. Type 1 AIH is found to be commonly associated with a human leucocyte antigen DR3 (also named as HLADRB1*0301) and DR4 (named HLADRB1*0401), with patients of type DR3 mostly recommended with liver transplantation (LT). On other hand, Type 2 AIH consists of anti-LKM1 antibodies expressing conditions such as dysplasia, candidiasis, etc. showing a strong correlation with Type 1 autoimmune polyglandular syndrome which typically occurs in children between the ages 2 and 4. Lastly, soluble liver antigen or LP antibodies are pertaining to Type 3 AIH, and the clinical features of this type are highly similar to Type 1 AIH and are quite indistinguishable (Washington 2007).

8.2.5 Treg Cells as Mediators in AIH

The main function of regulatory T cells in modulating the immunotolerance is by hindering the effector mechanisms of several cell types. Their improper functionality may be one of the causes of such autoimmune diseases. One such study conducted in London proved this in the case of paediatric autoimmune hepatitis. Another study proved the failure of Treg cells in inhibiting the IL-17 which is produced by CD+4 T cells. Till date the exact mechanism and role of Treg cell in AIH has not been understood. This may be due to the lack of understanding the true pathway of how Treg cells act by intracellular and extracellular markers (Sakaguchi et al. 2008; Longhi et al. 2010; Grant et al. 2014). Initially, Tregs were thought to be CD4+ and CD25+ T cells, but gradually, it has been proved that only cells having a high expression of CD25+ such as the transcription factor Foxp3 have the potential to suppress effector mechanism and used as a trustworthy marker. However, usage of

several Treg markers is efficient compared to Foxp3, and transient expression by the effector cells showed that a peripheral number of Tregs have not been impaired. It has been further elucidated and supported in the case of liver inflammation, where a tremendous increase in the number of Tregs was observed (Speletas et al. 2011; Peiseler et al. 2012).

8.2.6 Potential Triggers for AIH

One mechanism behind the major triggers for AIH is molecular mimicry, which is seen during hepatitis B/C infection. Here, autoimmunity plays a crucial role when a person's immune system instead of reacting against the foreign antigen in turn reacts with one's own immune components through structural homology and starts producing autoantibodies. In one of such studies investigating the generation of autoantibodies, it has been found out that in around 10 per cent of the patients diagnosed with hepatitis C virus infection, there has been a surge in the generation of anti-LKM-1 antibodies and direct correlation with disease severity. However, in Type 2 AIH, the body's own cytochrome CYP2D6 has been found as an autoantigen for anti-LKM-1 antibodies (Liberal et al. 2016). Apart from viral triggers, there are other environmental factors such as anti-TNF agents like infliximab and antibiotics like minocycline as depicted earlier in Fig. 8.1 of multiple hit hypothesis.

8.3 Primary Biliary Cirrhosis (PBC)

8.3.1 Epidemiological Characteristics

PBC results in a chronically progressive damage to the hepatic ducts as a consequence causing hepatic and portal inflammation along with hepatic dysfunction. Biliary cirrhosis is found to be more prevalent in females compared to males with a predominance ratio of 10:1, and among those over the age of 40 years, one out of a thousand women is likely to get diagnosed. Such a scenario has been observed in regions such as Britain and the United States. The incidence of PBC has bumped from nearly two cases per 1 lakh of population to nearly 3.2 within a span of 8 years in the United Kingdom starting from 1976 (Hirschfield and Invernizzi 2011; Bowlus and Gershwin 2014; Than and Oo 2015).

8.3.2 Disease Pathogenesis

As illustrated by the multiple hit hypothesis, apart from pathogens, etc., one study showed that in around 6 per cent of the cases, they had a minimum of one PBC diagnosed person in the near family. In the case of monozygotic twins, the percentage diagnosing for PBC having the same attributes among the two is nearly 63. Innate and adaptive immunity plays a major part in correlation with occurrence to

PBC because conventionally, class II human leucocyte antigens were thought to be associated. But after the advent of the genome-wide technology, apart from HLA, other risk loci like interleukin-12A, CTLA-4, etc. have identified. Another probable trigger might be through the anti-mitochondrial response as seen by the presence of self-reacting CD4 PDC-E2 T cells in hepatic cells and lymph nodes of the patient (Lindor et al. 2009; Hirschfield and Invernizzi 2011).

8.3.3 Clinical Features (Serum Autoantibody Studies)

After screening the patients who are diagnosed with biliary cirrhosis, raised levels of serum alkaline phosphatase have been observed, and some tend to exhibit symptoms such as fatigue, excessive bile salts and itching after a period of 2–4 years though some of the patients yet remain asymptomatic. UDCA as the first line of therapy, in a quarter percentage of patients, had no positive response over the span of 4 years, whereas nearly 30 per cent of the patients had shown significant improvement upon carrying out the liver biopsy and other enzymatic tests. One study showed that by treating patients of stage 1 and 2 with UDCA for an average time period of 8 years, they were showing features of a normal healthy population. On the other hand, the average time for the patients who underwent LT was found out to be nearly 9 years after treatment. Without any treatment, complete dysfunctionality of the liver was observed in an average of 5 years (Prince et al. 2002; Corpechot et al. 2005).

8.3.4 Stages of PBC (Histological)

Staging based on histological characteristics in the early stages of progress in this disorder is a challenge due to the fact that there might be hepatic duct loss with development of fibrosis. In the stage I PBC, damage to the lobes in the bile ducts is observed in the form of lesions without any fibrosis. In the case of stage II PBC, fibrosis and damage to periportal parts of the bile duct are visualized consequences of lobular bile duct damage. Stage III is also named as pre-cirrhotic stage with significant scarring of the liver being observed, whereas in the case of the cirrhotic stage (stage IV), total destruction of bile ducts and cirrhosis occurs (Washington 2007).

8.4 Primary Sclerosing Cholangitis (PSC)

8.4.1 Epidemiological Characteristics

This autoimmune disorder may affect the biliary components as a whole but mainly affects the biliary ducts which starts with inflammation and then progresses to fibrosis and shrinkage of the biliary system, thus leading to a chronically staged cholestasis resulting in hepatic cirrhosis. PSC is more prevalent in the European

male population with its incidence around an average age of 40 years, and a very low 0.4 cases have been reported in 1 lakh of people in the United Kingdom. Experts suggest that the quantitative incidence statistics may not be accurate due to the rarity of this disorder in people. Recent data has shown that around 75 per cent of the patients who have been diagnosed with IBD and 60–80 per cent of the people having ulcerative colitis have developed PSC (Eaton et al. 2013; Yimam and Bowlus 2014).

8.4.2 Disease Pathogenesis

An individual's parents or siblings who have been diagnosed with PSC have around 39% chance of developing sclerosing cholangitis. The risk loci of the human leucocyte antigens associated with PSC are HLA, DRB1 and Q1. Another hypothesis that aids in the development of an understanding regarding the pathogenesis of this disease is the 'gut and liver axis theory' where the microflora sustaining in the intestinal gut traverses to the hepatic and biliary system causing significant modifications to the immune system and metabolic pathways. This is through their metabolic products such as endotoxins, cell wall polymers, etc. resulting in severe hepatic inflammation. Apart from this, the movement of IELs (intestinal intraepithelial lymphocytes) having chemokine receptors such as CCR9 results in biliary damage (Hirschfield and Invernizzi 2011; Eaton et al. 2013; Karlsen and Boberg 2013; Tabibian et al. 2013).

8.4.3 Clinical Features (Serum Autoantibody Studies)

PSC in males generally develop at an average age of 30 years, and nearly 90 per cent of the stage II PSC patients tend to suffer from this disease for a span of 5 years or more. They have a propensity to exhibit features like strictures to biliary ducts, stones, acute bacterial cholangitis and sometimes bile duct cancers seen in 16 per cent of the patients. In around 80 per cent of the patients having sclerosing cholangitis, ANCA test for finding out anti-neutrophilic antibodies may be taxing due to its overlap with autoimmune hepatitis. Also, diagnosing the bile duct carcinoma tends to be highly challenging due to the fact that cytologic diagnostic tests may not give accurate results and performing cholangiogram may not yield the desired results due to the difficulty in distinguishing the tumour and the biliary strictures (Washington 2007).

8.5 Overlapping Syndrome/Variations in Autoimmune Liver Diseases (AiLDs)

In general, when a person exhibits disease characteristics, serum profiles and histological features either primary biliary cirrhosis (PBC) or primary sclerosing cholangitis (PSC) along with the symptoms of autoimmune hepatitis (AIH), such a

condition is called as an overlap syndrome. This is found in nearly 20 per cent of the AIH cases. Diagnosing such an overlap syndrome may be challenging since there are no specific diagnostic tests. Therefore, it is vital to check the patient's clinical characteristics multiple times or check the disease features once again before confirming. This is due to the fact that there is a confirmation yet lacking whether these overlap syndromes are unique or are variations of the original disease. The good part is that there might lie a possibility for developing a test by combining biochemical, radiation and immunological tests (Trivedi and Hirschfield 2012).

8.5.1 Overlap Features of AIH and PBC

AIH and PBC overlap occurs in around 8 per cent of the patients diagnosed with AIH or sometimes with PBC alone alongside having both the disease features of hepatitis and biliary cirrhosis. Furthermore, sometimes also for those patients for whom immunosuppressive drugs targeted towards a particular disease fail, treatment should be targeted to tackling both AIH and PBC simultaneously. As mentioned earlier with regard to the option of diagnosing with immunology, it is challenging due to the fact that sometimes there might be the presence of smooth muscle antibodies instead of anti-mitochondrial antibodies due to inefficacy of the immunofluorescence tests. One way of concluding this for a patient to be diagnosed with AIH/PBS overlap is by detecting elevated levels of immunoglobulins G and M, respectively, in the patient's serum along with higher levels of ALP and cholesterol in the serum of PBC patients. More specifically, for diagnosing this overlap syndrome as AIH/PBC, the patient must exhibit at least five times more level of ALT than the desired normal with the histological analysis of the liver exhibiting severe inflammation with respect to AIH.

Regarding PBC, the patient must have the level of gamma glutamyl transferase five times along with a biopsy report of the liver showing lesions on the biliary duct (Liberal et al. 2013).

8.5.2 Overlap Features of AIH and PSC

Similarly, in patients diagnosed with inflammatory bowel disease and autoimmune hepatitis, treatment may get affected as studies have shown nearly 40 per cent of the patients exhibit disease features of sclerosing cholangitis. This is especially significant in those people who developed either of the diseases mentioned above in their childhood to be susceptible to AIH/PSC overlap. In such an overlap, treatment should target PSC, and unless the patient still has IBD, the use of UDCA and immunosuppressive drugs should be encouraged. Yet, it is recommended that they be reduced in their usage prior to the liver's biopsy (Makol et al. 2011). In a study conducted taking 55 children with 16 years of age, cholangitis features were found in 27 of them, giving this syndrome another name called ASC (autoimmune sclerosing cholangitis). Histological reports in children may show irregularities in the formation

of bile ducts where there would be a malformation of the overt stricture with a high prevalence of intra-hepatic disorder (Gregorio 2001).

8.6 Potential Immunomodulators for Autoimmune Liver Diseases (AiLDs)

Toll-like receptors (TLRs) are some of the preeminent players in modulating the liver's immune system, thus making it one of the immunoprivileged tissues. The principal contestants accredited with modulating the liver's immune system making its environment non-reactive to the antigens that have crept in are the toll-like receptors (TLRs). But in some exceptional cases, these TLRs such as 2 and 4 might go rogue and be the sole cause for the pathogenesis of inflamed liver. One study showed that in the case of AiLD and primary sclerosing cholangitis, the signalling pathway involving toll-like receptor 4 and LPS (LPS/TLR4) was primarily responsible for the disease, and the inhibition of such toll-like receptors led to a downfall in the intensity of the disease pathogenesis (Pimentel-Nunes et al. 2010; Soares et al. 2010; Huebener and Schwabe 2013). The toll-like system induces immunotolerance against any foreign substance presented to the liver. Nonetheless, in case the host immune system is repressed, the probable effect would result in a long-standing infection of the liver. Therefore, by bringing the LPS/TR4 signalling pathway into play, a therapeutic response would be practicable. The subduing of the damage inflicted by AiLDs can be attainable by regulating the production of LPS, etc., bringing about the suppression of toll-like receptor 4 signalling (Broering et al. 2011).

In recent times, there has been extensive research in the field of the immunobiology of phages. So, by tapping the immunomodulatory potential of those bacterial viruses into creating a therapy for autoimmune liver diseases by maintaining homeostasis and preventing liver inflammation, they could be used to overcome problems caused due to the antibiotic's resistant bacteria, etc. By consolidating the data from earlier experiments, the bacterial viruses were found to play a part in maintaining a stable equilibrium environment in the GI region. Apart from this, they also curtail the T cells to divide and secrete cytokines and have also been found to decrease the detrimental immune reactions in mouse models with CIA (Górski et al. 2012; Międzybrodzki et al. 2017).

Apart from this, bacterial viruses have the potential to under-express those particular factors associated with AiLDs such as TLR4, ROS production, NF-kB transcription factor, IL-1, IL-10 and others. In patients with extremely low levels of monocytes and granulocytes, their levels can be brought back to normal by administering phage therapy, suggesting its therapeutic effect. Alongside its numerous advantages as an immunomodulatory agent, phages can help boost the generation of interleukin-10, an anti-inflammatory agent, and play a role against hepatic damage that is commonly observed in AiLDs. In the case of AIH, these phages help to ameliorate the inflammation and prevent the need for LT because of the end-stage liver disease. With respect to PBC, its central role would be to suppress autoimmune

reactions, which would be the same in PSC and fight against bacterial caused progression to end-stage LD (Van Belleghem et al. 2017; Górski et al. 2018).

8.6.1 Immunomodulation in Autoimmune Hepatitis (AIH)

Treatment against autoimmune hepatitis is by first suppressing the hepatic inflammation caused due to inflammatory cytokines. This can be achieved by using immunomodulators such as budesonide and prednisolone in combination with AZA (azathioprine) or sometimes individually.

Diabetes and osteopenia are some of the side effects generally observed in patients induced with steroids. In around 40 per cent of the treated patients, within the time frame of 365 days, such effects have been observed, and in nearly twice the percentage of patients, they have been seen in a biennial period. One way of circumventing the probable side effects caused by administering conventional steroids is by employing the synthetic immunomodulatory budesonide. This is due to its presystemic hepatic metabolism and a viable option for patients suffering from diabetes mellitus and those intolerant to prednisolone. Therefore, to alleviate such probable side effects, usage of AZA with azathioprine is preferred with patients diagnosed with AIH due to the lower percentage of them experiencing these as compared earlier (nearly 10 per cent). In the case of diagnosed patients intolerant to AZA, mercaptopurine (6-MP) is suggested which, after entering the metabolism, turns into thiouric acid (6-TU) or methyl mercaptopurine (6-MMP) (Strassburg and Manns 2011; Than and Oo 2015). Once the patients were treated, in a span of 14 days, a significant rise in the antibodies and hepatic enzymes in the serum of nearly 90 per cent of the patients has been found. In the case of patients where the AIH relapse occurred, there is a three times rise in the AT levels in nearly 50 per cent of patients after 6 months of ending the therapy (Manns et al. 2010; Makol et al. 2011).

8.6.2 Tac (Tacrolimus)

Tacrolimus is mechanistically similar to cyclosporine A, and both are calcineurin inhibitors. These are chemically compounds with a lactone ring bound to the deoxy sugar molecule binding to a unique immunophilin causing nephrotoxicity as its side effect. Very few studies were carried out on the use of Tac for patients with AIH; in one pivot study where 21 patients were administered with Tac for a period of 3 months, a drastic fall in the hepatic enzymes was found in nearly 80 per cent among them (Yeoman et al. 2010; Strassburg and Manns 2011).

8.6.3 MMF

Mycophenolate mofetil is an inhibitor of inosine monophosphate. The non-competitive mode of action plays a role in hindering the rate-determining process during the generation of purine nucleotides from R5P and lowering the proliferation of B and T cells. During the immunomodulatory therapy using MMF, many patients tend to exhibit side effects such as diarrhoea, dizziness and headache. In a study where MMF alongside prednisolone was given for a therapy period of 90 days to 59 AIH-diagnosed patients, significant positive changes in their immunological and biochemical characteristics were seen in nearly 88 per cent of them. The remaining percentage of patients showed partial yet positive responses. In a similar study conducted on 16 patients in Canada dosed with MMF or Tac, a positive response was seen in 8 of patients, with the other 12.5 per cent of them displaying no response at all, concluding that in many ways, this immunomodulatory therapy was better than conventional therapies (Hennes et al. 2008a; Zachou et al. 2011; Liberal et al. 2013).

8.6.4 Rituximab

Treatment of AIH with CD-20 targeting monoclonal antibody mainly results in the reduction of a number of B lymphocytes through cytotoxic pathways. Upon investigating the pathogenesis of AIH, it was seen that both B and T lymphocytes play a part. In one study conducted on rituximab's potential immunomodulatory role, patients showed no possible side effects and significant positive biochemical characteristic changes in those who previously showed no positive results during conventional therapy (Burak et al. 2013).

8.6.5 Recombinant Antibody

Infliximab is a humanized antibody and is generally used in AIH-diagnosed patients with LT and has also shown to treat patients diagnosed with IBD and RA successfully. In one study conducted for testing infliximab's potential on 11 patients, there was significant suppression of the hepatic inflammation along with a fall in the levels of antibodies in the patient's serum (Weiler-Normann et al. 2013).

8.6.6 Cyclic Peptides

The cyclic peptide is one immunomodulator with lipophilic nature. The T lymphocytes' functionality gets hindered with the help of the IL-2 gene by acting on Ca-dependent signalling pathways. A total of three experimental studies are reviewed here. In a study involving six AIH-diagnosed patients, cyclosporine homogenized the level of alanine aminotransferase in all of them. In yet another

study, out of five patients who were given conventional therapy, four showed significant improvements. Lastly, out of the eight patients to whom cyclosporine was administered, every one showed AIH remission. One disadvantage of using this immunomodulator is that it can cause side effects such as hypertension and induce cancer in some cases (Sciveres et al. 2004; Strassburg and Manns 2011).

8.6.7 SCO Immunomodulator for AIH

Schisandra chinensis is a fruit obtained from a plant employed in traditional Chinese medicine named Chinese magnolia vine and has shown high efficacy in treating diseases such as cardiovascular and intestinal diseases in the olden days. This fruit contains fatty acids, vitamins and essential oils such as *Schisandra* oil (SCO), which has nearly six types of lignans. These are mainly hepatoprotective in nature, and extensive studies on the metabolic activities of these lignans have been carried out which showed their role in upregulating cytochrome B5 and NADPH reductase activities and speeding up the proliferation of hepatocytes. Also, it modulates hepatic circulation and offers protection to hepatic cells during oxidative stress (Mocan et al. 2016; Szopa et al. 2017; Kortesoja et al. 2019). In the present study, SCO immunomodulatory effects on concanavalin A (Con-A)-induced AIH in mouse models are studied. Apart from its role in stimulating the T cells, this lectin helps activate the Kupffer cells and other monocytes, causing an overall hepatic inflammation due to the production of various interleukins and necrosis factors. Mentioned below are some of the other immunomodulatory properties of SCO against AIH. It reduces the levels of ALT and AST in the patient's serum. It also inhibits the activation of T cell in mediastinal lymph nodes and spleen induced by concanavalin A along with hindering the expression of cytokines by obstructing the activation of immune cells offering protection against hepatic inflammation in the liver (Takahashi et al. 2009; Zhang et al. 2010; Soares et al. 2011; Dong et al. 2019).

8.6.8 LDIL-2 as an Immunomodulator for AIH

Based on an experimental study conducted where low-dose interleukin-2 therapy was administered to patients who have autoimmune hepatitis, it was found that its usage was safe to the patient and played a part in upregulating the concentration of regulatory T cells in circulation. The experiment's predicted outcome was that it modulated the pSTAT5 response to an optimal level by increasing the sensitivity of regulatory T cells to interleukin-2, a characteristic hallmark of AIH, when used in two varying sets of LDIL-2 doses.

It was also observed that in the patients who were administered much higher or increased frequency of doses, the aforementioned effects sustained for a longer duration than in the patients who were given a lower dose. More so, by employing such variance in doses, no substantial changes were observed in other immune cells except the Treg cells. In another study conducted on animal models, it was found that

an increase in regulatory T cells was suppressed in the liver during severe hepatic disorders. This study proves the potential and viable immunomodulatory role of LDIL-2 in the case of AIH by pumping-up the number of lymphocytes and reducing the inflicted hepatic destruction (Hartemann et al. 2013; Liberal et al. 2015; Lim et al. 2018).

8.7 Immunomodulation in PBC

8.7.1 Ursodeoxycholic Acid (UDCA)

Ursodeoxycholic acid, also called UDCA, plays a major therapeutic and immunomodulatory role in PBC by increasing the BA secretions from hepatic cells and ducts. This gives protection against a cytokine and BA-induced injury increasing the BA pool's water-loving index, which in turn results in interference of hepatic circulation. A substantial drop in the toxic hydrophobic natured BA pools was observed in the hepatic environment. Usage of UDCA results in the rise of ROS levels and stabilizes the membranes of hepatocytes. Apart from this, inhibition of MHC class I and II expression in hepatic cells can be seen, thus preventing the severe damage inflicted during UDCA's immune response (Poupon 2012).

Obeticholic acid (OCA) majorly helps activate a nuclear hormone called FXR (farnesoid X receptor) and is derived as a semi-synthetic analogue of BA CDCA. Homeostasis of BA is regulated by apical sodium BA transporter, thus managing the expression. A significant rise in the expression of FGF-19 (fibroblast growth factor-19) is seen when FXR gets activated and hinders both ASBT and an enzyme involved in BA production (CYPA1). In hepatocytes, the uptake of BA could be limited by activation of FXR (Poupon 2012; Patel and Seetharam 2016). Other immunomodulators are fibrates (fibric acid derivatives) proven to inhibit the kappa light chain, which reduces cytokine expression (Lens et al. 2014). Recently, some studies have been conducted on the fibrates such as fenofibrate in association with UDCA and showed a reduction in ALP levels (Cuperus et al. 2014).

Glucocorticoids (budesonide) are corticosteroids having a very high binding affinity to its receptor, which can be attributed to the fact that it immediately gets absorbed in the small bowel due to the underlying internal metabolism compared to other glucocorticoids. By studying the data from the set of experimental studies performed, it can be observed that by administering budesonide in combination with UDCA, a significant improvement has been observed concerning the liver histology and biochemical characteristics in the case of PBC. Usage of budesonide may cause several side effects like osteopenia (Parés 2014; Patel and Seetharam 2016).

Rituximab (mAb) is another immunomodulator used for hepatic autoimmune diseases. In the case of regular and transformed B cells, the CD20 antigen is the target of rituximab. This chimeric monoclonal antibody destroys the B lymphocyte either through a cell-mediated or humoral pathway with a minimal noticeable effect on the biochemistry of the liver. Apart from the immunomodulatory role of this

monoclonal antibody in PBC, it has also shown a significant effect in cancers such as non-Hodgkin's lymphoma, etc. (Myers et al. 2013; Floreani et al. 2016).

Basically, an immunomodulatory therapy involving ustekinumab is administered only when the patient with PBC does not show any promising results on conventional treatment. Its main target is on the Th1/Th17 signalling pathway, which comprises interleukin-12 and interleukin-23 as their major cytokines, with experimental data showing zero effect on the patient's liver biochemistry (Floreani et al. 2016).

Liver transplantation (LT) is the only viable option to treat patients suffering from end-stage PSC because, as of today, no treatment has been found suitable to administer to patients with cholangitis. In some experimental studies, it has been shown that in patients with PSC, UDCA usage at high doses ranging from 17 to 30 mg/kg/day did not lead to any significant survival rate compared previously. But, by using a dose range of around 10 mg/kg/day, it showed a betterment in the hepatic biochemistry and histological findings. In some cases of patients with sepsis of bile duct and bacteraemia, antibiotics such as metronidazole have been used and have shown positive results in the treatment. Furthermore, vancomycin specifically reduced the chances of progression to end-stage LT (Imam et al. 2011; Triantos et al. 2011; Tabibian et al. 2013; Than and Oo 2015).

8.8 Conclusion

Diagnosis of different autoimmune liver diseases, understanding their mechanism in biochemical pathways and immunological modifications and studying their different stages are some of the likely emerging topics. Although current treatment methods are under trial, there is a significant need for further research to understand the treatment process better and post-treatment consequences. However, aspiration of present defiance requires enactment of biomarkers that help one make several solutions according to disease behaviour, pharmacological evaluation and prognosis of therapies.

Competing Interest The authors declare that there are no competing interests.

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Immuno-Modulatory Role for the Treatment and Management of Tuberculosis

9

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Abstract

Tuberculosis is caused by a bacterium called *Mycobacterium tuberculosis* (*M-tb*) which leads to major therapeutic challenges causing several immune dysfunctions by affecting various immune checkpoints. Over the past decades, many research efforts have been made to control tuberculosis infections. However, the etiology of tuberculosis reveals that *M-tb* has coevolved with human immune response and hijacks various defense mechanisms of natural and synthetic antimicrobial agents contributing to the development of multidrug resistance. Henceforth, the strategy of immunomodulation such as host-directed therapy (HDT) emerges as an important therapeutic modality in treating infectious diseases like tuberculosis. Moreover, the growing understanding of immune checkpoints of bacterial infections leads to the discovery of immuno-modulatory methods and novel drug targets. Thus, the present chapter discusses the efficacy of various immunomodulation against the etiology of *M-tb* infections and challenges in the development of different classes of immuno-modulatory agents.

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Antibiotic resistance · *Mycobacterium tuberculosis* · Immune checkpoints · Immuno-modulatory agents

9.1 Introduction

Infection caused by *Mycobacterium tuberculosis (M-tb)* causes pulmonary and extra-pulmonary tuberculosis (TB) which is the leading cause of death by a single microorganism (Chowdhury et al. 2018; Mvubu et al. 2018). Globally, around 1.7 billion people are affected by TB infection every year (WHO 2019). Among them, the majority is asymptomatic, and very few show symptoms of TB. The asymptomatic condition is attributed due to several factors associated with the host (Ahmed et al. 2020). Generally, innate and adaptive immunities are considered to be major controlling arms against the disease. But there is accumulating evidence to suggest that pathogenic *M-tb* evolved to bypass the host immune response to promote its growth and infection (Cruz et al. 2015). Besides, the pathogenic *M-tb* produces specific virulence factors which regulate the functioning of the immune system leading to its replication in the host and causing inflammation in the host (Johnson et al. 2017; Esin et al. 2013). Therefore, WHO recommends different classes of antituberculosis drugs for controlling the *M-tb* infection (Sacchettini et al. 2008). Conversely, long-term and indecorous drug prescription leads to drug resistance. Such drug resistances of *M-tb* disease are categorized as multidrug resistance (MDR) tuberculosis and extensively drug resistance (XDR) tuberculosis (Gygli et al. 2017). The MDR-TB is resistant to two first-line drugs (rifampicin and isoniazid), whereas XDR-TB displays resistance additionally to fluoroquinolone class and any one injectable second-line anti-TB drugs (Gygli et al. 2017; Shah et al. 2007). Concerning MDR and XDR, WHO recommends several treatment regimens with new drug combinations, novel drugs, and repurposed drugs, for controlling the disease (Ahmed et al. 2020). However, in resource-limited countries, high cost for TB detection, lack of timely modifications, and non-adherent treatment regimen lead to poor management for patients suffering from MDR and XDR tuberculosis (Morrison et al. 2008). The recent advance in the modulation of immune response will be the best strategy for the prevention and treatment of *M-tb* infections (Tsenova and Singhal 2020; Tobin 2015). Many of the available immuno-modulators are capable of correcting the congenital defect in the functioning of the immune system. Furthermore, immuno-modulatory therapy works by targeting the host instead of the pathogen, thereby impeding the evolution of microbial resistance (Gupta et al. 2016; Esin et al. 2013). Thus, the host-directed therapy will be effective and could speed up the treatment regimen by reducing the hyper-inflammatory response and TB pathology and promotes the memory that reduces the rate of relapse following therapy (Ahmed et al. 2020; Maiga et al. 2015). This chapter appraises the molecular mechanism of *M-tb* survival and pathogenesis on the progression of TB infection, highlights the recent updates on developing new and repurposed drugs

with antituberculosis property, and also addresses the necessity of immunomodulation in the prevention and treatment of TB infection with improved functionality.

9.2 Adaptation and Pathogenesis of *M-tb* Infection in Host

9.2.1 Regulation of Tuberculosis Granulomas

The formation of granuloma in host immune cells is a complex process that leads to either latency state or progression to a disease state (Salgame 2011). Usually, the inhaled *M-tb* enters the lungs via the trachea where they are engulfed by alveolar macrophages (AM) and subsequently degraded by phagosomes. AM could regulate the granulomas through their polarized form (M1/M2 phenotypes) (Tsenova and Singhal 2020). The M1 macrophage differentiation occurs by stimulation from T-helper type 1 (Th1) cytokines (IFN-γ and TNF) to produce several antibacterial agents (reactive oxygen species (ROS), nitric oxide (NO)) (Harriff et al. 2014). While the M2 macrophage differentiates into M2a, M2b, and M2c mediated by Th2 cells (IL-2 and IL-13), pattern-recognizing receptors (PRRs), and Treg cells (IL-10). Progression of TB is mainly suppressed by the M_1 phenotype, whereas the incorrigible inflammation is controlled by M_2 along with Th_2 type immunity (Tsenova and Singhal 2020; Tan et al. 2017). It was reported that after *M-tb* invasion, host cells restrict the pathogens through the formation of granuloma by the accumulation of immune cells such as granulocytes, dendritic cells (DCs), natural killer (NK) cells, and lymphocytes (B and T) surrounded by infected AM (Mvubu et al. 2018; Robinson 2017; Volkman et al. 2010). On the contrary, a major proportion of TB patients exhibit a quiescent state with the granulomas; thus, *M-tb* overcomes the host defense and hypoxic milieu. Moreover, *M-tb* like other mycobacteria is capable of persisting over a long period in the infected host cells without expressing any virulence (Yihao et al. 2015). During the granuloma stage, the innate (macrophage secreting cytokines and other anti-*M-tb* factors) and adaptive immune response (T-cell-mediated immunity) could control the intracellular *M-tb* during the latent phase of TB (Wang et al. 2012; Yadav and Schorey 2006). The latent *M-tb* turns to an active state in immune-compromised individuals, thus leading to the replication and triggering the necrosis of macrophages and facilitating the easy spread of infection (Cruz et al. 2015). Thus, the fate of TB progression is mainly regulated through a fine balance between the host and pathogen factors that influence the microenvironment within tuberculous granulomas.

9.2.2 Invasion of M-tb into Target Cells

The immune cells in which the invasion of *M-tb* takes place include epithelial cells, endothelial cells, fibroblast, and neuronal cells (Tsenova and Singhal 2020). Intracellular colonization of *M-tb* with unique adhesion molecules plays a prime role in disseminating TB infection to multiple organs (Tsenova and Singhal 2020; Franchi

et al. 2008). Several different host pattern-recognizing receptors (PRRs) intercede complex mechanisms of *M-tb* intake in the host cells. Of these, receptors such as Fc type, mannose receptor, C-type lectin receptors (CLRs), dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN), and macrophage-inducible C-type lectin have been identified from the host cells (Faridgohar and Nikoueinejad 2017; Pahari et al. 2017; Sancho and Reis e Sousa 2012; Yamasaki et al. 2009). Obviously, concerning *M-tb* surface, very few adhesion molecules were recognized such as heparin-binding hemagglutinin adhesin (HBHA), *M. tuberculosis* pili (MTP), Mammalian cell entry (Mce) family proteins, and ESAT-6 (Ryndak and Laal 2019; Chai et al. 2018). Among others, HBHA is a well-characterized adhesin of *M-tb* which plays a crucial role in extra-pulmonary dissemination of *M-tb* by binding with heparan sulfate proteoglycans (HSPGs) in the cell membrane and baseline membrane underlying the alveolar macrophage (Bartlett and Park 2011). Thus, inhibition of HBHA expression influences only the *M-tb* adhesion to the alveolar macrophage and not the macrophage. However, the MTP and Mce family proteins are an important surface molecule of *M-tb* which helps in the adhesion and invasion of host cells (Chai et al. 2018). Also ESAT-6, an adhesion molecule that binds to laminin of baseline membrane and cell membranes of alveolar macrophage, thereby facilitates macrophage-independent *M-tb* dissemination via alveolar wall. Another class of adhesin is PknD, a laminin binding molecule that facilitates *M-tb* binding and invasion of microvascular epithelial cells of the human brain but not to alveolar macrophage or other macrophage (Ryndak and Laal 2019).

9.2.3 Intracellular Survival Based on M-tb Balance with Host Immune Defense

M-tb has evolved with many different strategies to adapt to the hostile environment inside the macrophage. An intensive literature survey reveals that *M-tb* contributes a variety of mechanisms for survival in immune cells as mentioned in Fig. 9.1 (Khan et al. 2016c; Meena and Rajni 2010).

9.2.3.1 Cell Wall-Associated Virulence Factor

Pathogenic *M-tb* expresses a variety of surface adhesive proteins which helps in the internalization of *M-tb* by binding to the host-specific receptors. Lipoarabinomannan (LAM), a unique lipid molecule and cell wall active polysaccharide, especially mannose-lipoarabinomannan (Man-LAM) plays a vital role in *M-tb* virulence (Meena and Rajni 2010; Eddie Ip et al. 2009). It is mainly driven by the phosphatidylinositol moiety of LAM which contributes to non-covalent interaction between *M-tb* and host cell membrane. Studies suggested that LAM along with other lipomannan constitutes the immuno-modulatory glycol conjugate molecules capable of interacting with host cell receptors during *M-tb* disease (Bisht and Meena 2019). LAM intervenes in calcium signaling, thereby inhibiting the activation of CaMKII for phagosome maturation. Additionally, Man-LAM hinders apoptotic signals in their host cells through the stimulation of Akt protein kinase activity. Hence, all these consequences demonstrate that LAM, a major constituent of the *M-tb* cell wall,

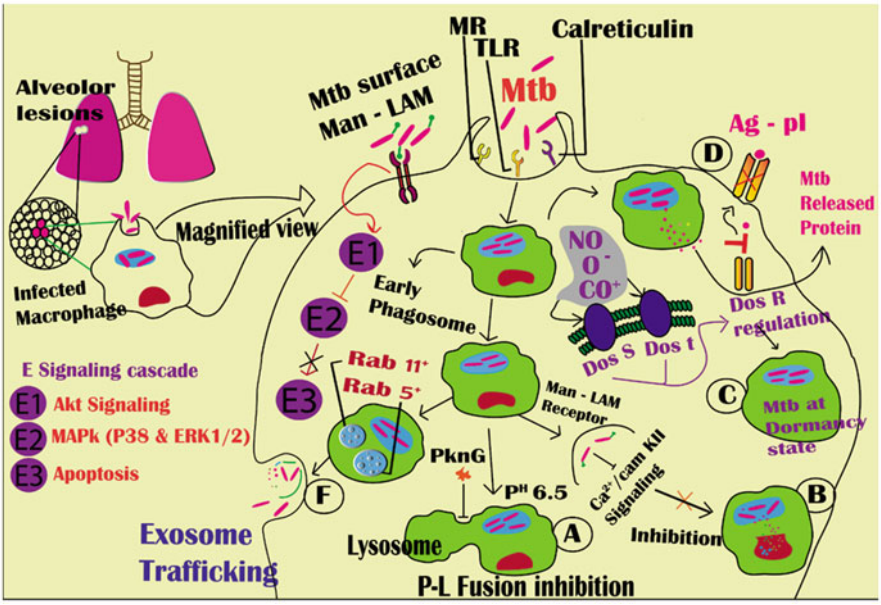

Fig. 9.1 *M-tb* adopted different strategies of evasion for its intracellular survival in infected macrophage. Within the early phagosomal development, *M-tb* remains confined to enzymatic degradation within the phagolysosomes. (**A**) *M-tb* evasion strategy blocks the phagosome-lysosome fusion by negatively regulating vesicle trafficking along with PknG autophosphorylation. (**B**) Within the nascent phagosome, *M-tb* inhibits phagosome maturation and acidification through the reduction in cytosolic Ca^{2+} levels, thereby inactivating CaMKII. (**C**) *M-tb* is capable of shifting between dormancy and active stage through the DosR regulon. The cytosolic CO activates *M-tb* DosS/DosR regulon and maintains its dormancy, whereas O_2 inhibits the DosS/DosR activation. (**D**) *M-tb* inhibits antigen presentation from the infected host by lipoprotein release. The released lipoproteins interfere in ligand-receptor interaction and so block antigen presentation. (**E**) *M-tb* inhibits intrinsic apoptotic pathway through its virulent receptor Man-LAM. Man-LAM interacts with the death receptor in the host cell and delivers the Akt signals and blocks the MAPK activation, which is responsible for cascade signaling of apoptotic pathway. (**F**) *M-tb* regulates host cell membrane trafficking and integrity by allowing $Rab5^+$ and $Rab11^+$ endosome recruitment. Also *M-tb* triggers mitochondrial membrane permeability transitions and allows ejectosome formation and exosome trafficking. Ag-pI, antigen presentation inhibition
Shows a schematic representation of the effective immunomodulation approach to revoke or sustain anti-*M-tb* immunity through HDTs. (**A**) Inhibition of PD-L1/PD-L2 immune checkpoints on *M-tb*-infected Mφ through anti-PD-L1/PD-L2 antibodies will allow T cells to involve in phagocytosis. (**B**) The use of anti-PD-1 antibodies will effectively kill the *M-tb*-infected Mφ. (**C**) Concurrent use of anti-CTLA-4 and anti-TIM-3 antibodies will prevent exhaustion and so enhance the immune responses against *M-tb*-infected macrophage. (**D**) Displays similar functions, like (II) B

is responsible for its prolonged survival inside the host for pathogenic dissemination (Bisht and Meena 2019; Meena and Rajni 2010). Similarly, *M-tb* is proficient in invading dendritic cells (DCs), through their interaction with DCs surface receptors such as TLRs and C-type lectins. Moreover, *M-tb* suppresses interleukin-12 (IL-12) production from DCs by blocking TLRs lipopolysaccharide signaling with its LAM (Aravindan 2019; Bisht and Meena 2019; Saiga et al. 2011).

9.2.3.2 Attenuation of Host Extracellular Receptors

In many cases, *M-tb* is internalized into the innate immune cells through the interactions with host surface receptor molecules such as complement (CR1 and CR3—an integrin family protein), mannose type, and Fc receptor (Bisht and Meena 2019; Meena and Rajni 2010). However, the mode of interaction varies among the strain type particularly during interactions with CR3, where the virulent strain prefers a different domain of receptor compared to avirulent strains. Similarly, the Fc receptor promotes different intracellular communication systems for the intake of virulent *M-tb*. However, there is no evidence on the role of other host cell surface receptors such as CD14, in intracellular *M-tb* survival (Meena and Rajni 2010).

9.2.3.3 *M-tb* Inhibition of Phagosome-Lysosome Fusion

Fusion of phagosome-lysosome (P-L) is the most significant step in the mononuclear phagocytosis of an intracellular pathogen (Meena and Monu 2016; Meena and Rajni 2010). Immediately after phagocytosis, many tubercle bacilli will be directed to phagolysosomes. But the evolutionary dynamics says *M-tb* bud out as vacuoles due to early endocytic pathway; thus, there is no interaction with phagolysosomes which results in the escape of *M-tb* from the phagosome digestion (Tan et al. 2017). Such mycobacterial vacuoles restrict the access of viable tubercle bacilli to lysosomal hydrolases of phagolysosomes. Vacuoles with killed mycobacteria will be rapidly transferred to lysosomal degradation (Meena and Rajni 2010). Moreover, *M-tb* can also mediate the anti-fusion effect through its constituents, sulfatides, and ammonia production to inhibit P-L fusion. LAM released by *M-tb* decreases the intracellular Ca^{2+} influx subsequently affecting the function of phosphatidylinositol 3-phosphate (PI3P) on the phagosomal membrane. Additionally, *M-tb* releasing lipid phosphatase into the cytosol of infected macrophage holds the ability to hydrolyze PI3P also responsible for delay in P-L fusion (Meena and Monu 2016).

9.2.3.4 *M-tb* Inhibition of Phagosome Acidification and Maturation

Within the phagosome, *M-tb* can produce secretory proteins that protect it from antagonistic conditions which arise in the phagosome-lysosome pathway. Many hydrolytic enzymes of phagosomes are responsible for digesting engulfed pathogens at low pH (Podinovskaia et al. 2013; Bruns et al. 2012). But *M-tb* infection fluctuates the pH above 6 and hampers the activity of lysosomal enzymes inside the vacuoles (Baker et al. 2019). This impaired phagosome acidification is associated with vacuolar ATPase exclusion and so obstructs the cathepsin D protease activation (Bruns et al. 2012). Such consequences negatively affect the antigen processing and presentation by host immune cells (Meena and Rajni 2010). On the other hand, for its extended intracellular survival, *M-tb* provokes modification of phagosomal compartments, thus influencing the maturation process. *M-tb*-infected phagosome stimulates anomalous expression of Rab5 protein and other molecular marker components, thereby affecting the maturation process at the early endosomal stage (Lala et al. 2014; Meena and Rajni 2010). Polymerization of actin filaments is key for the formation of pseudopodia and essential in phagosome maturation during infection. Conversely, *M-tb* with its abundant lipids triggers P2X7 polymorphism in

ATP/P2X7 axis (Biswas et al. 2008). Consequently, such changes destabilize the actin polymerization in the cytosol of *M-tb*-infected macrophages, thus delaying the maturation of phagosome and P-L fusions.

9.2.3.5 *M-tb* Survival Strategy Through TACO Retention over the Phagosomal Wall

Retention of TACO (tryptophan aspartate-containing coat protein) in the *M-tb*-infected phagosomal wall is another important mechanism associated with inhibition of P-L fusion and phagosome maturation (Dasgupta and Pieters 2018). Generally, the retention of TACO in the *M-tb*-infected phagosomal membrane makes it non-fusogenic with subcellular organelles such as lysosomes, thus helping *M-tb* to escape from bactericidal action of macrophage. Stimulation of TACO is mainly due to the interaction of host fibronectin with virulent factors of *M-tb* (Dasgupta and Pieters 2018; Meena and Rajni 2010). Such interactions activate a cascade of signaling pathways leading to the activation of phospholipase and recruiting TACO proteins. In many cases, TACO recruitment in a phagosomal surface is due to phagocytosis of bacterial clusters rather than a single bacterium (Carranza and Galan 2019).

9.2.4 M-tb Inhibition in Apoptosis

Apoptosis is the most preferred cleaning mechanism by host cell immunity to eliminate the infected pathogen. Neutralization of intracellular pathogens, by removing infected cells with phagocytosis, is observed in this process (Aleman 2015; Cruz et al. 2015; Kumar et al. 2011). Similarly, the induction of apoptosis for *M-tb*-infected host cells is triggered by inflammatory cytokine stimulation. Conversely, the development of *M-tb* infection is capable of suppressing apoptosis signals through the reputed *M-tb* proteins such as SecA2 and NuoG (Dasgupta and Pieters 2018). Additionally, *M-tb* promotes transcriptional repressor for avoiding host cell necrosis and phagosome induction. *M-tb* reduces the intracellular ROS generation by superoxide dismutase secretion using Sec2A-dependent pathway. Furthermore, the study on *M-tb* role in necrosis inhibition describes that type I NADH dehydrogenase subunits neutralize the ROS generation (Meena and Rajni 2010).

9.2.5 M-tb Transition from Latency to Active State: DosR Regulon

Dormancy survival regulator (DosR) is a major response regulator with 48 genes for the regulation of metabolic activity during *M-tb* dormancy state. In general, the state of *M-tb* latency is governed by a two-component regulatory system (DosS/DosR) (Dasgupta and Pieters 2018; Kumar et al. 2013). Hypoxia or other stress conditions will trigger autophosphorylation of DosS which subsequently phosphorylates DosR. Autoactivation of DosR is a result of up-regulation of the heme oxygenase enzyme responsible for the generation of carbon monoxide (CO) during *M-tb* dormancy state

(Dasgupta and Pieters 2018). Similarly, *M-tb* expresses another Dos response protein, α-crystallin, a chaperonin on activation inducing further 50 genes (Zhai et al. 2019). However, at the respiring conditions, *M-tb* maintains a basal level of DosR responsive genes and at non-respiring conditions, up-regulates DosR responsive genes (Yihao et al. 2015). Thus, *M-tb* maintains viability during the transition phase and also protects itself from oxidative stress during infection.

9.2.6 M-tb Interference in Antigen Presentation

Host immunity unveils antigen presentation as the prominent practice to eradicate intracellular *M-tb* via three routes as evident through literature support. In the first attack, the antigen-presenting cells will process the *M-tb* antigen and present it to MHC class II molecules which release inflammatory cytokine (IFN-γ, TNF-α) production from $CD4^+$ T cells for killing intracellular *M-tb* (Harriff et al. 2014; Meraviglia et al. 2011). The second attack on *M-tb* via the presentation of *M-tb* linked MHC class I antigen to $CD8^+$ T cells which release secreted granules for killing *M-tb* (Sreejit et al. 2014). On the third attack, CD1 molecules of NK cells and CD8+ T cells found the *M-tb*-associated glycolipids, thereby secreting cytotoxic granules for killing *M-tb* (Dasgupta and Pieters 2018). However, *M-tb* has evolved itself to escape the killing mechanism of cytotoxic immune cells by a transition to latency or by inhibiting antigen presentation (Yang et al. 2015; Sreejit et al. 2014). Furthermore, as a survival strategy, *M-tb* can inhibit antigen presentation on MHC class II molecules which is well documented in many kinds of literature: (i) *M-tb* could restrain IFN-γ production from antigen-presenting cells (APCs) and its MHC class II molecule expression for antigen presentation to T lymphocytes and (ii) *M-tb* ability on suppressing P-L fusion also impairs antigen presentation (Dasgupta and Pieters 2018).

9.3 Antituberculous Agents in TB Control: Benefits and Pitfalls

With the existing strategy of drug regimen, it takes several decades for sustainable treatment development to bring down the incidence of TB disease, since the intrinsic biological characteristics of the *M-tb* pathogen demand a long-term treatment with complex combinations of regimens to cure the disease (Ryndak and Laal 2019; Chai et al. 2018; Tobin 2015). Generally, the standardized regimens for TB control adhered with rifampin-based first-line drugs or fluoroquinolone-based second-line drugs (Sacchettini et al. 2008). Regrettably, based on the drug resistance profile (Table 9.1), due to lack of timely modifications, the advancement of TB disease progression and rapid emergence of drug resistance becomes a worldwide public health threat (Schwegmann and Brombacher 2008). Till now, based on WHO updated guidelines, several standardized or individualized treatments were performed under changing circumstances and evolving technologies (Shah et al. 2007).

Table 9.1 List of some of the important antituberculous drug and *M-tb*-associated resistant mechanism

Anti-TB drugs	Recommended dosage	Duration	Mechanism of actions	*M-tb*-associated resistance	References
Rifampin	35 mg/kg/day	2 months	Inhibits transcription	Mutation in rpoB	Sacchettini et al. (2008), Tiberi et al. (2018)
Isoniazid	10 mg/kg/day	2 months	Catalyzes peroxidase (inhibits mycolic acid biosynthesis)	Mutation in katC and katG	Tiberi et al. (2018)
Bedaquiline	400 mg/kg/day	2 weeks	Blocks proton pumps for ATP synthesis	Mutation in atpE	Chang et al. (2018)
Ethambutol	15 mg/kg/day	2 months	Inhibits arabinogalactan synthesis	Mutation in embB	Zumla et al. (2015)
Pyrazinamide	25 mg/kg/day	2 months	Inhibits FAS-1, thereby altering membrane energetics	Mutation in pncA	Zumla et al. (2015)
Fluoroquinolone	400 mg/day	2–6 months	Inhibits DNA gyrase	Mutation in gyrA/ gyrB	Tiberi et al. (2018)
Clofazimine	100 mg/day	2 months	Inhibits bacterial respiratory and ion transporter	Mutation in rv0678 gene	Chang et al. (2018), Zumla et al. (2015)

9.3.1 Controlling Drug-Susceptible TB

There are regularly updated guidelines for the management of tuberculosis which include antituberculosis drugs and treatment regimens. WHO has categorized the TB patients as new (no tuberculosis drug intake in <30 days) and retreatment cases (>30 days of tuberculosis treatment) (Tiberi et al. 2018; Chang et al. 2018; Zumla et al. 2015). New cases were prescribed with empirical regimens of quadruple drugs (isoniazid (H), rifampicin (R), pyrazinamide (P), and ethambutol (E)) for 6 months with intensive phase for 2 months (quadruple—HRPE) followed by continuation phase for 4 months (dual therapy—HR) (Zumla et al. 2015). Due to lack of superiority, a long treatment duration (>6 months) is not preferred. Based on the treatment choice, the antituberculosis drugs are available as fixed-dose combinations or loose formulations of a single drug. However, the available clinical trials display a lack of significant differences between the formulations. WHO strongly recommends that TB cases should undergo the universal drug susceptibility test (DST) for routine surveillance of TB drug resistance before prescribing the antituberculosis regimen (Shah et al. 2007). These data will help the physician to be aware of retreatment cases to give special attention, to avoid the risk of inducing drug resistance. Rather than conventional mode, the rapid test for drug susceptibility testing was suggested for prescribing appropriate treatment regimen which allows a low probability of rising MDR tuberculosis.

9.3.1.1 DOTS Strategy

Directly observed treatment, short-course (DOTS) strategy is one of the standardized approaches recommended by WHO for resource-limited countries with high TB burden (Morrison et al. 2008). This strategy established by the British Medical Research Council (BMRC) results in good recovery and improved health rates for infectious pulmonary TB in diverse ethnic groups (Shah et al. 2007). The treatment choice covers 6-month standard short-course regimen with the quadruple first-line (HRPE) drug in the clinical trial stage. However, this treatment explores consistent results only in the drug-susceptible TB patients (Zumla et al. 2015). Regardless of the global expansion of the DOTS strategy, only a 2% annual decline in TB incidence was reported in correspondence to the rapid emergence of resistance which necessitates the discovery of new drugs and novel treatment regimens for TB control (Shah et al. 2007).

9.3.1.1.1 Individually Tailored Regimen

The host factors, about secondary complications (diabetes mellitus, HIV), are capable of influencing the treatment outcome of drug therapy in a short-course regimen (Shah et al. 2007). Additionally, the intermittent treatment or lower dosage suggestions (WHO norms) on short-course regimens resulted in many failures at recent clinical trials (Chang et al. 2018; Shah et al. 2007). Henceforth, the treatment regimen tailored for individual needs was proposed with a possible outcome by tailoring the treatment duration and overall curing rate. Moreover, the genotypic and/or phenotypic drug susceptibility test (DST) makes the accurate choice of

tailoring made-treatment regimens for the declining emergence of MDR-TB (Zumla et al. 2015). However, the implementation of these personalized approaches with accurate detection in resource-limited settings is even challenging.

9.3.1.1.2 Alternate Regimen

Owing to inadequate clinical evidence for shortening the 6-month standard short-course regimen for drug-susceptible TB (DS-TB), searching for an alternate treatment strategy may help in improving the treatment with a higher chance of curing rate. In order to shorten the eight weeks course regimen, the first multi-arm, multi-stage (MAMS) studies were performed on DS-TB by administration of 35 mg/kg of rifampin instead of 10 or 20 mg/kg groups (Tiberi et al. 2018). Substitution of rifapentine for rifampicin (longer half-life in vivo), previously approved for continuation phase regimen once a week, explores inferior efficacy in patients at high relapse rate. However, the treatment shortening effects of rifapentine are procured only through daily dosing regimens studied in a murine model (Chang et al. 2018). Similarly, the investigation of new sparing combinations (metronidazole, and pretomanid, with moxifloxacin, and pyrazinamide) in the murine model was found to be more superior to the standard regimen (Srivastava et al. 2020). But due to the incidence of hepatotoxicity, this regimen was observed to be halted halfway through the treatment period.

9.3.2 Controlling Drug-Resistant TB

For the MDR-TB patients, the treatment options were recommended by WHO in 2011 with either monodrug or polydrug resistance (Gygli et al. 2017; Zumla et al. 2015). The management of drug-resistant tuberculosis highly relies on the combination of standardized and individualized regimens (Chang et al. 2018; Tiberi et al. 2018). In the former approach, the suspected MDR-TB cases were regularly suggested for analyzing drug susceptibility testing. However, the empirical standard treatment regimens were recommended for patients with sparsely analyzed data (one or two first-line drugs) or unavailability of DST data. In the latter approach, the design of treatment regimens consigned with the patient's past tuberculosis treatment history.

9.3.2.1 Treatment Regimen for MDR and XDR-TB

WHO categorized the recommended second-line antituberculosis drug against rifampicin and multidrug resistance tuberculosis into five groups (Zumla et al. 2015). The physician should adhere to WHO guidelines for designing the effective empirical regimen for the treatment of MDR tuberculosis, by including at least four potentially active drugs (Tiberi et al. 2018). During the intensive phase, the drug formulations comprise a first-line drug (purposely the susceptible drug—pyrazinamide), a later generation fluoroquinolone and aminoglycoside (injectable), and the addition of one drug from group 4. Moreover, if the outcome of the above

combinations shows intolerance or resistance or unavailable of potentially active drug, then the noncore drugs such as bedaquiline or delamanid might be preferred (Chang et al. 2018). The WHO recommends a total duration of at least 20 months, including 8 months (intensive phase) followed by 12–18 months of continuation phase (Zumla et al. 2015). The investigation of nonadherence factors, improper drug formulations, malabsorption, low quality of drugs, and other factors play a significant role in clinical response for *M-tb* positive patients (Zumla et al. 2015). Usually, the formulation of anti-TB drugs results in adverse effects, causes that may relevant to an increased attempt of failure, the emergence of resistance to second-line drugs leads to lower patient adherence (Tiberi et al. 2018). In such instances, recommended ancillary drugs are preferable. On the other hand, for patients suffering from XDR tuberculosis, the drug formulations are mostly associated with noncore drugs such as para-aminosalicylic acids and clavulanate with carbapenems. The XDR drug regimens depend on variable factors such as virulence, a pattern of resistance for infective *M-tb* strain, the extent of tissue damage, and status of host immunity (Tiberi et al. 2018; Chang et al. 2018; Sacchettini et al. 2008). The XDR treatment regimens are also allied with the outcome of the drug susceptibility test. The WHO recommends the programmatic management of drug-resistant tuberculosis on the evidence of a large cohort study which includes 9000 MDR and 400 XDR cases (Zumla et al. 2015). At the intensive phase, the drug combinations should include four and six drugs for MDR and XDR, while during continuation phase, three and four drugs for MDR and XDR are recommended (Zumla et al. 2015; Shah et al. 2007).

9.3.2.2 Treatment with the Repurposed Drug

During the interim period, the drugs used for other clinical aspects were repurposed for MDR-TB/XDR-TB. The most recommended repurposed drugs include fluoroquinolones, kanamycin, amikacin, clofazimine, linezolid, carbapenems, and amoxicillin/clavulanic acid (Chang et al. 2018; Zumla et al. 2015). However, over the past decades, the treatment regimen evidenced that linezolid and clofazimine were found to be frequently used repurposed drugs. Fluoroquinolones are being used in the treatment of many infectious diseases that are recurrently suggested for isoniazid-resistant TB and MDR-TB (Sacchettini et al. 2008). However, early intake of these second-line drugs leads to the development of resistance against fluoroquinolone, which can be managed with bedaquiline as a substitute drug (Zumla et al. 2015). Linezolid, evidenced with effective treatment choice for XDR-TB, demonstrated in randomized-prospective phase II clinical trial. However, a significant adverse effect was observed in a dose-dependent (600 mg/day) treatment process (Chang et al. 2018). In a randomized controlled trial, the heterogeneous administration of clofazimine for MDR-TB is an approved leprosy drug (Tiberi et al. 2018). Due to its intrinsic features such as wide tissue distribution and prolonged half-life with higher intracellular activity, it is selected as an active second-line drug. Similarly, carbapenems play a prominent role in the MDR tuberculosis regimen evidenced with phase 2b randomized control trial. The absence of active oral formulations with carbapenem class drugs (imipenem, ertapenem, and meropenem)

makes the treatment process difficult for MDR-TB and XDR-TB, as they are inefficient against *M-tb* β-lactamases enzyme. But the combination regimen along with amoxicillin and clavulanate hinders *M-tb* enzymes and promotes the action of carbapenem (Tiberi et al. 2018).

9.4 Immuno-Modulating Approach for Controlling the Propagation of *M-tb*

As an earlier view, innate immunity affords a primary line of protection against various pathogenic infections. But the existence of protection through the nonspecific response occurs only in the short term (Pahari et al. 2017). Nevertheless, over the past decades, several research findings uncover many significant features of innate immune response with prolonged immunity against pathogenic infections like *M-tb* (Pahari et al. 2017; Hoebe et al. 2004). For instance, innate immune cells such as macrophages, dendritic cells, natural killer cells, and others could secure against *M-tb* even in the absence of T-cell immunity in individuals with tuberculin negative (Verrall et al. 2014; Morrison et al. 2008). Such innate immune cells interact with *M-tb* through unique PRRs such as PAMPs (pathogen-associated molecular patterns) specific to mycobacteria. Several PRRs (i.e., TLRs, CLRs, and NLRs) directly induce *M-tb* phagocytosis through cytokine release, chemokines, and cascade activation of complement proteins for the opsonization of pathogens. Among the other organs, the lungs are being the primary site of infection for *M-tb* where it enters the nasal cavity and reaches the lungs through the respiratory tract. Inside the respiratory tract, *M-tb* were first encountered by neutrophils residing over the upper mucosa. Neutrophils trap the *M-tb* by phagocytosis and release specific chemokines, pro-inflammatory cytokines, and free radicals to trigger other immune cell (epithelial cells, macrophages, connective tissues, and DCs) activation (Pahari et al. 2017; Harriff et al. 2014; Meraviglia et al. 2011). Epithelial cells of a mucosal layer are capable of recognizing *M-tb* PAMPs via their PRRs and generate IFN-α, granzymes, and tumor necrosis factor (TNF)-α, mainly involved in *M-tb* elimination (Harriff et al. 2014).

DCs from alveolar tracts and lung parenchyma are other significant responders against *M-tb* (Pahari et al. 2017). Moreover, NK cells release cytokines such as type I IFN and IFN-α, for activating DCs, thereby killing infected macrophages. Besides, T cells function as APCs, thereby triggering CD4/CD8 T cells, and secrete IFN-γ and IL-17 for defending *M-tb* (Meraviglia et al. 2011). Generally, *M-tb* infects the lungs when it successfully evades the upper respiratory tract and nasal cavity immune responses. As a result, nasal defense works as a primary checkpoint for regulating TB infections. Alveolar macrophage plays a vital role in engulfing and killing *M-tb*. Along with it, neutrophils, DCs, and NK cells synchronize with each other in eliminating the bacteria. However, APCs, such as macrophages and DCs, create a bridge between adaptive and innate immunity (Pahari et al. 2017; Hoebe et al. 2004). Usually, DCs and macrophages exploit PRRs for detecting *M-tb* PAMPs and their agonists, such as zymosan, LAM, and other ligands. These interactions will

stimulate the expression of MHC, and release multiple soluble mediators, like chemokine, free radicals, and cytokines. Interestingly, PRRs can also stimulate the production of many reactive intermediates of oxygen and nitrogen and enhance inflammasome formation, apoptosis, and autophagy (Pahari et al. 2017; Kumar et al. 2011; Kleinnijenhuis et al. 2011). Thus, the activation of immuno-modulators creates different bactericidal mechanisms for treating TB.

9.4.1 M-tb Interactions with the Innate Immune System

Many receptor interactions with *M-tb* ligand incite the inflammatory responses, to clear the *M-tb* infection or granuloma formation. *M-tb* develops subversion strategies for survival and intracellular replication in macrophages and DCs. After engulfing, DCs allow intracellular *M-tb* replication, and explore the mechanism to deter migration (Ahmed et al. 2020). Regardless, *M-tb* interferes in DC maturation and cytokine secretion and antigen presentation over serine hydrolase Hip1 (Lala et al. 2014). Immediately after recognizing *M-tb* infection, a large influx of neutrophils get activated, and neutrophils start responding through a variety of antimicrobial polypeptides in their granules including defensins, lactoferrin, and lysozyme cathelicidin, for killing the bacteria (Ahmed et al. 2020; Martineau et al. 2007). Additionally, neutrophils can also abolish *M-tb* through NADPH oxidase and Ca^{2+} at the phagosomal membrane, by facilitating ROS generation in the phagosome (Ahmed et al. 2020).

Neutrophils are capable of releasing azurophilic granules and heat shock protein to activate macrophages through neutrophil extracellular traps (NETs). Similarly, NK cells release IFN-γ for activation of macrophages during *M-tb* infection (Ahmed et al. 2020; Braian et al. 2013). Moreover, NK cells destroy *M-tb*-infected macrophages, through an upsurge of pro-inflammatory response. Generally, the activation of NK cells and macrophage involves a wide variety of signaling events such as NKp44, NKp46, and NKp30, and inhibitory receptors' assassin cell CD94/NKG2 receptor, along with IL-12, IL-18, and IFN-α (Ahmed et al. 2020; O'Connor et al. 2007). Research findings emphasize that latent TB assists with a high frequency of NK cells, but in active TB, the population of NK cells is dramatically reduced (Chowdhury et al. 2018). However, several pieces of literature evidence suggesting the role of NK cells in *M-tb* infection are still inconclusive.

9.4.2 M-tb Interactions with the Adaptive Immune System

Mostly in adaptive immune response, $CD4^+$ T lymphocytes recognize the *M-tb* antigens from infected macrophages and dendritic cells and aggravate lymphocyte activation and proliferation. Similarly, *M-tb*-infected DCs present glycolipid and lipid antigens for triggering CD1-restricted T cells (Ahmed et al. 2020; Siddiqui et al. 2015). Also, the infected DC cells could produce cytokines such as IL-7, IL-12, IL-15, IL-23, and TNF-α for activating various leukocytes (Ahmed et al. 2020).

Usually, *M-tb* expresses multiple antigens, including antigens specific to BCG, that are recognizable by donor-unrestricted T cells, and CD1-restricted T cells. Hence, these immune cells act as potential targets for developing TB vaccine, while T cells can find *M-tb* phosphoantigen and defend against *M-tb* infection by releasing IFN-γ and TNF-α (Ahmed et al. 2020; Zhao et al. 2018).

Among the subsets of T helper cells, Th1 responds to *M-tb* through the production of pro-inflammatory cytokines like IL-18, IL-12, and IFN-γ thereby arouses NO and ROS secretion inside macrophages for *M-tb* killing (Ahmed et al. 2020; Tan et al. 2017). Alternatively, Th2 releases cytokines such as IL-5, IL-10, IL-4, and IL-13 for triggering anti-inflammatory macrophages and antibody production from B lymphocytes. However, in active TB patients, higher levels of IL-10 from regulatory T cells appraise IL-2 and TGF-β, consequently disturbing macrophages' microbicidal pathways (Kim et al. 2014; Rodrigues et al. 2006).

A study by Ahmed et al. (2020) demonstrated that $CD8^+$ cytotoxic T cells destroy *M-tb*-infected macrophages by producing perforin and granulysin. However, the preventive function of humoral immunity against tuberculosis infection remains unclear and challenging owing to its complex intracellular mechanisms. But still, there is consistent evidence on the role of humoral immunity in active and latent TB infection such as *M-tb*-based IgG Fc profiles on specific binding with glycosylation patterns (Lu et al. 2019; Lu et al. 2016). Nevertheless, antibodies of mucosal surfaces are effective in neutralizing or preventing invasion of an infectious pathogen, like *M-tb* (Ahmed et al. 2020; Reljic et al. 2006; Williams et al. 2004).

9.4.3 Regulation of Pattern-Recognizing Receptor (PRR)-Specific Molecules in M-tb Inhibition

9.4.3.1 Toll-Like Receptors (TLRs)

Toll-like receptors (TLRs) are a well-documented type of PRRs that are capable of recognizing *M-tb* directly through the extracellular and intracellular PAMPs (Akira 2006). So far, 10–12 associated functional *M-tb* were identified in both humans and mice. Remarkably, TLR2/4/8 and TLR9 were suggested to play a significant role in TB infection. Each of these TLR types can detect discrete PAMPs, derived from various classes of pathogens, i.e., bacteria, parasites, fungi, and viruses. Some of the PAMPs and its binding TLRs are as follows: CpG oligonucleotides (CpG ODNs) (TLR9), a single strand of RNA (TLR8/7), flagellin (TLR5), lipoproteins (TLR6/2/1), LPS (TLR4), and double-stranded RNA (TLR3) (Pahari et al. 2017; Faridgohar and Nikoueinejad 2017; Davila et al. 2008; Akira 2006).

9.4.3.1.1 Mechanism of TLR Interaction with *M-tb*

In *M-tb* infection, TLRs play a crucial role in stimulating both innate and adaptive immune responses (Faridgohar and Nikoueinejad 2017). Based on the research evidence, genetic polymorphism in latent TB infection promotes transition of *M-tb* from infectious to disease stage. Thus, gene polymorphisms in receptors like TLRs (TLR2, 4, 8, and 9), NOD-2, and others may induce alteration of an innate immune

response. Therefore, TLR polymorphisms are well correlated with mutated TB vulnerability among different populations (Aravindan 2019; Wu et al. 2015).

Initially, innate immune cells activate adaptive immune responses when leucine-rich repeats of TLR domains recognize *M-tb* (Faridgohar and Nikoueinejad 2017). Subsequently, its interactions trigger myeloid differentiation primary response 88 (MyD88), which is reported to play a vital role in all TLRs excluding TLR3 (Koets et al. 2010). MyD88 in turn activates the growth factors such as IRAK, TRAF-6, MAPK, and TAK1. These signaling pathways mediate nuclear translocation for initiating transcription of inflammatory mediators and adhesion molecules and apoptosis/activate polymorphic nuclear cells and dendritic cells (Faridgohar and Nikoueinejad 2017; Ahmad 2011). It reinforces that deficiency in MyD88 may increase the vulnerability to *M-tb* infections. Moreover, *M-tb* uses its cell surface protein (Rv1808, PPE family protein) to intrude such signaling by influencing the host cytokine profile through MAPK and NFκB from activated B cells (Deng et al. 2014).

At intracellular cytosolic regions, immunomodulation is mediated by the TIR domain through the downstream signaling pathways. As PAMP identifies TLRs, it recruits unique adapter molecules (i.e., MyD88 and TRIF) to adhere to TIR domains in the cytosol, and this in turn activates secondary signaling molecules such as chemokines, inflammatory cytokines, IFNs, and antimicrobial peptides (AMPs) (Faridgohar and Nikoueinejad 2017). Eventually, this process leads to the activation of macrophage, stimulation of IFN genes, and recruitment of neutrophils, which help in the killing of pathogens (Pahari et al. 2017). However, *M-tb* can intercede the cellular activation over TLR2 and TLR4 which signifies the role of TLRs in the control of *M-tb* infection.

9.4.3.1.2 TLR2

During *M-tb* infection, the role of innate immune response is mainly governed by the expression of TLR2 on macrophages. In one way, excessive expression of TLR2 on macrophages will aggravate the outcomes of *M-tb* infection through various mechanisms, such as the production of anti-inflammatory cytokines and providing signaling pathways (Wang et al. 2012; Faridgohar and Nikoueinejad 2017; Liu et al. 2016). In this regard, various *M-tb* components would elicit TLR2-dependent activation of macrophages to reduce several anti-inflammatory molecules and pathways (Rosales et al. 2011). For instance, *M-tb* lipo-glycoprotein (MPT83) operates as an agonist of TLR2 which stimulates MMP-9 from human THP-1 cells (Chambers et al. 2010). MMP-9 is an indispensable molecule involved in recruiting APCs such as macrophages and is prone to well-developed granuloma formation (Salgame 2011). Similarly, *M-tb*Ra (M. tuberculosis H37Ra) could augment TNF-α expression via stimulation of TLR2/ERK signaling and boosts MMP-9 and MMP-1 production in pleural mesothelial cells (Faridgohar and Nikoueinejad 2017). During the latent infection stage, *M-tb* secretes Rv2660c protein and activates macrophage secreting pro-inflammatory cytokines by engaging TLR2 and persisting in *M-tb* latency (Yihao et al. 2015). ESAT-6 is another *M-tb* surface molecule that affects

MHC class I presentation and promotes apoptosis mechanism in macrophages by activating TLR2/NFκB (Yang et al. 2015; Sreejit et al. 2014).

Mounting evidence emphasizing the role of TLR2 on innate immune cells are as follows: (i) interaction between dectin-1/LR2 triggers ROS production, thereby provoking neutrophil activation and apoptosis, (ii) peptidoglycan like components that can engage with TLR2, and (iii) IFN-γ production in resting NK cells (Aleman 2015; Esin et al. 2013).

9.4.3.1.3 TLR4

Next to TLR2, TLR4 is well documented for its role in controlling intracellular *M-tb*. It recognizes numerous *M-tb*-associated molecules like heat shock protein 65/60 (HSP65/60), 3/4-acylated lipomannan (LM), and 50S ribosomal protein for stimulating immune cells (Faridgohar and Nikoueinejad 2017). However, TLR4 is advantageous than TLR2 in promoting immune responses against *M-tb*. Briefly, studies on HSP60 interactions with TLR2 and TLR4 uncover many regulatory actions on immune responses. For *M-tb* control, TLR4 exhibits strong regulatory actions of the immune response through anti-inflammatory cytokine downregulation, not found with TLR2. TLR4 interaction mainly starts with phagosomal functions of macrophages and is responsible for DC maturation (Faridgohar and Nikoueinejad 2017; Podinovskaia et al. 2013).

Generally, TLR4 activation (by G1-4A polysaccharide), along with MYD88-dependent pathway, endorses multiple signaling molecules such as MHC-II, TNF-α, NO, IL-1β, IL-6, IFN-γ, CD-86, and IL-12 production in macrophages (Gupta et al. 2016). Similarly, TLR4 agonists may reduce the *M-tb* burden by upsurging the ratio of effector and memory T cells (CD8, CD4), in the lungs (Khan et al. 2016a). Surprisingly in mice studies, non-functional or deficient TLR4 shows a lack of susceptibility to *M-tb* unless it is exposed by aerosolized *M-tb* during chronic infection (Faridgohar and Nikoueinejad 2017; Heldwein et al. 2003; Abel et al. 2002). Among other factors in *M-tb*-Rpf (A-E), Rpf-E triggers TLR4-dependent pro-inflammatory cytokines (TNF-α, IL-6, IL-12p70, IL-23p19, and IL-1β) to mediate host immunity (Faridgohar and Nikoueinejad 2017).

9.4.3.1.4 TLR8 and TLR9

Unmethylated CpG motifs are pervasive in *M-tb* to provoke good immune responses in the host when interacting with TLR8 and TLR9 to activate DCs (Faridgohar and Nikoueinejad 2017). Plasmacytoid DCs, a subtype of DCs, play a prominent role in establishing inflammation and innate immune response (Guillerey et al. 2012). TLR9 can feasibly detect *M-tb*; as a result, it is selected as an effective vaccine target by researchers and is currently under randomized phase 1 b study (Bekker et al. 2020). So far, there are very limited studies performed on TLR8 and TLR9 in *M-tb* infection. Studies stated that the use of the BCG vaccine has enhanced the expression of TLR8 on macrophages, and such discovery reveals the role of TLR8 in susceptibility to *M-tb* and depends on its single nucleotide polymorphism (Faridgohar and Nikoueinejad 2017; Salie et al. 2015; Davila et al. 2008).

9.4.3.2 C-Type Lectin Receptors (CLRs)

Like TLRs, C-type lectin receptors are a broad protein superfamily encompassing one or more (C-category) lectin domains and in vertebrates that are diversified by almost 17 subclasses (Pahari et al. 2017). CLRs are expressed on numerous immune cells, i.e., macrophages, DCs, and NK cells, capable of detecting numerous pathogens like *M-tb* (Robinson et al. 2006). Many CLRs are soluble like mannose-binding lectin (MBL) and dectin-1. CLRs include single or multiple extracellular carbohydrate detection domains (CRD) of which some have Ca^{2+} binding sites. CRD helps in determining the specificity of carbohydrates on CLRs (Sancho and Reis e Sousa 2012).

9.4.3.2.1 Mannose-Binding Lectin (MBL)

MBL (present in serum and amniotic and synovial fluid) regulates the immune response through complement system by binding with a broad range of carbohydrate moieties during *M-tb* infection (Naqvi and Endsley 2020; Yamasaki et al. 2009). However, binding of ligand on MBL requires Ca^{2+} and is selective for terminal mannose, N-acetylgalactosamine, and fucose. Owing to its ubiquitous nature, MBL holds extensive ligand binding tendency and allows opsonization of *M-tb*, thus considered as a significant receptor in *M-tb* infections (Eddie Ip et al. 2009). Moreover, MBL ligation with *M-tb* is well related with *M. bovis* ligation inducing agglutination, lectin pathway activation, and augmented phagocytosis (Naqvi and Endsley 2020; Bartlomiejczyk et al. 2014). Polymorphism in the MBL2 gene impacts the expression and function of the MBL receptor in the susceptibility of TB in humans (Naqvi and Endsley 2020; da Cruz et al. 2013).

9.4.3.2.2 Dectin

Dectin is a CLR-type receptor found in immune cells such as neutrophils, Langerhans cells, macrophages, and DCs, which are efficient in recognizing wall-derived β-glucans in *M-tb* surface (Dennehy and Brown 2007; Yadav and Schorey 2006; Gross et al. 2006). Indeed, ligand binding specificity and intracellular signaling varied among different CLRs. In one way, dectin-1 recognizes *M-tb*-associated β-glucan to promote pro-inflammatory signaling such as Clec9a. Clec9a detects filamentous actin (F-actin) in the necrotized *M-tb*-infected immune cells and facilitates antigen cross-presentation in APCs (Pahari et al. 2017; Geijtenbeek 2012). Generally, the dectin-1 receptor enhances the *M-tb* uptake when interacting with alpha-glucan, and it is liable for regulating the innate immune response (Pahari et al. 2017). Moreover, dectin-1 can also trigger cytokine induction for regulating the adaptive immune response. Dectin-1 activates Th17 memory cells to secrete IL-17 (Brown 2006; Naqvi and Endsley 2020). Conversely, some of the early reports revealed the activation of dectin-1 and TLR4 promotes negative regulation of infected macrophage allowing intracellular *M-tb* growth by suppressing apoptosis (Naqvi and Endsley 2020; Cruz et al. 2015). However, a detailed study by Naqvi and Endsley (2020) clearly states that activation of dectin-1 and TLR4 does not influence *M-tb* growth. Moreover, dectin-2 receptors (observed mainly on tissue macrophages and specific DC subsets, such as peripheral blood monocytes and Langerhans cells)

are capable of recognizing several bacterial and fungal pathogens (Geijtenbeek 2012). Dectin-2 recognizes zymosan ligand such as Man-LAM of *M-tb* cell surface. Dectin-2 binding to Man-LAM activates pro-inflammatory (TNF-α, IL-6) and immune regulatory (TGF-β, IL-10) cytokines subsequently triggering T-cell response and Th17 differentiation (Naqvi and Endsley 2020; Decout et al. 2018).

9.4.3.3 NOD-Like Receptors (NLRs)

NLRs belong to the PRRs family and interact with nucleotides of *M-tb* by their oligomeric domain. So far, nearly 22 NOD molecules have been reported in humans. NLRs structure comprises a NACHT domain and a leucine-rich carboxy-terminal region (Pahari et al. 2017; Franchi et al. 2008). NLR proteins potentially recognize the peptidoglycan of *M-tb*. Except for a few NLRs (NOD-1 and NOD-2), the role of other NLRs on TB control is inconclusive (Pahari et al. 2017; Girardin et al. 2003). Both NOD-1 and NOD-2 hold amino-terminal caspase recruiting domain which helps in inducing NFκB signaling, leading to increases in the release of chemokine and pro-inflammatory cytokine followed by antimicrobial peptides (AMPs), nitrogen oxide, and co-stimulatory molecules on mononuclear cells (Uehara et al. 2007; Pahari et al. 2017). However, mutations in the gene responsible for NLRs expression will mediate several disease conditions. For instance, NOD-2^{-}/$^{-}$ *M-tb*-infected mice induce impaired cytokine production in DCs and macrophages, displayed abnormal bacterial population in the lungs, reduced survival rate, and had poor development of T cells (Saiga et al. 2011). Furthermore, NOD-2 along with TLR2/TLR4 receptors potentiates inflammatory cytokine release during *M-tb* infection (Khan et al. 2016b; Pahari et al. 2017). In particular, the combined action of NOD-2 and TLR4 on DCs inhibits the intracellular survival of *M-tb* (Khan et al. 2016a).

9.4.4 Endogenous Mechanisms Involved in M-tb Killing

Host immune response against *M-tb* infection starts when DCs and alveolar macrophage ingest the pathogenic *M-tb* by phagocytes in the lower respiratory tract. In reality, its distinct cellular process, including the development of reactive oxygen/nitrogen species (ROS/RNS), stimulation of host defense pathways, autophagy, and antimicrobial peptides (AMPs), decides the intracellular phagocytosis of *M-tb* (Ahmed et al. 2020).

9.4.4.1 Autophagy and TB

Autophagy is an innate housekeeping cellular mechanism that aims at the degradation of damaged organelles, old cells, and aggregated proteins by the double membrane vesicular autophagosome. Autophagy often plays a vital function in intracellular *M-tb* infection by managing a broad range of immune responses (Ahmed et al. 2020; Bah and Vergne 2017). The activation of the autophagy pathway is a complex process that includes three main components: autophagy-related protein complex (ATG), class III phosphoinositide 3-kinase complex 3 (PI3KC3), and Unc-51-similar kinase 1 complex (ULK1) (Singh and Subbian

2018). The autophagy mechanism starts with autophagosome formation with infected bacteria. This process is regulated by various autophagy-associated genes (ATGs), complex ULK/ATG1, and class III PI3kinase during the initiation stage of autophagosomes. Later, autophagosomes will come in contact with the lysosomes, forming autophagolysosomes, assisting intracellular degradation (Levine et al. 2011). In another way, ubiquitin, a cellular cargo protein, will recognize *M-tb* surface proteins and inhibit the intracellular growth of *M-tb* through the host xenophagy mechanism (Ahmed et al. 2020).

Mammalian target of rapamycin (mTOR) complex 1 and adenosine monophosphate-activated protein kinase (AMPK) are the two mediators of the host autophagy mechanism, which activates macrophages to kill *M-tb*. However, it is well known that virulent *M-tb* strains escape the host immune response by preventing autophagosome-lysosome fusion and subsequent acidification of autophagolysosomal part using secreted antacid and 1-tuberculosinyladenosine (Ahmed et al. 2020; Buter et al. 2019). As said earlier, *M-tb* inhibits autophagy activation in immune cells through several mediators such as ROS, ESAT6, and ESX-1 proteins (Ahmed et al. 2020; Romagnoli et al. 2012). The standard line of evidence suggests that *M-tb* DNA is known to cause direct ubiquitination of bacteria based on cyclic GMP-AMP synthase and stimulates the interferon (STING) gene-dependent pathway, thus activating the type I IFN, which leads to the development of inflammation in the infected cells. The autophagic receptors such as SQSTM1/p62 and nuclear-point protein (NDP52) are STING-dependent pathways as they play an integral role in the xenophagic elimination of *M-tb* (Watson et al. 2012). Since autophagy is a key protective mechanism to suppress the growth of *M-tb* in immune cells (Ahmed et al. 2020; Buter et al. 2019), it is most important to improve host-directed tuberculosis therapy with emphasis on autophagy activation.

9.4.4.2 Oxidative Stress and TB

Oxidative stress mainly affects nucleic acids and proteins sensitive to oxidation and also causes lipid peroxidation (Ahmed et al. 2020; Shastri et al. 2018). Generally, the *M-tb* infection induces respiratory burst and leakage of nitrogen intermediates and ROS from the infected macrophage. In humans, the first line of protection against *M-tb* is due to polymorphonuclear neutrophils which kill pathogens by producing ROS (Ahmed et al. 2020; Zhai et al. 2019). In another way, oxidative stress helps transform many first- and second-line antituberculosis medications from inactive to active state (Shastri et al. 2018).

As an example, conversion of isonicotinylhydrazide (isoniazid) into isonicotinic acyl radical by peroxidase enzyme/catalase (KatG) from *M-tb* is mediated through the oxidative process (Ahmed et al. 2020; Shastri et al. 2018). *M-tb* produces antioxidant enzymes such as catalase peroxidase (KatG) and superoxide dismutase (SOD) to maintain the unusual redox environment. Many *M-tb* strains are capable of generating increased intracellular survival proteins, namely, Eis (enhanced intracellular survival), for recognizing ROS (Ahmed et al. 2020; Shastri et al. 2018). Studies have also documented that *M-tb* has evolved various pathways for living with extreme oxidative stress of the host, including enhanced bacterial SODs,

peroxiredoxin, NADH/NAD^+ ratio, and catalases. Augmented expression of NADH/NAD^+ that encoded class II type NADH dehydrogenase (Ndh) by mutation of Ndh shows co-resistance to ethionamide and isoniazid (Vilcheze et al. 2005). Compared to wild-type strains (CB3.3 and CDC1551), isoniazid-resistant *M-tb* strains are reported to exhibit higher resistance to ROS (Ahmed et al. 2020; Idh et al. 2017). Additionally, these strains displayed improved resistance to peroxide and acidified nitrite molecules. *M-tb* modulates peroxidase mechanisms to keep its virulence by the expression of the alkyl hydroperoxide reductase subunit C (Ahmed et al. 2020; Shastri et al. 2018; Jamaati et al. 2017). Moreover, the production of NO and ROS helps humans to protect against *M-tb* infection. In healthy persons, induction of NO from AM is associated with the inhibition of intracellular *M-tb* growth (Jamaati et al. 2017). These factors suggest that host immunity plays a major role against *M-tb* infection.

9.4.5 Host-Directed Therapies (HDTs)

TB is proved to be associated with immunomodulation of the host immune system. The inappropriate or inadequate treatment regimen in the TB infections particularly with MDR and XDR *M-tb* strains aggravates TB-associated morbidity in immune-compromised patients. The goal of HDTs is to enhance or revoke the functions of host immune cells against *M-tb* with reduced inflammation and increased bacterial killing together with traditional anti-TB drugs (Tobin 2015). To do so, HDTs should work through a short treatment regimen, with minimal doses of existing drugs to strengthen its effect. The use of HDTs and their clinical outcome in TB are given in Table 9.2. The following sections discuss the management of TB and its treatment approaches through HDTs:

9.4.5.1 Drugs

9.4.5.1.1 Metformin

This drug was officially approved to treat type II diabetes, but subsequent studies confirmed that it can stimulate host autophagy for *M-tb* clearance (Bento et al. 2015). Many research reports evidenced that metformin promotes immunomodulation by increasing immune cells such as monocytes, macrophage precursor cells, as well as lymphocytes and neutrophils. Such stimulation creates oxidative stress through mitochondrial ROS generation and stimulates the population of naive T cells. In vivo studies performed on mouse models have shown that metformin is capable of improving the function of conventional anti-TB drugs in both chronic and acute stages. Overall, metformin reduces TB-associated tissue pathology, regulates inflammatory gene activity, and improves $CD8^+$ and $CD4^+$ T-cell population (Bento et al. 2015; Singhal et al. 2014).

Table 9.2 HDTs-associated molecules and their biological activities along with its clinical outcome in *M-tb* treatments

Category	Therapy/inhibitor or supplement	Biological activity	Clinical outcome or reports in TB	Reference
Repurposed drug	Metformin	Stimulates host autophagy	Increase in T cells and reduces TB pathology	Singhal et al. (2014)
	Imatinib	Develops phagosomes	Increase in monocytes and neutrophils	Napier et al. (2011)
Vitamin	Vitamin D3	Inducer of cathelicidin peptide	Improves host immunity and inhibits *M-tb* growth	Mily et al. (2015)
Immune checkpoint	PD-1 inhibitor (pembrolizumab or nivolumab and others)	Brakes immune cell functions	Improves phagocytosis and intracellular killing	Wang et al. (2018)
	PD-L1 inhibitor (atezolizumab or durvalumab)	Brakes immune cell functions	Improves phagocytosis and intracellular killing	Shen et al. (2016)
	CTLA-4 inhibitor (tremelimumab or ipilimumab)	Hinders immune cell functions	Increased in CTLA-4 and activation of Treg cells	Wang et al. (2018), Shu (2019)
Cytokine therapy	IFN-γ	Induces immune responses	Immunogenicity indicator for a new TB vaccine	Lalvani and Millington (2008)
	GMCSF	Stimulates host defense	Reduces *M-tb* burden	Robinson (2017)
Enzyme	Phosphodiesterase inhibitors (CC-11050, CC-3052, roflumilast)	Inhibits cAMP and TNF production	Shortens lung inflammation, TNF production	Subbian et al. (2016)
	Matrix metalloproteinase inhibitor	Delays granuloma formation	Abrogates granuloma formation	Volkman et al. (2010)

9.4.5.1.2 Imatinib

This drug is generally regarded as a kinase inhibitor, which inhibits the tyrosine kinase enzyme. Therapeutic administration of imatinib facilitates the acidification and development of phagosomes in *M-tb*-infected macrophages, thereby shortening *M-tb*-CFUs. Moreover, imatinib together with another anti-TB drug could restrain drug-resistant *M-tb* strains and their propagation (Kim and Yang 2017). For instance, the application of imatinib and rifampicin usage drastically reduced the granulomatous lesions. Imatinib, in sub-therapeutic doses, improves host defenses through accelerating monocytes and neutrophil cells. Imatinib was also found to be effective against both susceptible and resistant *M-tb* strains. Some adverse effects of imatinib usage are reported against T-cell response (Bruns et al. 2012; Napier et al. 2011).

9.4.5.1.3 Vitamin D3

It acts as an important dietary supplement by stimulating an innate immune response. Conversion of vitamin D3 from inactive form 5 (OH) D to its active form 1,25-(OH) 2D3 (1,25-dihydroxyvitamin D3 (VD3)) is essential for the development of antimicrobial peptide cathelicidin for indirect TB control. At the early pre-antibiotic stage, VD3 improves innate immunity and controls intracellular *M-tb* growth. Accelerating expression of cathelicidin is confirmed through the actions of vitamin D3, while 4-phenyl butyrate in macrophages also stimulates autophagy against *M-tb* infections (Pahari et al. 2017; Montoya et al. 2014). Moreover, several clinical studies suggested that VD3 can be considered as supplementary for the treatment for TB patients (Pahari et al. 2017; Mily et al. 2015).

9.4.5.1.4 Ibuprofen

Ibuprofen is an anti-inflammatory drug widely used as an antipyretic and painkiller and is also known as an indiscriminate cyclooxygenase inhibitor. It was reported that ibuprofen does not have a direct effect on *M-tb*, albeit it lowers bacterial infections by controlling tuberculous lesions, thereby rendering an increasing survival rate in a mouse model (Ahmed et al. 2020). *M-tb* infections in mice and humans reduce cytokine levels, i.e., IL-1, besides enhancing type I IFN, leading to an eicosanoid inequality, which promotes the spread of TB (Barber et al. 2014).

9.4.5.2 Immune Checkpoints

The immune checkpoints are another significant target of HDTs, which are widely approved in cancer studies. Remarkably, recent clinical studies have shown a potential role of immune checkpoints in the development of TB pathogenesis such as PD-1/PD-L1 or PD-L2 pathway (Shen et al. 2016). Briefly, programmed death ligands such as PD-L1 or PD-L2 from *M-tb*-infected macrophages were capable of interacting with PD-1 receptors, thereby modulating T-cell functions as mentioned in Fig. 9.2. Expression of PD-L1 is often seen on *M-tb*-infected macrophages. Nevertheless, blocking the PD-1/PD-L1/2 pathway allows T-cell interaction in the phagocytosis process for effective killing of *M-tb*-infected cells (Pahari et al. 2017; Shen et al. 2016). PD-1 blockades, for example, pembrolizumab and nivolumab, have often been used in the treatment of several cancers and have recently shown some hope for TB control (Wang et al. 2018; Pahari et al. 2017).

Apart from macrophages and DCs, Tregs, NKT cells, and neutrophils also express PD-L1 on their surface, which regulates the inflammatory response, preventing lung tissue damage in patients. Conversely, PD-L1 inhibitor promotes the development of IFN-γ by T cells and helps to regulate the progression of *M-tb* in patients suffering from pulmonary TB (Jurado et al. 2008). In addition to PD-1/PD-L1 expressions, other immune checkpoints capable of weakening immunity against TB are CTLA-4, LAG-3, and TIM-3. Therefore, inhibition of lymphocyte activation gene 3 (LAG-3), T-cell immunoglobulin, and mucin domain 3 (TIM-3) prevents exhaustion of T cells and modulates their immune response against *M-tb* infection (Phillips et al. 2015; Ngiow et al. 2011). A comprehensive study of TB uncovers higher CTLA-4 expression in active TB cases, whereas PD-1 expressed peaks at

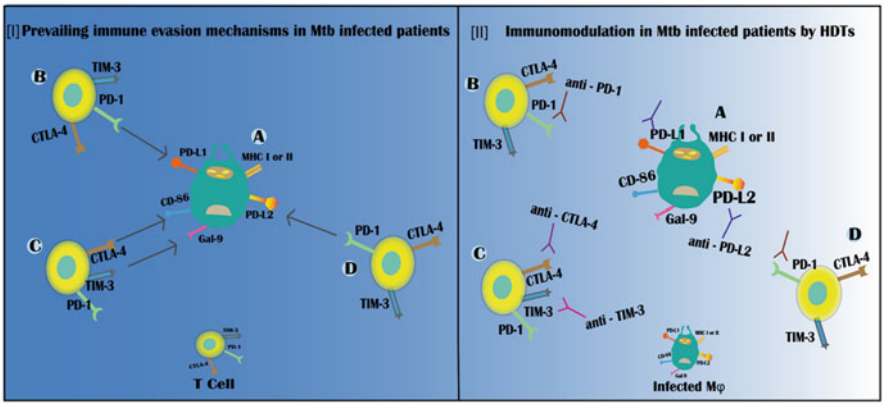

Fig. 9.2 Immune checkpoint inhibitors as effective host-directed therapies (HDTs) against *M-tb* [I], [II]. (**A**) *M-tb*-infected macrophage (Mφ) displayed multiple immune checkpoints on its surface such as PD-L1/2, CD-86, and Gal-9, which impedes T-cell functions. (**B**) T-cell PD-1 receptor interaction with *M-tb*-infected Mφ will block their immune response, and lead to apoptosis. (**C**) T-cell response shows the concomitant interaction of CTLA-4 and TIM-3 receptors toward CD-86 and Gal-9 ligands of *M-tb*-infected Mφ affecting the T-cell functions, thereby facilitating loss of functions leading to apoptosis or autophagy. (**D**) Similarly, PD-1 receptor of T cell interacts with PD-L2 ligand of *M-tb*-infected Mφ affecting the function of T cells

latent TB infection (Shu 2019). This study demonstrates that blocking the immune checkpoint molecules will greatly enhance host immunity and might be a potential target.

9.4.5.3 Cytokine Therapy

Cytokine therapy is an alternative method for treating TB by using immunomodulators to improve the immune system, such as interferon-γ (IFN-γ), granulocyte-macrophage colony-stimulating factor (GM-CSF), and IL-2. Cytokines trigger a variety of cellular responses for control of TB, like antimicrobial activity and pro-inflammatory immune response. During *M-tb* infection, the production of IFN-γ acts as infection markers for the development of a new TB vaccine (Lalvani and Millington 2008; Millington et al. 2007). IFN-γ is a key molecule responsible for the induction of cytokine IL-12 level, which is essential for Th1 differentiation, subsequently upregulates cell surface MHC class II and I on APCs, and develops antimicrobial activity. Literature survey reveals that IFN-γ produced by immune cells as well as through recombinant cells enhances the role of macrophages against MDR-TB (Khan et al. 2016c). However, mutations in the IFN-γ gene or its receptor may restore susceptibility of *M-tb* infections. GM-CSF expressed in lung epithelial cells triggers several macrophages and T cells in human TB granulomas. The mice model studies clearly explained the significance of GM-CSF in reducing the burden of *M-tb* in the infected macrophage. Besides, co-administration of IFN-γ and GM-CSF creates a robust impact on *M-tb* reduction (Mvubu et al. 2018; Robinson 2017).

Excessive GM-CSF existence enhanced inflammatory response and manifested antimycobacterial activity. However, balance in both the cytokines will affect the outcome of *M-tb* disease (Kim and Yang 2017). IL-2 another potential biomarker can restore normal T-cell function in active TB in higher concentrations. Emerging drug resistance in TB due to long treatment regimens leads to impaired expression of IL-2, which results in poor development of T-cell differentiation, owing to treatment failure. In such instances, exploiting IL-2 may restore the T cell's activation and maturation. On the contrary, rather than T-cell activation, an increase in IL-2 level promotes negative consequences in the expansion of $CD4^+$ and $CD25^+$ regulatory T cells, thereby exhausting T-cell response during TB treatment (Liu et al. 2019). Thus, the present knowledge about cytokine therapy uncovers the precise mechanism of IFN-γ, GM-CSF, and IL-2 for designing sustainable development in HDTs against *M-tb* infections.

9.4.5.4 PDEs and TNF Inhibitors

Cyclic AMP (cAMP) is the principal component involved in the development of inflammation, and modulation of TNF, which is regulated by phosphodiesterases (PDEs) (Tobin 2015). Concerning TB control, TNF plays a vital role in the activation of macrophage and granuloma formation (Lin et al. 2007). Adenylyl cyclase from *M-tb* stimulates the production of cAMP in infected macrophages, which is released into the intracellular phagosome complex of macrophages, thereby interfering with host cell signaling and cytokine responses. Henceforth, *M-tb* subverts its infected macrophage environment as an important residence for its survival and growth (Maiga et al. 2015). Many research findings reveal that a rise in cAMP at the onset of *M-tb* infection consequently elevates TNF-α level and facilitates granuloma formation. Concurrently, cAMP induces PKA-CREB, hence upregulating the transcription of nuclear factor-kappa B (NFκB) (Tobin 2015).

However, NFκB activation intercedes many pro-inflammatory responses of macrophages indispensable for intracellular *M-tb* growth. Thus, inhibition of NFκB subsequently decreases the viability of intracellular *M-tb* in human macrophages by stimulating autophagy and apoptosis (Bai et al. 2013). Another recent study performed on the effect of V-58, evidenced with inverse regulation of *M-tb*, interrupts TNF secretion in macrophage, but in turn, promotes cAMP overexpression in TB (Johnson et al. 2017). In such an instance, a multinucleated giant cell (MGC) secretes TNF-α, which helps in granuloma formation. Subsequent research identified TNF-α inhibitor (etanercept) effectively blocked macrophage population within the granuloma and also repressed the MGCs development (Mezouar et al. 2019).

In TB, PDE-4 inhibitors (i.e., CC-3052, CC-11050, and roflumilast) have reduced lung inflammation as well as TNF production and macrophage activation. It also improved antibiotic responses, while etanercept usage delayed granuloma formation. Hence, these checkpoints and inhibitors might be considered as potential targets in HDTs (Li et al. 2018; Subbian et al. 2016; Maiga et al. 2015).

9.4.5.5 mTOR Inhibition

mTOR signaling cascade is another important target that alleviates host immunity for enhanced pathogen protection. Thus, inhibition of mTOR signaling facilitates improved host immune response against *M-tb* pathogens (Singh and Subbian 2018). The FDA-approved mTOR inhibitors are rapamycin and everolimus. Rapamycin can establish enhanced host immune responses against *M-tb* infection by upsurging antigen presentation on DCs and inducing autophagy as evident from the BCG-vaccinated mice model (Ahmed et al. 2020; Jagannath et al. 2009). Similarly, everolimus evokes autophagy by hindering the mTORC1 complex and thus enhancing the cellular immune response, which is evident through clinical studies (Saran et al. 2015).

9.4.5.6 Statins

Statin drugs especially maintain the lipid and cholesterol levels in human macrophages. *M-tb* expresses cholesterol-binding protein on its surface, which helps in binding to cholesterol-enriched domains on macrophage surface allowing pathogen invasion (Tahir et al. 2020). Studies have shown that depletion of membrane cholesterol prevents the entry of TB pathogen in host macrophages as mentioned in Fig. 9.3. Thus, statin usage in a chronic diabetic condition abridged the incidence of *M-tb* infection. Subsequently, the entry of TB pathogen into the

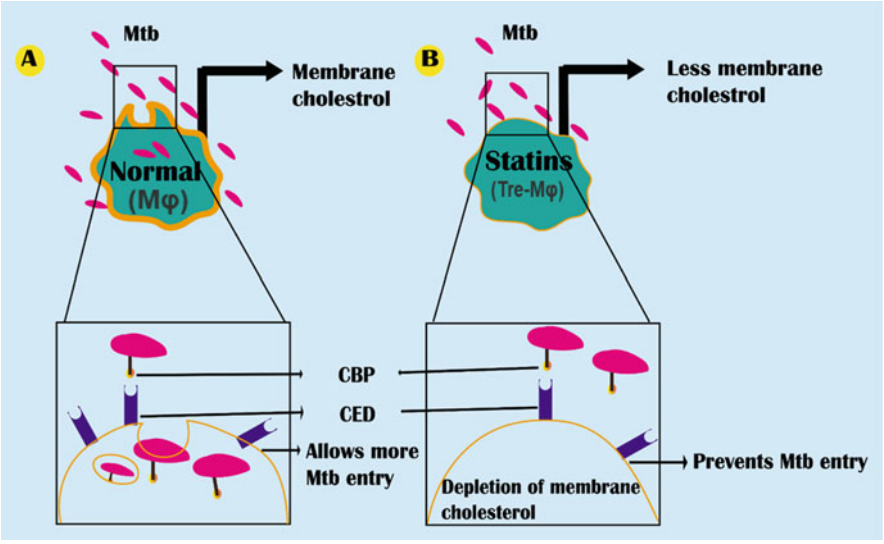

Fig. 9.3 Schematic representation of *M-tb* entry into normal macrophage (Mφ) and statin-treated macrophage (Tre-Mφ). (**a**) Displays the mode of *M-tb* entry into normal Mφ. The enlarged view of *M-tb*-macrophage surface clearly states that a ligand of *M-tb* cholesterol-binding protein (CBP) interacts on cholesterol-enriched domain (CED) of normal Mφ with its surface cholesterol, thereby allowing *M-tb* entry. (**b**) However, in HDTs approach, there is a prevention mechanism of *M-tb* entry into (normal) Mφ. It is evident through the statin-treated (Tre-Mφ) regimen, which largely decreases the membrane cholesterol of macrophage, and thus blocks *M-tb* entry into the Mφ

macrophage is decreased due to a lack of membrane cholesterol (Tahir et al. 2020; Gries et al. 2020).

9.4.5.7 Matrix Metalloproteinases

Matrix metalloproteinases (MMPs) are enzymes involved in human lung remodeling and encompass a significant role in TB. Hitherto, 24 MMPs have been identified from mammals, and they can break down all the extracellular matrix components, including vitronectin, laminin, fibronectin, proteoglycans, and collagens (Tsenova and Singhal 2020; Salgame 2011; Elkington et al. 2011). Mounting evidence affirmed that *M-tb* implicitly induces the production of MMP-9 by its ESAT-6 in epithelial cells and incites MMP-9 from lung tissue and macrophage. Besides, MMP-9 upregulation also recruits newer macrophages and monocytes, for granuloma maturation (Volkman et al. 2010). But the use of batimastat (BB-94 is an inhibitor for MMP-9, MMP-1, and others) postponed the induction of granulomas, which inferred the granuloma formation onset or after *M-tb* infection (Pando et al. 2000). For instance, MMP-9 deficient mice had shown diminished recruitment of macrophages in the lungs and developed smaller granulomas (Salgame 2011). Conversely, MMP-9 knockdown with altered antisense oligonucleotide abrogates granuloma formation and restricts bacterial growth, thus demonstrating the key role of MMPs in *M-tb* infection (Volkman et al. 2010).

9.4.5.8 Benefits of Host-Directed Approaches

Antibiotic resistance is a significant public health issue for TB. MDR-TB and XDR-TB are severe global problems occurring due to prolonged drug therapies. In reality, accretion of alleles was reported to impart resistance to multiple drugs such as rifampicin, amikacin, and isoniazid during infection (Gygli et al. 2017; Sacchettini et al. 2008; Shah et al. 2007). Mutation rates were found to be higher in active TB as compared to latent TB patients. Hence, HDTs warranted avoiding pathogen-directed therapies as HDTs target only the host proteins not *M-tb* protein (Tobin 2015; Schwegmann and Brombacher 2008). For that reason, HDTs are regarded as better and more efficient therapy in restoring or evoking host immunity.

9.5 Conclusion

Mycobacterium tuberculosis is an intracellular pathogen evolved with a diverse mechanism to escape host immunity. With prolonged treatment duration, the emergence of drug resistance and adverse effects due to drug toxicity are some of the limitations associated with the existing antituberculosis drugs. These limitations necessitate the urgent need for an alternative regimen for the treatment of tuberculosis. Furthermore, treatment management in randomized clinical trials reveals the strengthening of research capabilities in low-resource countries with high TB burden. Several clinical trials with immunomodulation therapy like HDT enhance the immune response with reduced hyperinflammation and protect from the host tissue damage. HDT associated with existing anti-TB drugs may potentiate TB control by a

short treatment regimen. However, it is to be stated that much clinical practice is required to evaluate the efficacy of immunomodulation therapy against MDR-TB and XDR-TB management.

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Conflict of Interest We hereby declare that we do not have any conflict of interest with anyone.

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Immunomodulation in Autoimmune Disorders

10

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Abstract

The immune system comprising an intricate network of various specialised cells and associated molecules is crucial to extermination of pathogens from the host's body, thus vital to human survival. Along with the generation of immune response, the immune system is also responsible for maintenance of tissue homeostasis in a continuously fluctuating environment. Although maintenance of tissue homeostasis is a tightly regulated process, multiple factors including pathogen prevalence, environs, diet, immune health, genetic defects, etc. and predisposition to other factors occasionally cause an imbalance, leading to a state of either hyperactivity or hypoactivity. Autoimmunity is a consequence of one such imbalance where the immune cells lose 'self-tolerance' and is hyper-reactive against self-antigen causing damage to one's own organs. Autoimmune disorders have many manifestations and can either be localised such as rheumatoid arthritis or be systemic such as systemic lupus erythematosus to name a few. Current therapies for most of the autoimmune disorders include immunosuppression in general, aiding to the reduction of exaggerated immune response and inflammation. However, suppression of the overall immune response has chances of increasing susceptibility to other infections and toxic effects. Since therapies targeting specific autoimmune disorders are limited, immunomodulation approaches are being considered in the present era. Some of the strategies involving immunomodulatory agents include targeting specific antigens, modulating signalling pathways, inhibiting antigen presentation, activating regulatory T cells, etc. Although some of them have shown tremendous potential,

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adverse effects have been seen in others. Despite their side effects, immunomodulation strategies seem to be a better approach for targeting autoimmune disorders. Here, we discuss the current strategies of immunomodulation along with their advantages and disadvantages.

Keywords

Immunomodulators · Immune response · Autoimmunity · Anti-inflammation

10.1 Overview of the Immune System

The current understanding of immunity is derived from a long history of disease and pestilence, affecting various civilisations across the globe. Although the earliest documentation of disease can be traced as early as 2000 B.C., the concept of *immunity* emerged only around 430 B.C. with the observation that a person once infected with the disease does not succumb to any future episodes of it (Doherty and Robertson 2004). In its most fundamental form, immunity refers to the protection from an infectious disease. And the complex organisation of specialised cells and molecules responsible for protection against infection is known as the *immune system* (Delves and Roitt 2000).

Every day, we come in contact with millions of invisible pathogens which can either be inhaled, swallowed or populate our skin and mucous membranes. However, their exposure and persistence does not necessarily lead to an infection or disease. Whether a person suffers from an infection or not is determined by both, the pathogenicity of the organism and the integrity of the host immune system (Parkin and Cohen 2001).

10.1.1 Classification of the Immune System

The host defence mechanism is primarily organised into three stages for the effective clearance of pathogen. The first line of defence comprises the skin and mucous lined surfaces which do not serve as an ideal habitat for the pathogens. Only when there is a breach of this barrier, the pathogen encounters the innate and adaptive immune systems. The innate immune system encompasses various cells and other chemical and microbiological elements capable of providing the immediate response to pathogen. However, these cells are devoid of a memory phenotype or recall response, though current studies indicate the presence of 'trained immunity', a phenotype akin to the memory cells of adaptive immunity (Netea et al. 2011, 2020; Riera Romo et al. 2016). The adaptive immune system, on the other hand, is a complex arrangement of cells and associated soluble factors responsible for the generation of an antigen-specific response and is characterised by the presence of a distinct memory of host-pathogen interactions (Kellie and Al-Mansour 2017; Medzhitov and Janeway Jr. 1997). Amongst each of these innate and adaptive

Table 10.1 Cells of the immune system (Parkin and Cohen 2001)

Cells	Key function
Innate	
Mast cells	Homeostasis of tissues and organs, wound healing and tissue repair, functions as effectors of chronic allergic inflammation, fights helminths and microbial infection
Neutrophils	Phagocytosis of microorganism and other foreign particles, mediated the early phases of inflammatory response
Basophils	Regulation of Th2 immune response by releasing Th2 cytokines, induces B cells to synthesise IgE and initiates allergic inflammation through granzyme B
Eosinophil	Degranulation in an inflammatory response, aids in negative selection, type 1 and type 2 immune response and helminth clearance and prevents from reinfection
Macrophage	Recognises a wide range of pathogens through TLRs and initiates inflammatory response, serves as APC to activate both B- and T-lymphocytes and clearance of debris and dead cells and helps to synthesise different varieties of proteins
NK cells	Recognises and kills damaged cells and those missing or having very low levels of self-antigens
γδT cells	Interacts and kills tumour cells by releasing inflammatory cytokines or by antibody-dependent cellular cytotoxicity, helps in antigen presentation and Th1 response
Adaptive	
NKT cells	Activated NKT cells produce huge quantities of IFN-γ, IL-4, GM-CSF and other cytokines to kill foreign particles. Amplifies humoral immunity and supports B cells to fight infections
$CD4^+$T cells	Recognises peptides presented on class II MHC molecules by APCs, polarisation of immune response depending on the immunological niche, influences effector T-cell response, role in autoimmunity
$CD8^+$T cells	Recognises peptides presented on class I MHC molecules, cytotoxicity towards cancerous and infected cells through cytotoxins that leads to apoptosis
B cells	Produces antigen-specific immunoglobulins against harmful pathogens, helps in antigen presentation and T-cell response

immune systems, there are multiple types of cells, with specific phenotype and function (Table 10.1).

10.1.2 Cardinal Features of an Adaptive Immune Response

In spite of the innate immune response having a significant role in activating the T cells of adaptive immune system, the cells of the adaptive immunity are the ones primarily implicated in autoimmune disorders. Therefore, here we are focussing on the general characteristics associated with an adaptive immune response.

The adaptive immune response is characterised by at least three distinct characteristics: *selectivity*, *specificity* and *memory*. As the cells of the adaptive immune system can distinguish between the types of antigen and generate a response accordingly, it confers these cells with the property of selectivity. Specificity is defined by the presence of a pre-existing clone of antibody which specifically binds

to its cognate antigen and leads to the expansion of that particular clone of antibody. This process corresponds to the primary immune response associated with the first interaction between antigen and antibody. Upon subsequent exposure to the same antigen, a more robust immune response of a higher magnitude is observed, known as the secondary immune response. The significantly higher immune response is a consequence of immunological memory, generated by the memory cells after their primary encounter.

10.1.3 Hyper- and Hypo- immune Response and Their Consequences

During an early infection, a primary immune response is activated which is responsible for clearance of the pathogen in a certain period (depending on the type of infection). During this period, there is a state of hyper-inflammation due to the activation of neutrophils, associated cytokines and chemokines. This hyper-inflammatory state facilitates the effective elimination of pathogens. However, once the infection is subdued, it is crucial that the immune homeostasis be restored to normal. And the regulatory T cells play a crucial role in downgrading the inflammation. Maintenance of balance between the hyper- and hypo-immune response is of critical importance as dysregulated hyper-response may lead to tissue injury, whereas hypo-immune response may not be able to clear an infection due to immunosuppression (Tsirigotis et al. 2016).

10.1.4 Immunological Tolerance

For proper maintenance of the immune homeostasis, it is important that only those immune cells are selected, which either are incapable of generating an immune response or mount a very mild immune response to self-antigens. This is maintained by a series of complex mechanisms, known as the immunological tolerance. When immature lymphocytes encounter self-antigen(s) in the primary lymphoid organs, only those are selected which have an optimum affinity for self-antigens. Most of the lymphocytes having higher binding affinities to self-antigens die (some develop into Tregs), the process being known as *central tolerance*. However, some mature lymphocytes escape from the negative selection of central tolerance and encounter self-antigens in the secondary lymphoid organs. These cells eventually undergo anergy, suppression or deletion and this process is known as *peripheral tolerance*. Additionally, a heterogeneous population of regulatory T (Treg) cells have been accredited towards the maintenance of immunological tolerance in normal physiology. However, in case there is a breakdown of either central or peripheral tolerance, it leads to a severe immune response, known as autoimmunity. The precise cause of such an event is unknown and can be a consequence of various genetic or environmental factors. However, the common factor governing almost all autoimmune disorders is a dysregulated immune homeostasis due to a weakened Treg response (Romagnani 2006).

10.2 Immunomodulation

Immunomodulation is a wide-ranging field, inclusive of all the therapeutic interventions by which immune system can be modulated to ameliorate specific diseases. In immunodeficiencies and infections such as AIDS, a certain order of enhancement is required in the dynamics of the immune system. Apart from that, treatment of genetic defects of the immune system has been challenging; therefore, a shift in the present approach of therapeutics was crucial which led to the consideration of immunomodulation by gene therapy or transplantation (Gea-Banacloche 2006).

In the case of infectious diseases, specifically bacterial diseases, a shift towards Th1 response is desirable, while for virus, fungi and worms, it is Tc1, Th17 and Th2, respectively (Anthony et al. 2007; Li et al. 2018; Oran and Robinson 2004; Spellberg and Edwards Jr. 2001). So, use of vaccinations is the primary approach of immunomodulation, which is given to elicit a specific pathogen-type response so that the infection can be controlled.

In case of cancer, the immune cells are mostly exhausted, particularly in the CD8 T-cell compartment, so antibodies directed against co-inhibition markers such as PD-1/PD-L1 have efficiently modulated the immune response in specific cancers such as melanoma (Sahni et al. 2018). Other approaches that have been used to modulate cells in cancer are cytokine therapy, anti-CTLA4 and other tumour-specific antibodies (Feldmann 2008; Zhao et al. 2018).

In allergy and autoimmunity, the major goal of immunomodulation is to downregulate the hyper-immune reaction. With respect to allergy, it is mainly done by desensitising the receptors for histamines and leukotrienes (Cobanoglu et al. 2013), whereas in case of autoimmunity, various approaches such as IL-2 therapy, agents that interfere with antigen presentation (anti-CTLA-4), T-cell activation (tacrolimus), T-cell proliferation, or B-cell depletion (mycophenolate mofetil, rituximab) have been approved. In conjunction with these therapies, antigen-specific immunotherapy (antigen-SIT) is being examined in certain autoimmune diseases such as type I diabetes (Duddridge and Powell 1997; Karim et al. 2002; Musette and Bouaziz 2018; Rosenzwajg et al. 2019; Tan et al. 1993).

10.2.1 Goals of Immunomodulation

The need for immunomodulation arises from the fact that the most effective strategy to combat diseases is to stimulate the host's own adaptive and innate immune responses. With this approach as the basis, immunomodulation can essentially involve passive and active approaches. In the passive approach, ex vivo generated antibodies and immune cells can be administered to patients, while in the active approach, the patient's own immune system is induced to produce immune effector cells such as antibodies, antigen-specific T cells, etc. The passive approach does not induce a long-lasting memory phenotype in direct contrast to the active approach. As of now, various therapies capable of immunomodulating the host's response have

been approved in cancer, infectious diseases, autoimmunity, allergy and graft-versus-host disease.

10.2.1.1 Stimulation of Immune Response

In infections or immunodeficiencies, the immune system has to be stimulated to mount a robust immune response. In case of immunodeficiencies which are genetic, the road to enhance the activity of immune cells is very limited. In certain diseases such as X-linked agammaglobulinemia (XLA) and common variable immune deficiencies (CVID), there are genetic defects in the B-cell compartment that lead to a deficiency of antibodies in the patients. This in turn results in the high rate of bacterial infections. The only viable treatment till now is immunoglobulin replacement therapy, where intravenous immunoglobulins (IVIg) from healthy donors are administered to the patients (Wood 2012). The scenario becomes even more challenging if genetic defects are linked to T cells or B cells, as effective therapies are not viable till date and gene therapy and allogeneic stem cell transplantation is under consideration since long (Cole and Cant 2010).

Additionally, there are certain autoimmune disorders where genetic defects hinder the function of phagocytes. A classic example is the case of chronic granulomatous disease, which is an X-linked recessive genetic disorder. Here, the patients are unable to generate a respiratory burst during phagocytosis due to a mutation in the gene gp91phox. Administration of interferon-γ (IFN- γ) has been shown to ameliorate the symptoms to a large extent. However, immunomodulation in genetic immunodeficiencies has been unsatisfactory till date, and ongoing clinical trials with gene therapy and stem cell translation may provide better resolution of the diseases.

In infections, the major pathway of immunomodulation is through administration of vaccines. These vaccines usually contain attenuated (measles, mumps, rubella), inactivated (polio) or certain products of the pathogen such as toxoid (tetanus) to activate the T-lymphocytes and antibodies without inducing serious diseases. Essentially, there are five major strategies for vaccination: live attenuated, inactive, toxoid, subunit and conjugate vaccines. In attenuated vaccines, a weakened pathogen is introduced to the body which stimulates CD4, CD8 and B-cell responses. Since these pathogens are live, APCs can directly phagocytose them and present their antigenic peptides to CD8 T cells via MHC I pathway, which is not possible to achieve through inactive vaccines. The CD8 cytotoxic response is essential for eliminating intracellular viral and bacterial infections (World Health Organization 2019). At times, the attenuated version cannot elicit a proper immune response against the pathogen such as in the case of *Haemophilus influenzae*. Their polysaccharide capsule is the major antigen but the capsule alone is insufficient for provoking a strong immune response. So, a conjugate vaccine has been designed containing the polysaccharide capsule along with a carrier protein which is capable of inducing both T-cell and antibody responses (Wasserman et al. 2018).

In toxoid vaccines, toxins from the pathogens are processed and weakened such that they are rendered incapable of causing the disease. This weakened toxin is known as toxoid and tetanus toxoid vaccine against *Clostridium tetani* is one of the widely used toxoid vaccine (World Health Organization 2018).

In subunit vaccines, only a part of the pathogen is used to create the vaccine. Since the vaccine contains only the essential antigens and not the whole pathogen, there are significantly lesser side effects. The pertussis component of the DTap (diphtheria, tetanus, pertussis) is an example of subunit vaccine (World Health Organization 2018). These vaccines are given to prevent infection, but in case where the infection has already established, there is a shift in the goal, i.e. from inducing a Th1-Tc1 response as opposed to Th2-Treg response. In infections such as *Mycobacterium*, administration of IFN-ϒ has better outcomes in animal models. Similarly, in animal models of *Leishmania*, prevention of IL-4 expression and induction of Th1 cytokines such as IL-12, IFN-γ and IL-18 has ameliorated the disease (Kim et al. 2016; Palic et al. 2019). In hepatitis B and hepatitis C, PEGylated forms of interferonα is used, which modulates the expression of cytokines and MHC I molecules, preventing viral replication and inducing Th1-Tc1 pathways. So the major goal of vaccines and cytokine therapy is to stimulate the immune response towards a Th1-Tc1 effector and long-lasting memory phenotype for life-long immunity (Palumbo 2011; Tamai et al. 2017).

The immunomodulation of cancer is much more complex and depends on the type of cancer. It primarily occurs as a collective result of uncontrolled proliferation of malignant cells. The immune cells detect these transformed cells in most cases. However, sometimes they bypass the immune surveillance based on their antigen expression, location and associated processes of necrosis or inflammation. The recent developments in cancer immunomodulation are chimeric antigen receptor (CAR) T-cell therapy. Here, altered T cells are associated with a recombinant receptor, comprising a scFv (a fusion protein containing variable regions of the heavy and light chains of immunoglobulin connected with a short linker peptide of 10–25 amino acids) that redirects the specificity of effector T-lymphocytes, fused to a transmembrane and signalling domain that mediates T-cell activation without MHC engagement. The therapy involves ex vivo proliferation of the patient's peripheral T cells only to genetically modify and express CAR in them. Subsequently, these cells are infused back into the patient. As of now, two CD19-targeted CAR T-cell therapies, tisagenlecleucel (second generation) and axicabtagene ciloleucel (third generation), have been approved recently for treating B-cell lymphomas (Naran et al. 2018; Neelapu et al. 2017; Schuster et al. 2017).

CAR T-cell therapy has been impressive in treating haematological malignancies but in solid tumours, it has shown lesser efficacy. This may be due to the tumour microenvironment being immunosuppressive in nature, presence of extracellular matrix, lack of tumour antigens and most importantly, the incapability of CAR T cells to infiltrate the tumours. Targeting immune checkpoint inhibitors has been one of the most noteworthy immunomodulatory therapies for solid tumours in the recent times. The interaction between receptor and ligands can be easily blocked by monoclonal antibodies (mAb) or by using a recombinant form of the ligand and receptor. Ipilimumab, an anti-CTLA-4 mAb, which acts by blocking the binding of CTLA-4 expressed on T cells to CD80/86 expressed in tumour cells, has successfully treated advanced melanoma and has received FDA approval. Other mAbs such as pembrolizumab and nivolumab target PD-1 and block its interaction with its

ligand, PD-L1 and PD-L2, thus restoring the cytotoxic potential of exhausted CD8 T cells completely or partially, and have been used with reasonable success in melanoma, renal cell carcinoma and non-small lung cancer. mAbs such as atezolizumab, durvalumab and avelumab, targeting PD-L1, have also shown efficacy in lung, bladder and urothelial cancers(Balar et al. 2017; Hsu et al. 2017; Kyi and Postow 2014; Massard et al. 2016; Rittmeyer et al. 2017; Robert et al. 2015).

In addition, cytokine therapy has also been used to treat cancers. Cytokines provide essential signals in normal physiological processes, and its dysregulation is implicated in a number of cancers and infectious diseases (Richter et al. 2013; Said et al. 2010). TGF-β and IL-10 are the most extensively studied immunosuppressive cytokines involved in cancer, and it has been shown that their neutralisation has improved the outcome of multiple cancers and other chronic infectious diseases. TGF-β is overexpressed in multiple malignancies and is known to promote their progression, while their targeted inhibition by small molecules, antibodies and synthetic peptides has shown restoration of the immune function and improved outcome in combinational therapies in cancer (Drabsch and ten Dijke 2012; Tian et al. 2011).

10.2.1.2 Suppression of Immune Response

Unlike conditions of immunodeficiencies, cancer and infection, cases of hypersensitivity and autoimmunity essentially require the suppression of hyper-immune response that occurs in these diseases. Allergy (hypersensitivity) is mostly caused by an external agent known as allergen, to which the body mounts a disproportionately high immune response. In certain extreme cases, it could also lead to an anaphylactic shock, eventually leading to death. On the other hand, in cases of autoimmunity, there is a disruption in the central and/or peripheral tolerance, and the immune system acts against self-antigens such as one's own DNA. However, in certain cases, it is reported that external agents including pathogen(s) can trigger autoimmunity even after the pathogen is eliminated. This may be due to the molecular mimicry of pathogen-associated molecular patterns (PAMPS) with certain self-antigens (DNA along with cationic peptides in neutrophil traps knows as NETs) (Granger et al. 2019; Saeki and Ishihara 2014).

Allergy or immediate hypersensitivity is one the most common immunological disorders found in developed countries. Allergic rhinitis, asthma, eczema and food allergy are clinical manifestations of certain allergen in genetically predisposed individuals. In atopic allergy, there is an increase of IgE on exposure to the allergen. These IgE molecules bind to the Fc receptors on mast cells. Subsequent exposure to the allergen causes crosslinking of IgE on the surface of the mast cells, causing degranulation and release of various mediators of allergies such as 11B-prostaglandin F2α (11B-PGF2α) and/or leukotriene E4 (LTE4). These mediators may cause vasodilation, increased vascular permeability, smooth muscle contraction, mucus production and inflammation. The most severe atopic reactions may lead to anaphylactic shock where there is a release of histamines, bradykinins and other allergic mediators causing a sudden cardiovascular collapse and

bronchospasm. If left untreated for sometime, anaphylactic shock may lead to fatal risk (Galli and Tsai 2012; White and Kaliner 1992).

The main cause of allergy is a biased Th2 response in certain individuals. Th2 cytokines such as IL-4, IL-5 and IL-13 cause an isotype switch from IgM or IgG to IgE. The first signal is received from cytokines such as IL-4, the subsequent signal being the interaction of CD40L on T cells and CD40 on B cells for the generation of IgE. Other Th2 cytokines such as IL-5 and IL-13 promote eosinophilia and mucus production, respectively (Akdis et al. 2011). The immunomodulatory therapy in allergy is mostly inclined towards desensitising the individuals by administering low doses of antigen to shift the immune paradigm towards a Th1 response (Rosenblum et al. 2012). Other agents such as nasal and oral corticosteroids are used to reduce swelling, itching and redness. Antihistamines and mast cell stabilisers prevent the mediators such as histamines and granules released from mast cells to cause swelling, itchiness, running nose and watery eyes. Decongestants are used to reduce the stuffiness of the nose by shrinking swollen membranes in the nose. Auto injector, epinephrine, is a potent lifesaving drug for the treatment of anaphylaxis. For all its benefits, one of the expected side effects of immunosuppressive drugs is that they make the recipient susceptible to infections, apart from other toxicity issues (Cobanoglu et al. 2013; Shapiro 1983; Wood et al. 2013; Xu et al. 2014).

Autoimmunity occurs as a consequence of breach of tolerance in the body. This breach of tolerance can occur, either in the thymus during central tolerance or in the secondary lymphoid organs as a part of peripheral tolerance (Xing and Hogquist 2012). In context of peripheral tolerance, during T-cell activation, MHC-peptide interaction with TCR along with co-stimulation is required for T-cell expansion, proliferation and cytokine production. This co-stimulation occurs via interaction between CD28 (co-activator) present on T cells and CD80/86 (B-7 family) present on APC. Another molecule known as CTLA-4, which is a co-inhibitor, interacts with the same CD80/86 and negatively regulates T-cell activation. The absence of a co-activator or the presence of a co-inhibitor and their subsequent interaction with APCs can downregulate the self-reactive T-cell clones in the periphery (Buchbinder and Desai 2016).

Along with the processes of central and peripheral tolerance, regulatory T cells play a crucial role in suppressing an autoimmune response. These suppressor or regulatory T cells (Treg) are unique with respect to their mode of action, as in response to antigen binding, they prevent the activation of helper and cytotoxic T cells, instead of proliferation. Tregs are capable of suppressing the CD4 and CD8 T-cell proliferation and the production of effector cytokines regardless of TCR specificity. This is mediated, at least in part, by inhibition of IL-2 transcription within the target cell. Tregs constitutively express CTLA-4 and secrete inhibitory TGF-β and IL-10. Although the mechanism of action is not very clear, Tregs may constitute a specialised T-cell subset optimised to reduce the activity of autoreactive T cells. Primarily, Tregs leave the thymus as $CD4^{+}CD25^{+}$ cells. However, the demonstration of in vitro Treg generation by a variety of stimuli leaves the possibility that there may be additional in vivo mechanisms for their generation as well (Sakaguchi et al. 2008).

Autoimmune disorders are usually chronic in nature and once established, they are nearly impossible to resolve. Even if the autoimmune reaction develops as a result of infection, removal of the pathogen does not reverse the reaction, and if it is against self-antigen, it evidently becomes a chronic disease. The outcome of autoimmune disorder varies from disease to disease and is also dependent on the individual's immunogenetic makeup. For some, it may be mild but for others it may lead to the complete destruction of organs, leading to mortality. The classification of autoimmune diseases is roughly based on whether it is local or systemic. However, we must keep this in mind that apart from giving us a general perspective, this stratification provides no insight to the disease mechanism or to its severity.

Treatment of autoimmune disease essentially depends on immunosuppression, but long-term effects of these therapies result in increased susceptibility to various other infections. Over the years, corticosteroids have remained the gold standard for treatment of autoimmune disease. They are derivatives of glucocorticoid hormones and act as anti-inflammatory agents in low doses. Corticosteroids, such as prednisone, bind to the steroid receptor present in cytoplasm and then form a complex with heat shock protein 90 (Hsp90), followed by translocation to the nucleus. Here, it binds to the promoter of certain steroid responsive genes causing their activation and suppression of the genes that activates inflammatory cytokines such as IL-1, GM-CSF, TNF-α, etc. (Fauci 1983; Harati and Patten 1979; Strehl et al. 2019). Immune checkpoint inhibitors of CTLA-4 and PD-1 (mAbs) have also shown efficacy in treating rheumatoid arthritis, psoriatic arthritis and type I diabetes. Presently, Treg therapy is in clinical trial, where $CD4^{+}CD25^{+}Foxp3^{+}$ T cells from patients are expanded ex vivo and then re-infused to control the pathogenic T cells. Inhibition of T-cell proliferation and activation by targeting IL-2 pathway, MTOR, pyrimidine and purine synthesis have shown efficacy in different types of autoimmune diseases (Gosselin et al. 2017; Mimouni and Nousari 2002; Rosenzwajg et al. 2019; Zhao et al. 2019).

10.2.1.3 Immunomodulating Agents

The therapies based on the approach of immunomodulation can be broadly known as the immunomodulating agents. Although immunomodulation therapies can be targeted against multiple immune cells, most of the therapies are directed towards T and B cells and the associated soluble proteins and pathways. Additionally, it has been found that a particular immunomodulatory drug can intervene in multiple pathways, thus affecting various disorders. There is a vast range of therapies essentially based on immunomodulation, and we have addressed a few of the major ones as an overview of the strategies and their mechanisms (Table 10.2).

10.3 Immunomodulation in Autoimmunity

We now understand that the autoimmune disorders are essentially a manifestation of the disruption of balance created within our own immune system. More often, it is a consequence of the breach of immunological tolerance to self-antigens

Table 10.2 Immunomodulating agents (Bascones-Martinez et al. 2014)

Immunomodulators	Mode of action	Uses
Inhibiting T-cell activation		
Azathioprine	Purine analogue; inhibits purine synthesis in lymphocytes due to absence of salvage pathway	Treatment of RA, pemphigus and vasculitis
Rapamycin (sirolimus)	Inhibits mTOR pathway, reducing IL-2 sensitivity of lymphocytes	Systemic lupus erythematosus (SLE)
Methotrexate	Folic acid analogue prevents synthesis of tetrahydrofolate	RA, Behcet's disease and in transplantations
Mycophenolate mofetil	Non-competitive inhibitor of IMPDH prevents purine synthesis in lymphocytes	Behcet's disease, pemphigus vulgaris, lupus nephritis
Basiliximab	Chimeric mouse-human monoclonal antibody to the α chain (CD25) of the IL-2 receptor in T cells	Kidney transplants, lichen planus
Interference with T-cell activation		
Cyclosporine A	Binds to cyclophilin of T cells, inhibits calcineurin, responsible for activating the transcription of IL-2	RA, psoriasis, in transplantation, Crohn's disease, nephrotic syndrome
Tacrolimus	Binds to FKBP12 complex, inhibits calcineurin hindering both T-cell signal transduction and IL-2 transcription	Lupus nephritis, vitiligo, psoriasis, eczema, allogenic organ transplant
Inhibition of antigen processing by APC		
Abatacept	Composed of Fc region of IgG1 fused to CTLA-4; binds to B7 protein on APC, blocks co-stimulation	RA, psoriatic arthritis and juvenile idiopathic arthritis
Natalizumab (anti-α4 integrin)	Humanised mAb against α4, integrin, reducing immune cell migration via intestinal lining and blood-brain barrier	MS and Crohn's disease
Anti-CD40 (CFZ533)	Block CD40L reducing autoantibodies' formation	Clinical trial for RA, SLE, Sjogren's syndrome, myasthenia gravis
Other agents		
Glucocorticoids	Blocks transcription of IL-2, preventing T-cell activation	RA, Sjogren's syndrome Crohn's disease, SLE, asthma
Rituximab	Chimeric mAb against CD20, triggers cell death	RA, myasthenia gravis, Non-Hodgkin's lymphoma
Adalimumab	Monoclonal antibody against TNF-α	RA, ankylosing spondylitis, Crohn's disease
Tocilizumab	Humanised mAb against the interleukin-6 receptor (IL-6R)	RA and systemic juvenile idiopathic arthritis
Secukinumab	Human IgG1κ monoclonal antibody against interleukin (IL)-17A	Psoriasis, ankylosing spondylitis and psoriatic arthritis

(Theofilopoulos et al. 2017; Yang et al. 2018). Although autoimmune disorders can be broadly classified as systemic or tissue-specific in nature, most of the traditional therapies have been following the general use of non-specific immunosuppressive therapies to dampen the immune response (Chandrashekara 2012). While these medications are highly effective in most of the cases, persistent high dose may have serious repercussions such as increased susceptibility to lethal opportunistic infections and the risk of developing malignancies (Lallana and Fadul 2011). Therefore, in the current scenario, there is a requisite for target-specific immunomodulation strategies which are capable of reducing the side effects while improving tolerability. The preferable strategy for targeting autoimmune disorders should encompass (1) directed targeting of aberrant cells or pathways without affecting the normal physiological processes, (2) restore the normal immune tolerance in due course of time, (3) have low side effects and toxicity and (4) must be cost-effective in its approach. Along with the traditional concept of using immunosuppressive therapies, present approaches are directed towards inhibiting the generation of the pathogenic cells or augmenting the pathways capable of naturally suppressing these cells (Rosenblum et al. 2012).

10.3.1 Strategies of Immunomodulation in Autoimmune Diseases

Immunomodulation therapies can have various mechanisms specifically aimed at weakening the aberrant immune response. Therefore, classification of the immunomodulating agents can have multiple frameworks. One way of categorisation can be based on the nature of autoimmune disorder, i.e. whether the therapy targets a specific tissue or is systemic. Mostly, this method of classification is followed due to its easier approach. In this respect, we have outlined the major autoimmune disorders and associated immunomodulation strategies (Table 10.3). However, certain disorders are found to be predominantly affecting one organ with lower grades of pathology affecting other organs as well. Therefore, this basis of classification is misleading to some extent (Gea-Banacloche 2006). We have therefore extensively described a classification system based on the target effector cell and the mechanism associated with the pathology of the specific disorder. In certain conditions, multiple effector pathways are implicated simultaneously, so here we have defined each of the effector pathways associated with the disorder.

Pathogenesis of most of the autoimmune disorders is mediated mostly either by T cells, B cells, dendritic cells or combined immune complexes of these cells and their pathways. Therefore, we have essentially outlined the immunomodulation strategies targeting each of these effector cells and inhibiting the related pathways.

10.3.1.1 Immunomodulation Strategies in T Cells

Present approaches in immunotherapies revolve around the concept of understanding the various checkpoints of tolerance which reflect an opportunity for therapeutic intervention. Broadly, the immunomodulation strategies target a particular phase of the T-cell immune response such as (1) antigen presentation to T cells, (2) T-cell

Table 10.3 Example of autoimmune diseases (Gea-Banacloche 2006)

	Antigen/autoimmune mechanism	Immunomodulatory therapy
Multisystem diseases		
Rheumatoid arthritis	Immune response against unknown antigen in the joint synovium that results in destructive arthritis	Corticosteroids, anti- IL1, cyclosporine, azathioprine, etanercept, infliximab, methotrexate, leflunomide
Systemic lupus erythematosus	Pathogenic autoantibodies against nucleic acids and their binding proteins that results in vasculitis, arthritis and renal disease	Corticosteroids, cyclophosphamide, azathioprine, rituximab
Polyarteritis nodosa	Hepatitis B/C surface antigen Vasculitis of small and medium arteries results in systemic disease	Corticosteroids, cyclophosphamide
Sarcoidosis	Unknown antigens in genetically susceptible individuals. Interplay between DC and $CD4^+$ T cells facilitates sarcoid immune response	Chlorambucil, azathioprine, cyclosporine, thalidomide cyclophosphamide, infliximab
Organ-specific autoimmune diseases		
Type 1 diabetes mellitus	Autoantibodies against β-cells of the pancreas. T-cell response and Abs resulting in β-cell destruction	Vaccination with molecules and peptides carried out to prevent killing of β-cells
Psoriasis	Ag in keratinocytes. Interplay between keratinocytes and immune cells resulting in psoriatic plaques	Corticosteroids are used to reduce inflammation and pain
Multiple sclerosis	T-cell response against myelin basic protein resulting in repeated episodes of CNS demyelination	Corticosteroids, daclizumab, IFN, natalizumab
Graves' disease	TSH receptor antibodies generated, binding to receptor causing continuous release of thyroid hormones	No drugs for hyperthyroidism, corticosteroids for ophthalmopathy
Guillain-Barre syndrome	PNS damaged by antibodies so muscles have trouble responding to the brain	Plasma therapy and administration of intravenous Ig are beneficial
Immune thrombocytopenic purpura	Platelet membrane proteins become antigenic. Autoantibodies attack and lower the platelet count by opsonisation and phagocytosis	Corticosteroids, intravenous Ig
Anti-GBM antibody disease (Goodpasture's syndrome)	GBM protein (alpha-3 chain of type IV collagen). Ab and complement-mediated inflammation	Removal of antibody from plasma by plasmapheresis with cyclophosphamide and corticosteroids
Addison's disease	21-Hydroxylase in adrenal gland. Adrenal failure with decreased glucocorticoids and increased ACTH and plasma renin levels	Hormone replacement therapy along with corticosteroids

(continued)

Table 10.3 (continued)

	Antigen/autoimmune mechanism	Immunomodulatory therapy
Hashimoto thyroiditis	Ab against antigens on thyrocytes causing lymphocyte infiltration and apoptosis of thyroid epithelial cells	Corticosteroids
Sjogren's syndrome	Unknown Ag causes inflammation and lymphocyte aggregation in exocrine glands, results in reduced levels of tears and saliva	Cyclosporine ophthalmic along with corticosteroids
Myasthenia gravis	Ab targets components of postsynaptic membrane causing impaired neuromuscular transmission and results in weakness and fatigue of skeletal muscles	Corticosteroids, azathioprine, cyclosporine A, cyclophosphamide, mycophenolate mofetil, methotrexate
Pernicious anaemia	Intrinsic factor protein or parietal cells and results in poor absorption of vitamin B12 from the food	Vitamin B12 derivatives like cyanocobalamin and hydroxocobalamin
Coeliac disease	Transglutaminase acts as autoantigen; T and B cells coordinate to recruit killer lymphocytes and cytotoxicity to enterocytes	Glucocorticoids, hydrocortisone
Antiphospholipid syndrome	Membrane phospholipids bound to a plasma protein. Antiphospholipid antibodies produced results in deregulation in blood coagulation	Anticoagulants, cyclophosphamide, corticosteroids and intravenous immune globulins
Achalasia	Motility disorder of the oesophagus. Ab against unknown Ag of myenteric neurons which help in oesophageal peristalsis	Intravenous methylprednisolone, intravenous immunoglobulin (IVIg) and plasmapheresis
Amyloidosis	Deposition of misfolded proteins. Activation of inflammasomes, pro-inflammatory cytokine release, phagocytosis, degradation of amyloid deposits	Chemotherapy and steroids with melphalan + dexamethasone
Autoimmune hepatitis	Presence of autoantibodies against liver hepatocytes. Immune response involves Th1/Th17 polarisation and dysfunctional Tregs	Corticosteroids, cyclosporine A, anti-TNF alpha therapy-infliximab
Autoimmune oophoritis	Autoantibodies to ovarian Ag. Premature ovarian failure manifests as PCOS, infertility and endometriosis	Corticosteroids, cyclical hormonal therapy with oestrogen and progestin
POEMS syndrome	Paraneoplastic syndrome due to plasma cell neoplasm. Chronic inflammation by cytokines results in vascular leak and polyneuropathy	Corticosteroids, thalidomide

(continued)

Table 10.3 (continued)

	Antigen/autoimmune mechanism	Immunomodulatory therapy
Scleroderma	Inflammation and fibrosis of skin. Autoantibodies against endothelial and fibroblast cells, inducing ROS production	Methotrexate, cyclosporine, corticosteroids
Uveitis	Intraocular inflammation. Abs against retinal antigens like IRBP and arrestin, causing impaired vision	Corticosteroids, methotrexate, cyclosporine A
Haemolytic anaemia	Acquired haemolysis by Ab against own RBC antigens. Destruction of RBC through complement fixation, phagocytosis or ADCC	Corticosteroids, prednisone, monoclonal antibody-rituximab
Devic's disease	Autoantigens of the heart. Increased anti-myocardial antibodies targets pericardium	Corticosteroids, indomethacin, prednisone
Alopecia areata	Antibodies against hair follicles. T-cell mediated disease with poorly defined pathogenesis	Cyclosporine, methoxsalen, anthralin and glucocorticoids

activation, (3) proliferation and downstream events of T cells and (4) migration of T cells (Gea-Banacloche 2006). Along with this, there are certain immunomodulating agents which play a distinct role and cannot be directly categorised under this framework. We have discussed each of these approaches and the associated therapies for immunomodulation in autoimmune disorders.

10.3.1.1.1 Modulation Strategies Targeting Antigen Presentation to T Cells

The foundation of T-cell activation is dependent on its interaction with the antigen-presenting cells (APCs). Along with antigen recognition through T-cell receptor (TCR), costimulatory signals from APCs serve a crucial role in T-cell activation and in maintenance of tolerance (Sharpe 2009). In normal physiology, if TCR stimulation is not accompanied by strong costimulatory signal, T cells lead to a state of suboptimal activation, eventually resulting in anergy. It is only during the episodes of infection or inflammation that the costimulatory signals in APCs are upregulated and cause T-cell activation. However, in case of an autoimmune disorder, the self-reactive T cells receive costimulatory signals resulting in an aberrant activated T-cell therapy (Mueller 2010). Moreover, persistent use of this therapy led to susceptibility to multiple infections, as it prevented activation of naïve T cells in general. Therefore, costimulatory blockade can be considered as an immunomodulatory therapy favourable for the early stages of an autoimmune disorder.

It is now evident that the costimulatory signalling is critical for T-cell activation, thus intervention at this step can be a promising target for treatment of autoimmune disorders. The underlined ideology behind costimulatory signal blockade involves enforcing the self-reactive T cells towards anergy. Based on this approach, a new wave of immunotherapieshas surfaced. One of the pioneering ones includes the

generation of a chimeric CTLA-4-Ig protein, which is responsible for blocking co-stimulation through CD28 (Lenschow et al. 1992). Potent activation of TCR signalling cascade is highly dependent on the interaction of CD28 on T cells and its ligands, CD80 or CD86 on APCs. CTLA-4 is a structural homologue of CD28, responsible for binding with the same ligands, but delivering an inhibitory signal to T cells. This process eventually leads to the attenuation of T-cell signalling and inhibits further activation (Rowshanravan et al. 2018). Based on the concept of competition between CTLA-4 and CD28 for binding with the same ligands with differing outcomes, fusion protein CTLA-4-Ig (clinically known as abatacept) was developed. It is composed of the extracellular domain of CTLA-4 fused to Fc domain of human IgG (Vincenti 2008). Abatacept has been approved for the treatment of moderate to severe cases of rheumatoid arthritis and psoriatic arthritis and has shown potential in declining the rate of β-cell reduction in type I diabetes (Orban et al. 2011). Additionally, the use of abatacept for multiple sclerosis (MS) and systemic lupus erythematosus (SLE) is also going through phase II of clinical trials.

In spite of its short-term efficacy, costimulatory blockade did not show any significant long-term consequences. The drug dosage required repetitions and self-tolerance was not attained over time. One probable explanation could be the inhibition of costimulatory pathway was only responsible for activation of naïve T cells. It was not capable of suppressing the pathogenicity of the self-reactive T cells which were activated prior to this.

10.3.1.1.2 Immunomodulation Therapies Targeting T-Cell Activation

The generation of drugs responsible for suppression of T-cell activation can be attributed to transplantation immunology, as the inhibition of this process is directly involved in immunosuppression (Allison 2016). Since immunosuppression was equally important in blocking a hyperactive immune response, these immunosuppressants found a major role in some of the autoimmune disorders. The most important class of drugs in this category are the calcineurin inhibitors. Their general mechanism of action includes binding to a type of protein, known as immunophilins which inhibit the calcium-calcineurin pathway, crucial for T-cell activation. Once calcineurin is blocked, it restricts the transcription of many cytokines such as IL-2, TNF-α, IFN-ϒ, IL-1β, etc. critical for T-cell activation and their clonal expansion (Yoon 2010). Primarily, there are two types of calcineurin inhibitors, cyclosporin A and tacrolimus. The mode of action for both drugs is similar and involves inhibition of calcineurin. The difference lies only in the type of immunophilins they bind to, i.e. cyclosporin A binds to cyclophilin, whereas tacrolimus binds to FK binding protein (Mok 2017). Both drugs have shown comparable efficacies in treating systemic lupus erythematosus (SLE), specifically lupus nephritis (Mok 2016).

10.3.1.1.3 Immunomodulation as an Approach Inhibiting T-Cell Proliferation

Amongst the cytokines responsible for ensuring T-cell proliferation, IL-2 remains of significant importance. Therefore, intervention of this pathway is considered for

suppressing the clonal expansion of T cells. Monoclonal antibodies targeting the IL-2 receptor (CD25) have been developed with the aim of inhibiting the interaction of IL-2 and IL-2 receptor. However, it is now known that IL-2 plays a pleiotropic role and has been shown to have contradictory effects. On one hand, IL-2 is responsible for augmentation of an immune response through increased proliferation of activated T cells; on the other hand, it is accountable for decreasing the immune response by upregulating the regulatory T cells (Tregs) (Mitra and Leonard 2018). The optimal therapy for targeting autoimmune disorders would essentially require both the suppressed proliferation of activated T cells and an enhanced Treg response at the same time. Therefore, researchers developed immune complexes of recombinant IL-2 bound to anti-IL-2 antibody [rIL-2-IL-2 monoclonal antibody (mAb)] (Boyman et al. 2006). This therapy has been shown to preferentially stimulate the proliferation of regulatory T cells while suppressing the excessive proliferation of effector T-cell population. This therapy has shown convincing results in mice models of haemophilia, experimental autoimmune encephalomyelitis and myasthenia gravis (Liu et al. 2011; Liu et al. 2010; Webster et al. 2009).

Certain drugs such as sirolimus (rapamycin) also block T-cell proliferation by leading to cell cycle arrest. The target site for this drug is same as that of tacrolimus, i.e. FK binding protein (FKBP). However, it does not intervene in the calcineurin pathway and binds to mTOR protein, which is crucial for cell cycle. This eventually leads to cell cycle arrest at G1 phase (Augustine et al. 2007). Similarly, immunomodulatory agents such as leflunomide are also responsible for G1 arrest by inhibition of pyrimidine synthesis. This therapy has been approved for immunosuppression in rheumatoid arthritis (Zhang and Chu 2018).

10.3.1.1.4 Immunotherapies Interfering with T-Cell Migration

T-cell migration to secondary lymphoid organs and site of inflammation both play significant roles in the dynamics of inflammation in autoimmune disorders. Therefore, certain immunomodulation therapies restrict the migration of pathogenic T cells to the site of inflammation. One of the most successful therapies in this respect includes the anti-integrin-based monoclonal antibody, natalizumab, approved for the treatment of multiple sclerosis. It is a humanised recombinant monoclonal antibody targeting the α4 and α4β7 integrins, present on the surface of all leucocytes, except neutrophils. Binding of natalizumab to the α4 and α4β7 integrins is responsible for inhibition of leucocyte migration to the central nervous system (CNS), thereby limiting inflammatory response in the brain (Delbue et al. 2017; Shirani and Stuve 2017).

Additionally, some immunotherapies are responsible for inducing tolerance through T-cell trafficking to lymph nodes, leading to subsequent generation of antigen-specific regulatory T cells. Towards this, monoclonal antibodies targeting LFA-1 integrins have shown considerable increase in regulatory tolerance along with blockade of lymphocyte trafficking (Nicolls and Gill 2006). Studies in the context of anti-LFA-1 therapies are under clinical trials for disorders such as psoriasis, type I diabetes, etc.

10.3.1.1.5 Strategies Involving Upregulation of Regulatory T Cells

Regulatory T cells (Tregs) are amongst the key players responsible for maintaining immune tolerance. With respect to autoimmune disorders, it has been shown that the Treg population either decreases or is functionally challenged (Goschl et al. 2019). It is thus obvious that strategies revolving around increasing these populations of cells are of primary interest. One of the major approaches is based on isolation, ex vivo activation and expansion of the Treg population to adoptively transfer them in patients with autoimmune disorder. For adoptive transfer, the purity of cells is of utmost importance. Additionally, it has to be ensured that these cells retain their capability of immune suppression. Due to lack of clear understanding of markers representing regulatory T cells, adoptive transfer therapies have shown very conflicting results in most of the autoimmune disorders. However, Treg therapy has proven to be successful in acute graft-versus-host disease (aGVHD) (Elias and Rudensky 2019).

10.3.1.1.6 Corticosteroid-Based Therapies in Autoimmune Disorders

Corticosteroids are the most traditional therapies being used for multiple autoimmune disorders. They are derivatives of glucocorticoid hormones and have variable effects, based on their dosage. In smaller doses, corticosteroids are used as anti-inflammatory agents, whereas at intermediary doses, they function as immunosuppressive agents. At very high doses, they are responsible for lymphocyte depletion. The mechanism of action of glucocorticoids initiates with its diffusion through the membrane lipids. Once inside the cell, it binds to its receptors present in the cytoplasm and nucleus and forms a glucocorticoid-receptor (GCR) complex. This complex enters the nucleus and binds to specific sequences of DNA in the promoter region of steroid responsive genes. This subsequently results in the activation and suppression of multiple genes. The consequence of this signalling cascade is downregulation of pro-inflammatory cytokines such as IL-1, TNF-α, IL-3, GM-CSF and other chemokines. Alongside, glucocorticoids are also responsible for altering cellular structure and activity (Coutinho and Chapman 2011; Ferreira et al. 2016).

10.3.1.1.7 Therapies Interfering with Pro-inflammatory Cytokine-Receptor Signalling

Since inflammation plays a critical role in aggravating the pathology of autoimmune disorders, specific targeting of pro-inflammatory cytokines and their receptors can result in improvement in clinical parameters. Additionally, blockade of this signalling cascade has been shown to restore tolerance in some cases leading to reversal of the balance between the pathologically aberrant and Treg population. Towards this approach, TNF-α antagonists have played a significant role in reducing inflammation in joints, gut and skin. Blockade of TNF-α signalling regulates multiple mechanisms such as downregulation of pro-inflammatory cytokines, adhesion of molecules, reduction in vascular permeability and angiogenesis, increase in expression of Tregs and mitigated recruitment of inflammatory cells to the target tissue. TNF-α

targeting drugs are approved for rheumatoid arthritis, psoriasis and Crohn's disease (Sfikakis 2010).

Similarly, studies suggest the role of IL-1 inhibition in alleviating the clinical parameters in patients having type II diabetes and rheumatoid arthritis. However, this perspective requires further analysis and validation for consideration of therapeutic application (Ruscitti et al. 2019).

10.3.1.1.8 Therapies Based on Allergen-Specific Immunotherapy

Using allergen-specific immunotherapy (ASIT) is a concept used historically in the case of allergy. The proof of concept in this approach is that when an allergen is repeatedly administered in increasing doses to an allergic patient, the consequence is the generation of a high robust immune response stable over time (Larche et al. 2006). Based on this approach, similar strategies are being considered for autoimmune disorders as an option for combination therapies. Here, researchers are exploring the combination of disease-causing autoantigen with an immunomodulatory agent. If this approach of autoantigen-specific destruction is successful, it may alter the complete perspective on therapies related to autoimmune disorders (Northrup et al. 2016).

10.3.1.2 Immunomodulation Strategies in B Cells

Along with T cells, B cells play a crucial role in exaggerating the immune response in autoimmune disorders. Their role in autoimmunity broadly encompasses antibody production and acts as an aid to the T cells and dendritic cells and hyper-secretion of pro-inflammatory cytokines. The autoreactive B cells found in some autoimmune disorders are capable of generating autoreactive plasma cells. The IgG autoantibodies produced by these plasma cells bind to target antigens and lead to the generation of complexes which subsequently activate the complement system. Additionally, effector B cells are responsible for regulation of lymphoid tissue structure and serve in antigen processing and presentation (Ronchese and Hausmann 1993). Therapies in context with B cells mainly lead to its depletion by targeting the surface proteins with monoclonal antibodies, or cytokine and costimulatory blockade (Craft 2012).

10.3.1.2.1 B-Cell Depletion Strategies by Targeting Surface Proteins and B-Cell Receptor

Monoclonal antibodies targeting CD20 have shown varying degrees of efficacies, based on the disorder concerned. Currently, this approach of therapy is used in rheumatoid arthritis, pemphigus, systemic lupus erythematosus (SLE), granulomatosis with polyangiitis, microscopic polyangiitis and relapsing multiple sclerosis (Cohen and Keystone 2015; Thaunat et al. 2010; Townsend et al. 2010). Additionally, monoclonal antibodies directed against CD19 and CD22 surface proteins are considered for treatment of multiple sclerosis and SLE. However, these studies need further validation before approval.

Although B-cell depletion strategies have shown to contribute in reduction of clinical parameters, their efficacy is highly variable with respect to multiple

autoimmune disorders. Major challenges associated with this therapy include the simultaneous depletion of regulatory B cells, thus affecting the tolerising capabilities.

Apart from targeting the surface proteins associated with B cells, downstream signalling processes required for its activation can also be targeted. Amongst the proteins downstream of B-cell receptor, Lyn, Syk, PI3K and BTK are potential therapeutic targets for silencing of autoreactive B cells. Ibrutinib, a BTK inhibitor, has shown convincing results in graft-versus-host disease and is approved for its treatment (Musette and Bouaziz 2018).

10.3.1.3 Immunomodulation Strategies in Dendritic Cells

Although cells of the adaptive immune system are the key players of autoimmunity, dendritic cells (DCs) are indirectly involved in providing aid to T cells. Additionally, DCs are also responsible for maintaining immune tolerance to self-antigens (Ganguly et al. 2013). Therefore, current strategies are focussed towards adoptively transferring tolerogenic DCs in autoimmune disorders. Multiple experimental studies have been able to demonstrate the amelioration of symptoms in tissue-specific autoimmune disorders using tolerogenic DCs; however, their effect in systemic autoimmunity needs to be addressed in greater detail. Moreover, it needs to be kept in mind that all these studies are only in the preliminary stages and require extensive clinical trials before they can be declared to have therapeutic application.

10.4 Conclusion

Traditional therapies in autoimmune disorders are dependent on the concept of immunosuppression and/or immunomodulation. However, with the increasing complexities of various autoimmune disorders, a more target-specific approach is of crucial importance. Therefore, the primary objective in research based on autoimmune therapeutics should encompass discovering the specific antigen responsible for driving an autoimmune disorder. Apart from this, we must understand the mechanisms responsible for enhancing the regulatory T-cell populations in terms of numbers and functionality while suppressing the pathologically aberrant ones.

Additionally, the focus of therapeutics for autoimmune disorders must shift to the simultaneous use of multiple therapies, i.e. combinatorial therapeutics for enhancing therapeutic efficacy. Each of the autoimmune disorders has specific dynamics and it is crucial that a suitable combinatorial therapy is selected.

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11 Psychology, Epigenetics, Immunomodulation, and Immune Dysfunction: Understanding the Connection

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Abstract

Immunomodulation is a process by which the body's immune response is regulated, and the cells and molecules involved in the process are called immunomodulators. In conventional medical science, there are a group of drugs, termed as immunomodulators, which acts by activating, suppressing, or regulating the immune response according to the therapeutic need. All these medications are designed and administered based on the ideology that the cause of any disease is limited only to the physical body. And by doing so, we are creating a chaotic interaction between the natural immunomodulators and artificial immunomodulators unknowingly and unintentionally, and the result is constant damage to the overall homeostasis, adverse drug reactions, and drug-induced immune dysfunction. This is the result of incomplete understanding about the concepts relating to health and immunomodulation. This chapter explains in brief the essential concepts relating to health and diseases that are important for having a complete understanding of immunomodulatory processes and immune dysfunction. The immune dysfunctions that are observable in the physical body are the direct reflection of psychology and the surrounding environment. This chapter aims in addressing the knowledge gap by pointing out the importance of considering the existence of natural immunomodulators and their relevance in regulation of immune system functioning while designing and administering artificial immunomodulators.

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Rheumatoid arthritis · Autoimmune disorder · Adverse drug reaction · Drug-induced immune dysfunction · Mind-body medicine

11.1 Introduction

The incidence and prevalence of diseases like cancer, hypersensitivity reactions, and autoimmune disorders are increasing worldwide. In general, all these conditions are grouped under the immune system disorders. The National Cancer Institute defines immune system disorders as a condition that affects the immune system (National Cancer Institute n.d.). The word *affect* is derived from the Latin word *affectus* which means *to act upon* or *a state (of mind or body) produced by external influence* (Etymonline n.d.-a, n.d.-b, n.d.-c). Thus, in simple terms, immune system disorders can be defined as the *dysfunction of the immune system caused by certain external factors*. The word *dysfunction* in Latin means *abnormality or failure of normal functioning* (of the immune system) (Etymonline n.d.-a, n.d.-b, n.d.-c). The immune system is the defense system of our body composed of various types of molecules, cells, and organs working together in a synchronized manner (Abbas et al. 2004).

In the majority of the cases, the conventional medical science is not aware about the actual cause of the immune system dysfunction, and in some cases (e.g., cancer and autoimmune diseases) the genes or the gene products (faulty proteins) have been attributed as the underlying cause (Bollinger 2016; Lipton 2016; Otto 2020). The diagnostic tests that the conventional medical science implements only identify the result (i.e., the measurable abnormal physical parameters) of the immune dysfunction and not the actual cause (because the cause is not known). These abnormal physical parameters are then considered and treated as the cause (even if they are not). Based on the assumption that the measurable abnormal physical parameters are the cause, various kinds of chemical drugs are being synthesized industrially that specifically regulate the abnormal physical parameters, bring them back, and maintain them at their normal level (by force). This is accepted as an evidence-based treatment for diseases of the immune system (Bollinger 2016; Hegde 2014; Null 2011).

One such group of chemical drug is called the immunomodulators. The word immunomodulators can be divided into two parts: the first part is "immune" and the second part is "modulators." The word *modulator* means any component that has the *ability to modify or regulate* (Etymonline n.d.-a, n.d.-b, n.d.-c). In this context, the immunomodulators are industrially synthesized chemical drugs that have the ability to modify and regulate the immune response by physically manipulating the molecules, cells, and organs that make up the immune system. Suppression and activation are two basic ways by which an immunomodulator modifies and regulates immune response. The immunomodulators that act by suppressing an immune response are known as immunosuppressants, while those that act by stimulating an immune response are known as immunostimulants. Industrially synthesized

immunomodulators are promising while addressing the medical conditions where emergency quick fixes are absolutely necessary.

Simply put, industrially synthesized immunomodulators are extremely necessary for acute medical conditions and certain surgical procedures but not for the chronic diseases (Bollinger 2016; Hegde 2014; Null 2011; Otto 2020). All these processes have been developed and implemented only keeping the physical body and its components (that can be measured by conventional technology) under consideration. But here are the few fundamental drawbacks because of which industrially synthesized immunomodulators are not appropriate for treatment of chronic diseases:

- What if the phenomenon of health is multidimensional and not one dimensional (as perceived by conventional medical science)? There are factors beyond the physical body that conventional science does not acknowledge because they do not fit into the ideology of conventional Newtonian physics.
- What if by not acknowledging health as multidimensional phenomenon the researchers are developing a faulty perception and incomplete understanding about how nature actually functions? As the definition states, immune dysfunction means abnormality or failure of the normal functioning of the components of the immune system caused by certain external factors. If observed carefully, the mutated genes, faulty proteins, and molecules are the (internal) components of the immune system and thus the part of the physical body. Scientifically, none of these components can be attributed as the External Factors. Thus, the external factors that are regulating these internal components are coming from outside the physical body.
- Not willing to acknowledge the environmental immunomodulators and body's inherent immunomodulatory abilities while designing a treatment and developing therapeutics because of the faulty perception and incomplete understanding of the actual definitions may lead to serious health consequences (e.g., adverse drug reactions or drug-induced immune dysfunction). Nature along with its components (human body) knows how to fix itself, wants to fix itself, and constantly trying to fix itself using the ways natural to them.
- There are external factors, present in nature, that modulate our immune response either by suppression or by stimulation. It is because of the immunomodulatory effects of these external factors the overactivity and underactivity of the molecules, cells, and organs of the immune system are observed and diagnosed in the physical body. Now imagine the consequences if synthetic immunomodulatory drugs are administered to address the underactivity and the overactivity of immune system that are being modulated by external natural forces.
- Even if it is viewed from the perspective of Newtonian physics, that matter impacts matter, then also the molecules, cells, and organs of the immune system (that are under natural immunomodulation) are subjected to be impacted by forceful action of artificial immunomodulators (opposing the action of natural immunomodulation). Imagine the potential disharmony this process creates on

the homeostasis of the body, and if continued for a significant period of time, this may lead to drug-induced immune dysfunctions.

The chapter explains in brief certain essential concepts relating to health and diseases that are important for having a complete understanding of immunomodulatory processes and immune dysfunction. The immune dysfunctions that are observable in the physical body are the direct reflection of psychology and the surrounding environment. In this chapter, the *natural immunomodulators* are described as *immunomodulators*, while the *industrially synthesized chemical immunomodulators* are described as *artificial immunomodulators*. This chapter aims at addressing the knowledge gap by pointing out the importance of considering the existence of natural immunomodulators and their relevance in regulation of immune system functioning while designing and administering artificial immunomodulators.

11.2 Understanding Science

Science is a medium for understanding how nature functions. This understanding can be achieved in two ways, the reductionistic approach and the holistic approach. Reductionism views the universe (e.g., nature or human body) as a machine made out of various parts that are discretely functional, and each of these component parts can be isolated and studied separately. This ideology believes the addition or removal of component parts does not produce a significant effect on the overall functioning of the system. In case the system breaks down, efforts are made only to detect and fix the part that appears to have malfunctioned. It does whatever it takes to fix the part that appeared to have malfunctioned and does not consider the overall effect the approach makes on the system. The ideology of reductionism is based on Newtonian physics which separates the universe into matter and invisible energy. According to the Newtonian physics, the components that are made out of matter are only affected by matter and not by the invisible energy. Conventional medical science strictly follows reductionistic ideology, and all the textbooks (of medicine and technology) have been written from the point of view of reductionism (Goswami 2011; Hegde 2014; Lipton 2016).

On the contrary, wholism views the universe (e.g., nature and human body) as a system made out of interrelated and inseparable parts that function in a synchronized manner. Each component part is inseparably interconnected with each other and thus cannot be studied in isolation. The ideology believes the addition or removal of component parts produces a dynamic effect on the overall functioning of the system. In case the system breaks down, detecting and fixing the part that appeared to have malfunctioned will not solve the issue. The emphasis is made to identify the actual cause that resulted in the malfunctioning of the component part and restoring the overall balance. The ideology of wholism is based on quantum physics which states that matter itself is made out of energy (the concept of aduality) and it is impossible to eliminate the direct influence of energy in the physical (biological) expression of the body (Goswami 2011; Hegde 2014; Lipton 2016).

So, it is clear that both approaches allow us to understand the universe from a different perspective and are equally important in scientific studies. It is naïve and completely unscientific to embrace the one approach and completely exclude the other one. For example, when the discipline of physiology is taught in the context of medicine, it is taught from the reductionistic point of view but the same discipline when taught in a nonmedical context, the same science is taught from the wholistic point of view. And it is because of this bias incomplete understanding of the science persists in our community. Not being able to perceive nature as it is is our own fault. One needs to realize that manipulating the experimental outcomes under laboratory settings is not going to change the ways nature is meant to function. It is wise to acknowledge the fact that the wisdom of nature is much greater than all the combined knowledge that human beings have gathered through time. This is the essence of understanding science and it is time to implement this mindset practically. This unbiased mindset will make scientific studies more elaborate, error-free, complete, and evidence-based.

11.3 Health, Understanding the Definition

Before moving on to understanding the relation between psychology, epigenetic control, and immunomodulation, let us first understand the meaning of the word "health." Health is not just a medical terminology but have a deeper meaning. Understanding the actual meaning of health is important to realize the need for considering the existence and direct influence of natural immunomodulators on immune system functioning while designing artificial immunomodulators. According to the World Health Organization:

> Health can be defined as a state of complete physical mental and social wellbeing and not merely the absence of any disease or infirmity.

As per the standard definition, health consists of three dimensions, the physical dimension, mental dimension, and social dimension. The definition also points out to the fact that health cannot be defined just by considering the presence or absence of abnormalities observable in the physical body. Unfortunately, conventional science acknowledges only the physical dimension and presence and absence of abnormalities observable in the physical body while treating illnesses. So the question arises what mental and social dimension is and how they relate to and influence physical dimension. There is another dimension that is now taken into consideration, that is, the spiritual dimension (Dossey 1995, 2000; Goswami 2011; Hegde 2014; Lipton 2016). Let us understand each of these dimensions and how they influence the state of health.

11.3.1 Physical Dimension

The first dimension of health is the physical dimension. Physical dimension represents the physical body and its component parts. The components of this dimension can be evaluated in terms of matter and can be measured and analyzed. This dimension is affected by factors such as physical activity, diet, chemicals and drugs, pathogens, injury, and aging. Conventional medications and surgical procedures are effective if and only if the actual cause of the disease is present in the physical body. The molecules, cells, and organs of the immune system are part of physical body, thus belonging to the physical dimension.

11.3.2 Mental Dimension

The second dimension of health is the mental dimension. It is very important to understand that mind is not present inside the brain. Human brain belongs to physical dimension. Thought, intellect, emotion, and perception are four basic attributes of mental dimension. The signal generated by combined action of these four attributes controls the behavior of components of physical dimension and thus strongly influences physical health. Disharmony in mental dimension creates disharmony within the components of physical dimension. So when the actual cause lies in the mental dimension, treating the result observable in the physical dimension is not going to restore the state of health.

11.3.3 Social Dimension

The third dimension of health is the social dimension. The word society means aggregate of people living together in a community. Thus, the social dimension is created by collective mental dimension of each individual who are the part of the society. One's lifestyle, occupation, education, and financial condition and interaction with people in the society are some of the attributes of social dimension. Disharmony in social dimension creates disharmony in one's mental dimension, results of which are observable in the physical dimension. So, when the actual cause is in social dimension, addressing only the physical dimension is not sufficient.

11.3.4 Spiritual Dimension

The fourth dimension of health is the spiritual dimension. Just like mind is not brain, spirituality is not religion. In Latin, the word spirituality means act of breathing, a process that makes life possible. Thus, in the context of science, spiritual dimension means an individual's interconnectedness with the environment and its components. So spiritual dimension (in a way) can also be called as the environmental dimension. The interaction with environment and its components and responding to natural

energies that controls life are the attributes of spiritual dimension. Interconnectedness with environment and its components influences social, mental, and physical dimension. Thus, disharmony in spiritual dimension creates disharmony in social and mental dimension, results of which are observable in the physical dimension. So if the actual cause is in the spiritual dimension, only addressing the results observable in the physical dimension is not going to restore the state of health. One health concept is the evidence-based example of importance of spiritual dimension of health.

11.4 Immunomodulation and Immune Dysfunction: Conventional Perspective

Reductionism is the process of breaking a "system" into small pieces, studying the pieces in isolation, and assuming that the small pieces of the system are discretely functional. Reductionism which follows Newtonian physics believes that the things that cannot be seen or measured by technologies available do not exist. Our conventional medical science is a firm believer in reductionism and Newtonian physics, and this is the reason they only acknowledge the physical dimension and overlook the other three. Based on that assumption, conventional system has created specialized disciplines like cardiology, neurology, nephrology, endocrinology, etc., and not only that, each of these disciplines has their subdisciplines (Glidden 2012).

So, in conventional system of medicine, every organ or component of a body is regarded or viewed as discrete units working in an isolated manner. When a particular organ gets affected, the treatment procedures are performed and medications are prescribed with the intention of providing relief to the particular organ under consideration. What are the effects and consequences that the treatment and medications impose on the other components of the body and overall health (homeostasis) of an individual are beyond the scope of the treatment (Glidden 2012)?

Let us understand the concept with an autoimmune disorder, rheumatoid arthritis (RA) as an example. Rheumatoid arthritis is a chronic inflammatory disorder that affects the joints in our body. Tenderness of the joints, swelling, stiffness, fatigue, fever, and loss of appetite are some of the common symptoms of rheumatoid arthritis. Rheumatoid arthritis may affect many non-joint structures like eyes, skin, lungs, heart, blood vessels, bone marrow, etc. According to the conventional medical science, inflammation of the synovium and subsequent destruction of the cartilage and bones within the joint are the hallmarks of the disease.

However, the actual cause of inflammation of the synovium is not known although several genes have been linked to this disease. Some of the complications that follow rheumatoid arthritis are osteoporosis, rheumatoid nodules, infections, cardiac diseases, pulmonary diseases, cancer, etc. There are no blood tests or physical findings that confirm the diagnosis. Some blood tests are performed to detect the erythrocyte sedimentation rate (ESR) or the presence of C-reactive protein (CRP) that only indicates the presence of inflammatory process, while the other

blood tests are also performed to detect the presence of rheumatoid factor and anti-cyclic citrullinated peptide (anti-CCP) antibodies. According to the conventional medical science, there is no cure for rheumatoid arthritis, but there are medications such as nonsteroidal anti-inflammatory drugs (NSAIDs), steroids (corticosteroids), disease-modifying antirheumatic drugs (DMARDs), and biologic response modifiers that are administered to address the symptoms. And when the medication fails to control the symptoms, surgical procedures are performed to repair the damaged joints. Surgical procedures include synovectomy, tendon repair, joint fusion, and total joint replacement. These are the standard operating protocols used worldwide as the treatment for rheumatoid arthritis (Abbas et al. 2004; Lipton 2016; Null 2011; Walker 2014; Otto 2020).

When considered from the reductionistic point of view, health is only limited to the physical body. Based on the perspective, the measurable abnormal concentration of cells and molecules (the mediators of inflammation) is considered as the possible cause and is detected using the diagnostic procedures. The idea is based on the comparison between the blood test results of a healthy person and a person having rheumatoid arthritis. The elevated levels of ESR, CRP, and rheumatoid factor are present in the patient having rheumatoid arthritis and are normal in healthy person; thus, by definition, these measurable abnormal cells and molecules are causing the discomfort and thus the medications and surgical procedures are designed to address those selective components. A range of industrially synthesized chemical drugs (grouped as immunomodulators) like NSAIDs, steroids, DMARDs, and biologic response modifiers are prescribed to suppress the body's immune response by blocking the signal transduction pathways and neutralizing the active immune mediators in the bloodstream (Abbas et al. 2004; Lipton 2016; Null 2011; Walker 2014; Otto 2020).

11.5 Immunomodulation and Immune Dysfunction: Overcoming the Knowledge Gap

The drawback mentioned in the above sections can be addressed by integrating the holistic approach with reductionistic ideology. In holistic (wholistic) system of treatment, each and every component of the body is regarded as functional whole and is structurally and functionally dependent on each other. They view the body as a complex combination of various extremely specialized systems functioning in two major ways. First, each and every system performs their functions at an individual level, and second, they work together with other systems of the body, thus making life possible. The ultimate goal of these specialized systems is to maintain the homeostasis of the body. As long as the homeostasis is maintained, the body is protected from diseases. So in case of a particular disease, the efforts are made to identify the root cause that actually disturbed the homeostasis and resulted in the disease under consideration. Treatment procedures are performed and medications are administered keeping in mind that to cure the disease, the root cause must be eliminated. And it is also kept under consideration that the medication and treatment

procedure must not cause disturbance in homeostasis. For understanding the scientific basis and importance of wholistic system in treating diseases, it is essential to understand three fundamental concepts:

1. Connection between psychology and immunomodulation.
2. Immune response is epigenetically controlled and not genetically determined.
3. Molecular movements are guided and regulated by invisible energies.

Sound knowledge about the ways by which external factors like psychology, epigenetics, and invisible energy influence and modulate the immune response is essential to realize the ways nature actually functions and designs drugs and strategizes treatment protocols accordingly. Let us understand each of these concepts sequentially in brief.

11.5.1 Psychological Regulation of Immune Response

The function of human mind is to create connection between a person's belief and the reality. If an individual believes that there is a stressful situation, the perception of stress generates signals which are transmitted to the different parts of the body through the nervous system. The chemicals that prepare cells for protection response are then released into the blood by direct influence of signal generated. One's thought and perception directly modulates the immune response using the nervous system and endocrine system. This ideology is not an unscientific notion but an evidence-based multidisciplinary branch of science known as psychoneuroendocrinoimmunology (PNEI). An individual's perception to a situation determines the type of signal generated. But what if the situation an individual is perceiving as a stressful situation is not real? Cells of the body only respond to the chemicals triggered based on one's perception. When an individual believes that he is under stress, then the stress is manifested on physical body in the form of chemicals, indicating the role of perception in the process of immunomodulation. The psychoneuroendocrinoimmunology (PNEI) states that the chemistry of one's body and behavior of cells are based on the individual's thought or perception. Faulty behavior of cells and organs of the body leads to disease condition. The behavior is the result of signal (energy) from the environment and the proteins (matter) present in the body. So, either the protein or the signal can be considered as the cause of the disease. Scientific studies showed that defective genes cause less than 1% of all diseases, while faulty signal can cause up to 90% or more of disease. The most important cause of the faulty signal is not present in the physical dimension but is beyond that. Our thought becomes translated into chemistry. If an individual have a disease due to faulty perception or unhealthy thought, it's not because the body is different but because the signal is inappropriate. And acknowledging this aspect is really important because it is recognized now that our consciousness is the primary problem in relation to health and plays important and direct role in

modulating the immune response (Dossey 2000; Hegde 2014; Lipton 2016; Pert 1999).

11.5.2 The Concept of Epigenetics

Conventionally, it is a common trend to establish a direct relation between genes and disease condition. When health is viewed as one dimensional (i.e., considering only the physical dimension) and not acknowledging the influence of mental, social, and spiritual dimensions, then the common approach of finding out the cause of a disease is to look inside cells (or in the specific organ). In present time, the cause of the majority of the chronic diseases (like cancer, autoimmune disorders, diabetes, hypertension, etc.) has been attributed to different genes. People are made to believe that "genes control biology" and thus people are the victim of the genes that they have inherited from their parents. And the only way to health is through the use of industrially synthesized chemical drugs and/or surgical interventions. Emphasis is made on the ideology that DNA produces RNA and from those RNA proteins that are responsible for the structural and functional activities of a cell are produced. So, faulty gene produces faulty proteins that ultimately results in disease conditions (Bollinger 2016). The Darwinian ideology that "genes control biology" is still prevalent in the medical research. Charles Darwin in 1876 acknowledged and admitted that genes are controlled by the signals from surrounding environment (Darwin 1876). In the year 1990, Dr. H. F. Nijhout, in a paper titled Metaphors and Role of Genes and Development, presented evidence that the notion that genes control biology has been so frequently repeated for such a long period of time that the scientists have forgotten that it is actually a hypothesis and not a proven truth (Nijhout 1990). Dr. Bruce Lipton, a medical doctor and cell biologist, in his book *The Biology of Belief*, provided the experimental evidence that human beings are not the victim of their inherited genes, based on the studies performed on stem cells. Dr. Lipton concluded that while studying a diseased cell, the researcher must first look to the cell's environment and not inside the cell for the cause (Lipton 2016).

The 50 trillion individual cells that make up a human body have their own consciousness. Each of the cells performs its own functions and also works in synchronization with the other cells of the body, thus making life possible. The biochemical processes inside each cell of the body are inseparably interconnected with each other. The consciousness of the body control regulates all the biochemical reactions that are occurring inside each cell with the help of the brain, the nervous system, and the endocrine system. Keeping the homoeostasis of the body maintained at desired state is the sole aim of human consciousness. The control over the biochemical pathways gets disturbed when the mind is burdened with stressful thoughts. This loss of control over the biochemical pathways results in the production of a faulty molecule. This "single" faulty molecule holds the potential to disturb all the associated biochemical pathways just like the "domino effect." This disturbance in the synchronization among the biochemical pathways occurring in the body creates a condition inside the cell that forces a certain gene or group of genes to

mutate and express the abnormal protein and thus result in a clinical condition. Thus, a faulty signal from the environment forced the genes to express the abnormal protein. So, if the environmental signal is corrected, the brain, the nervous system, and the endocrine system get back their control, and the synchronization of the biochemical pathways gets restored, which results in cessation of expression of gene that was producing the abnormal protein. In scientific studies, the genes are "correlated" with a particular disease. It is to be understood that the word "correlation" does not mean "causation." Thus, having the gene does not mean that the person will get the disease. It is one's lifestyle and consciousness that determine whether that gene will be expressed or not. If the person does not have faulty lifestyle and negative consciousness, he may possess the gene but it will never express itself. Thus, it is clear that environmental dimension influences social and psychological dimension which modulates the body's immune response (Bollinger 2016; Dossey 2000; Hegde 2014; Lipton 2016).

11.5.3 Energy Controls Our Body

The conventional medical science believes that health is one dimensional and considers only the visible and measurable physical dimension (as the reality). Based on the belief, the abnormal measurable physical parameters are considered as the cause of any disease. All the diagnostic procedures detects only the abnormal physical parameters (based on predefined standards), and medications are used to bring the abnormal physical parameters into their defined desired range. So with the idea that abnormal physical parameters (symptoms) are the problem (disease), then by definition bringing back and keeping the physical parameters at their desired range should resolve the issue. But in reality, the issue is never resolved and the medications never stop. This is because the abnormal physical parameters (symptoms) are the result and not the cause of the disease. In conventional medical science, there is no cure for a disease, but only the symptomatic relief. The actual cause of the disease is beyond the physical dimension.

According to the conventional science, anything that is made out of matter is affected only by matter and not affected by energy. So that is why there is a concept of treating every abnormal physical parameter with industrially synthesized chemical drugs and/or surgery. The idea is that if a person is having an illness, then medicine and drug which is a form of matter should go into the system and adjust the physical body (thus the concept of "matter adjusting matter"). But what conventional science has left out of the equation is the energy. But according to wholistic science, the physical body is made up of energy (and not matter), because when an atom is taken apart, proton and electron are obtained. Electrons and protons are further broken down into bosons, quarks, and smaller particles and these particles are made out of energy.

The Nobel laureate Sir Hans Peter Duerr, in his theory of "aduality," demonstrated that matter is not made out of matter, but is composed of energy (invisible). According to this concept, the matter is not of particle nature

(reductionism) but is of energies or waves (wholism). Unlike matter, the energy cannot be separated, and thus we are not particles colliding with each other but energy waves interacting with each other, the phenomenon known as the quantum entanglement. And there is either positive energy or negative energy. When two energies interfere, one can change the power from destructive interference (state of disease) to constructive interference (state of health). So, when we leave the energy out of the equation we automatically looking at half of the influence, we are only looking at that "negligible" portion of energy that appears as matter. According to quantum physics and Sir Albert Einstein, the field "the physicist's term for energy" is the aquarium in which we are living in. It is impossible to eliminate the role of energy in the physical (biological) expression of the body. Thus, the state of health or disease is completely dependent on energies present in the environment (Dossey 1995, 2000; Goswami 2011; Hegde 2014; Lipton 2016).

11.6 Understanding the Immunomodulatory Effect of Psychology, Epigenetic Factors, and Natural Energies

In the above sections, we learned that regulation of immune response is not limited to our physical body, instead is strongly influenced by psychology and the environment. The psychological state of an individual and the response to surrounding environment influence the composition of blood (the components of physical dimension). When an individual has unhealthy perception or is under stressful situation (e.g., fear; anger; ego; greed; envy; lack of empathy; tendency to bring others down; suppression of emotions; feeling of disgust toward fellow beings; tendency to exploit, cheat, and take advantage of others; a constant sense of negative competition; as well as the tendency to escape from the unpleasant situation), he/she releases "toxic chemicals and mediators of inflammation" in the blood. The toxic chemicals spread into the whole body and begin to modulate the immune responses. This toxic chemistry created by the person's psychology and response to the environmental factor brings the entire system in a state of "fight or flight" and blocks the maintenance and repair mechanisms of the body (Peacefulness 2014a, b; Hegde 2014; Lipton 2016; Pert 1999).

In the state of spiritual (environmental) and psychological tranquility, the blood is concentrated toward the components of the body that provide the condition for the body to grow, repair, and maintain itself. So the energy (both in the form of matter and invisible energy) present in blood maintains and nurtures one's state of health. But when the spiritual and psychological homeostasis gets disturbed by one or combination of abovementioned factors of stress, the adrenal system starts taking over and maintains a "fight or flight" state at a systemic level. This state of systemic fight or flight redirects the blood flow into those organs that could be used to escape the situation or deal with the stress factor. So, when the "chemicals of stress and mediators of inflammation" are released into the blood, the blood vessels supplying blood to the gut shut down. When faced with a "stress factor" that is present outside

the body, the maintenance, growth, and repair mechanisms of the body become less important (Hegde 2014; Lipton 2016; Pert 1999).

When the adrenal system creates a state of systemic "fight or flight," the consciousness of the body starts conserving energy by shutting down all the mechanisms that are not necessary for a fight or flight response. One of the major mechanisms that get shut down is the "activities of the immune system." And this is very vital because the usage of the energy by the immune system is so high it would interfere with fight or flight response. So, not only does the "negative psychological state" redirect the flow of blood away from the components, which maintains our body but also suppress the immune system which is protecting the interior of the body (natural immunomodulation—immunosuppression) (Peacefulness 2014a, b; Dossey 2000; Goswami 2011; Hegde 2014; Lipton 2016; Pert 1999).

The human body is made up of about 50 trillion individual cells that came together and created a community where they survive together in harmony and maximize the functional capabilities and intelligence of the system. So an individual is actually a community of 50 trillion cells working in a synchronized manner. It is very important that one must let go of the individuality (the feeling of "I") and acknowledge the fact that we are all cooperative, integrated communities coming together in the bigger structure called society (the feeling of "We"). Just like 50 trillion individual cells made one human body in the same way 7.8 billion people make up the bigger body called society (social dimension). There have been scientific studies that show what we do in our society, the same behavior is reflected by the cells that make up our individual body. For example, human beings have been fighting and trying to destroy each other for a long time; this mindset is progressively destroying the society or social well-being. Now if we observe the physical dimension of an individual, the cells of their body try to destroy other cells of the body (self, attacking self), a condition which in medical terminology is known as autoimmunity (natural immunomodulation – hyperactivity or hypersensitivity). The way we are destroying the society (even after being a fundamental part of it), in the same way the cells that is the fundamental part of our body is destroying us. And all these processes are guided by the way each individual thinks. So when we diagnose a patient, we must understand that what we are looking at is a community (of cells) and not a single person (Luucid 2015; Dossey 2000; Goswami 2011; Hegde 2014; Lipton 2016; Pert 1999).

It is evident that unhealthy perception or unpleasant situation (collectively called as stress) is the cause of 90% of the chronic diseases. So changing and replacing these kinds of thoughts with the sense of love, honesty, integrity, compassion, kindness, humility, respectfulness, and above all the feeling of "We" produces the positive psychological state. Positive psychological state reverses the ill effects of negative psychological state back to a healthy vital system, where the blood flows back into the components maintaining the body and the immune system is activated again (natural immunomodulation–positive immunoactivation). And following this process, we can then have a life of full health and happiness (Luucid 2015; Dossey 2000; Goswami 2011; Hegde 2014; Lipton 2016; Pert 1999).

11.7 Conversion of Acute Immune Dysfunction into Chronic State

From the concepts presented in the previous sections, it is evident that psychological and epigenetic factors directly regulate the immune response. It is now important to understand the ways by which a state of acute immune dysfunction gets converted into a chronic state. When a person escapes from the situation of acute or momentary stress that is triggering the negative psychological state (like anger, fear, envy, ego, etc.), then after a certain amount of time, the system returns back to normal. But in cases where a person is forced to be in the situation, that is, triggering the negative psychological state (as mentioned earlier) as a part of regular activity like a routine (i.e., the chronic stress), then chronically it is shutting down the maintenance of the body which opens us up to the infections and inflammations and all of the other problems. If the growth and maintenance of the body are shut down for a short duration of time (as occurs in cases of acute stress), it does not cause any major issue, but the negative psychological state (results in chronic stress) in the present society is present "24/7/365." Consequently, the body becomes sick and starts to fall apart, and now turns out that stress, the problem that a negative psychological state generates, is responsible for up to 90% of all diseases. So, the thinking that genes were causing the disease is not true; abnormal gene expressions are the consequences of the stress (caused by negative psychological state) and chemical consequence of that shutting down growth, maintenance, repair, and immune system and open up the body for failure. This clarifies the fact that a positive psychological state nurtures the state of "health," and the negative psychological state nurtures the state of "disease." Thus, chronic illnesses are the direct reflection of the psychological state of the individual, and when the cause of the disease is in the mental dimension, treating the results observable in the physical dimension with industrially synthesized chemical drugs or surgery will never be able to eradicate the actual "disease" and restore the state of "health" (Hegde 2014; Lipton 2016; Pert 1999).

11.8 Reanalyzing the Example of Rheumatoid Arthritis (RA)

Let us now understand the example of rheumatoid arthritis (RA) by taking the influence of energy, epigenetics, and psychological aspects under consideration. The hallmarks of rheumatoid arthritis (RA) are inflammation of joints, tenderness, swelling, morning stiffness, fatigue, fever, loss of appetite, and weight loss followed by the destruction of cartilages (Walker 2014). The consequences of forceful and opposing interaction between natural healing mechanisms (Natural Immunomodulation) and artificial immunomodulators are as follows:

11.8.1 Inflammation

It is fundamentally a protective response (Abbas et al. 2004). It is the body's defense mechanism. Anti-inflammatory and immunosuppressive drugs suppress the fundamental protective response of the body by acting against the natural healing processes. While addressing one disease, the body is made defenseless unknowingly (Bollinger 2016; Hegde 2014; Otto 2020).

11.8.2 Tenderness

It means sensitivity to pain. Tenderness is another feature of inflammation. Pain is a protective mechanism of the human body which indicates that the organ needs rest and extra care. Pain response is meant to protect us from causing further damage to the organ under consideration (Abbas et al. 2004). Multiple doses of painkiller suppress the pain and provide a false idea that the condition is cured. Since there is no feeling of pain because of the action of drug, the person keeps on putting pressure on the injured organ, thus making the injury worse, converting the reversible damage to the irreversible state (Bollinger 2016; Hegde 2014, Otto 2020).

11.8.3 Swelling

It means abnormal enlargement of the body part. Swelling is a feature of inflammation. It is the accumulation of mediators of inflammation at the site of injury and indicates the human body is trying to heal itself (Abbas et al. 2004). Anti-inflammatory and immunosuppressive drugs block inflammatory response and inactivate circulating immune mediators. The swelling disappears because of the action of drugs, and at the same time, the immune system is kept inactive forcefully (Bollinger 2016; Hegde 2014, Otto 2020).

11.8.4 Morning Stiffness

It is a condition created as a result of pain and swelling that forces the restriction of movement. Momentary restriction of movement is a mechanism to remind the human system that the healing process is in progress and restriction of movement is needed. Again in this situation, the painkillers are administered which gives a false idea that the injury is cured because the patient no longer feels the pain. The patient makes the movements freely but the thing that we do not realize in doing so is we have disturbed all the ongoing healing (Bollinger 2016; Hegde 2014; Lipton 2016; Otto 2020).

11.8.5 Fatigue

The entire energy of the body is being utilized to repair and maintain the damaged organs. Very few energy is spared for other activities that are not necessary for the repair and maintenance mechanisms. It is indicative that the person must take adequate rest as the internal healing is in progress (Bollinger 2016; Hegde 2014; Lipton 2016; Otto 2020).

11.8.6 Fever (Hyperthermia)

It is not a disease but a protective mechanism. Fever means acute systemic inflammation. The human body's intelligence triggers fever to neutralize possible threats. Fever means the immune system is active. Providing anti-inflammatory, anti-pyretic drugs and antibiotics not only suppresses the immune response but also destroys the gut microflora leading to increased susceptibility to opportunistic infections (Bollinger 2016; Hegde 2014, Otto 2020).

11.8.7 Loss of Appetite and Weight Loss

Loss of appetite is also the body's protective mechanism. Digestive processes require a huge amount of energy. When the human body is in repair and maintenance mode, all the energy is directed toward the healing process. The body intelligently turns off all those responses that may take away a huge portion of energy unnecessarily. Hunger is not a priority, survival of the organism is. It is to be noticed that there is "increased thirst" indicating nutrients and food to be consumed in liquid form that does not need to be digested at the same time are readily absorbable by the body. This may lead to momentary weight loss but is very much essential for healing. During fever when there is a loss of taste, consuming medications to increase hunger causes immunosuppression indirectly (Lipton 2016).

11.8.8 Destruction of Cartilage

It is the pathological condition which is the result of our incomplete understanding about the disease condition and inappropriate treatment methods like forced immunosuppression. Prolonged use of anti-inflammatory, immunosuppressive medications and biological response modifiers nurtures the disease insidiously by forcefully shutting down the body's natural healing mechanisms (Bollinger 2016; Glidden 2012; Hegde 2014; Lipton 2016; Null 2011; Otto 2020).

Administration of multiple industrially synthesized chemical drugs (artificial immunomodulators) like NSAIDs, steroids, DMARDs, and biologic response modifiers suppresses the body's immune response (forcefully) by blocking the signal transduction pathways and neutralizing the active immune mediators in the

bloodstream. Because of the action of artificial immunomodulators, the patient does not feel the pain or the discomfort and thus believes that the disease is cured. But as soon as the effect of medications fades away, the pain and discomfort returns and with a much higher intensity as compared to pain and discomfort felt before starting the medications. The power and dosage of the medication are increased after the subsequent visit and the process continues. It is to be understood (as mentioned earlier) that inflammation is not a disease but a body's natural defense response. It is a natural process that the body supposed to perform if it encounters any situation or substance that might harm the body system. The elevated levels of molecules and cells of the immune system are only the result, the actual cause of which is not present in the physical body. So, the "artificial" immunomodulators are only suppressing the results, while the actual cause remains undetected and unaddressed. These "artificial" immunomodulators are known to cause gastrointestinal, cardiac, and renal dysfunction, thinning of bones, weight gain, diabetes, liver damage, bone marrow suppression, severe pulmonary infections, abnormal blood clots, and a constant state of forced immunosuppression. The chronic diseases that follow rheumatoid arthritis like osteoporosis, rheumatoid nodules, infections, cardiac diseases, pulmonary diseases, cancer, etc. are the direct result of the adverse drug reactions caused by the "artificial" immunomodulators. For treating one clinical condition, a patient is subjected to all these kinds of life-threatening diseases. When the medications fail to suppress the symptoms, the surgical procedures are performed.

Let us understand the consequences of surgical procedures. Removing a part from the body leads to disturbance in the chain of functioning, and replacing certain parts with prosthetics made up of metal or plastic creates additional inflammation because the system never accepts that as self. To make the transplanted prosthetics acceptable, another range of immunosuppressive and anti-inflammatory drugs are prescribed. These processes do not solve the issue but replace one set of anti-inflammatory and immunosuppressive medications with another set. The inflammation that occurred as a result of the pathological state is replaced with inflammation induced by surgical procedures, and the medication continues for a lifetime. Unnecessary prescription of multiple industrially synthesized chemical drugs to a patient at a given point of time to manage symptoms, during which the dose and amount of chemical drugs keep increasing exponentially with time, interferes with normal biochemical pathways in patient's body by creating unwanted molecular crowding and thus damaging the organs in the long term. Considering critical factors that relate health with the environment can help minimize the adverse drug reaction and create drugs that are less toxic to the body when taken for a long period of time (Bollinger 2016; Glidden 2012; Hegde 2014, Lipton 2016; Null 2011; Otto 2020).

11.9 The Conclusion

From the discussions made in the above sections, it can be realized that administering artificial immunomodulators to a patient with immune dysfunction such as rheumatoid arthritis (autoimmune diseases) without considering the

influence of energy, psychological, and epigenetic factors leads to derangement of the overall homeostasis of the body. For developing effective treatment procedures and safe medicines, it is essential to consider the system as a whole and not in bits and pieces. It is very important to analyze a situation from both a reductionistic and holistic point of view; only then it is possible to understand the root cause of the ailment. From the concepts mentioned, it is also clear that health is not one dimensional but multidimensional, and all the dimensions are inseparably interconnected with each other. By not acknowledging the mental, social, and spiritual dimensions, we are performing scientific studies with incomplete knowledge, and because of the incomplete knowledge, we are treating the results as the cause of the disease. The understanding and implementation of concepts like epigenetics, psychoneuroimmunology, and quantum physics (or the science of energy) along with the conventional ideology are very much essential not only for the practice of medicine but also for designing and administration of immunomodulatory drugs.

Thus, it can be concluded that health is not one dimensional but a multidimensional component. There are factors beyond the physical body that directly regulate the functioning of the immune system. By considering health as a multidimensional concept, one can develop a complete understanding of the ways by which nature actually functions. Through careful observation, it will be clear that the mutated genes, faulty proteins, and molecules are the (internal) components of the immune system and are the part of the physical body, and the external factors that are regulating these internal components are coming from outside the physical body. Acknowledging the existence and importance of natural immunomodulators while designing a treatment and developing therapeutics will not only reduce the chances of forceful suppression of natural healing mechanisms but also minimize the chances of adverse drug reactions or drug-induced immune dysfunctions. The aim should be the production of immunomodulatory drugs that acts by supporting the natural healing mechanisms of the body and not against it, because healing is done only by the body's intelligence and not by any drugs or surgery. As the Father of Medicine, Great Hippocrates said:

> Natural Forces within us are the true Healers of Disease. The Physician treats, but Nature Heals.

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Herbal Immunomodulators and COVID-19

12

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Abstract

Novel Coronavirus (COVID-19) globally affects the people's health and social life, and it became a challenging task for pharma and research communities. Several medical research or scientific institutions are trying to develop potent antiviral vaccine/drugs against the coronavirus. There are urgent needs to explore all the possibilities against the pandemic disease, and, among that, it is well cited in many literature that Ayurveda has an important role since ancient time against many viral diseases. The Ayurvedic medicinal system is mainly based on herbal formulations, which boost the immune system or work synergistically to protect our body against invading harmfull micro-organisms. The herbal medicinal system has identified several herbs used in various home remedies. It is thought to effectively fight corona and improve health immunity; the current chapter describes the therapeutics of plants *Phyllanthus emblica*, *Azadirachta indica*, and *Swertia chirata* in the current scenario against COVID-19.

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12.1 Introduction

Immunity is an intricate cell biological process and is also known as the body's natural defense system. It describes the composition of different immune cells such as white blood cells, being capable of distinguishing between proteins or cells of the body and foreign entities (Baxter 2007), and also neutrophils, lymphocytes, macrophages, and monocytes, as well as cytokines (Oberlies and Kroll 2004; Baxter 2007; Moradali et al. 2007; Vesely et al. 2011; Lai et al. 2020; Shereen et al. 2020; Pal et al. 2021). According to research, the immune system can be divided into two different categories: innate (non-specific), immune (non-specific), and adaptive immune system (Oberlies and Kroll 2004; Baxter 2007; Jantan et al. 2015; Shereen et al. 2020; Pal et al. 2021; Subhash et al. 2021).

Innate immunity is linked with several obstacles or barriers, including bacteria, chemicals, and physics. Pattern recognition receptor (PRR) protection biochemical cytokines, acute-phase proteins, macrophages, monocytes, complement, and neutrophils are among the other critical defense system mediators that are rapidly distributed, even if pathogens came into the human body (Jantan et al. 2015; Kumari et al. 2021). The pathogens express very specific molecular patterns known as pathogen-associated molecular patterns (PAMPs) (Subhash et al. 2021), which are recognized by the host to trigger or activate the immune system by releasing different resistant molecular immune system components (another type I interferons, chemokines, and cytokines) to alarm the body health system. Other immune system moieties including cytokines and nitric oxide (NO) are secreted after the activation of the immune system (Subhash et al. 2021; Kumari et al. 2021).

Every community of the world faces unprecedented health social and economic problems and shocks in the form of a novel COVID-19 caused by the coronavirus called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Kanika et al. 2021). In Wuhan City, Hubei Province, China, COVID-19 was first observed, along with an outbreak of respiratory sickness. COVID-19 is animal-communicable disease, and it is prevalent since it spreads from a single source to almost every corner of the globe (Wuhan, China). According to doctors and experts, the signs are very similar to pneumonia and are known as novel coronavirus 2019 (Mohamad et al. 2020). On December 31, 2019, it was first reported to the WHO, and on January 30, 2020, it was announced COVID-19 was pandemic. COVID-19 is currently spreading around the world in less than 90 days. It has wreaked havoc on significant health facilities (vaccination, drug screening, identification, and diagnosis), as well as disease prevention and management (Van Paassen et al. 2020; Khanna et al. 2021).

SARS-CoV has a similar nucleotide sequence to bat coronavirus. Its spike glycoproteins have the highest affinity toward human angiotensin-converting enzyme 2 (ACE-2) receptors (to conquer and transmit through humans to humans). Reports have shown that viral infection, or COVID-19, only affects those with a weakened immune system, making them more vulnerable to the disease and its worst consequences (Tooze and Tooze 1985; Gallagher and Buchmeier 2001). As of May 11, 2021, COVID-19 had infected more than 220 countries and territories across the world, resulting in more than 160,080,091 confirmed cases and 3,324,912 deaths (Ren et al. 2020; Kumari et al. 2021).

According to some phylogenetic evolutionary studies, coronavirus shares a nucleotide with SARS-CoV, and MERS-CoV bats (Banik et al. 2020; Moradali et al. 2007; Kanika et al. 2021). As a result, there are no effective drugs available in the market for the treatment of COVID-19. However, scientists and researchers are working incredibly hard to find the best way to relieve symptoms and avoid COVID-19, including the use of herbal medicine. Patients' immune system plays a significant role in infection with COVID-19, an herbal immunomodulatory drug that may have a possible therapeutics on patients with COVID-19 disease, according to recent research (Ren et al. 2020; Zhou et al. 2020).

12.1.1 General Overview of Coronavirus and Its Structure

The coronaviruses (CoVs) belong to the family *Coronaviridae* (two subfamilies: *Coronavirinae* and *Torovirinae*) and genus *Coronavirus* and the order *Nidovirales* (Zhou et al. 2020; Kumari et al. 2021). They are enclosed and have a non-segmented, single-stranded, positive-sense ribonucleic acid (ssRNA+) as their genetic material (Kumari et al. 2021). Several scientific studies have been found after electron microscopy investigation viruses have a characteristic look that resembles a crown (Latin corona which means crown) due to the presence of club-shaped surface protein projections (Ben-Efraim 2001; Durrani et al. 2008; Chowdhury et al. 2012; Lai et al. 2020). The CoVs are pleomorphic, measure between 80 and 160 nm in length, and feature a tiny genome of 27–32 kilobytes (KB) with a unique replication method (Zhou et al. 2020).

Although the *Coronavirinae* subfamily includes four genera of viruses (*Alphacoronavirus* (α-Cov), *Betacoronavirus* (β-Cov), *Gammacoronavirus* (γ-Cov), and *Deltacoronavirus* (δ-Cov)), which have been grouped primarily based on serology and phylogenetic clustering (divisions based on the genetic relatedness), the former two genera (α-Cov and β-Cov) typically infect mammals, whereas the latter two (γ-Cov and δ-Cov) mainly infect birds and animals (Kumari et al. 2021). In addition, there are seven CoV species reported to infect human people (Kumari et al. 2021). Among these, only MERS-CoV and SARS-CoV have been able to cause severe human disease. The rest are related to moderate respiratory disorders such as the common cold. However, they may produce catastrophic repercussions in immunocompromised patients. At present, the medical severity of the nCoV-2019 (nCoV-19) is precisely unclear but life-threatening, and deaths have been related to the infections (Kumari et al. 2021).

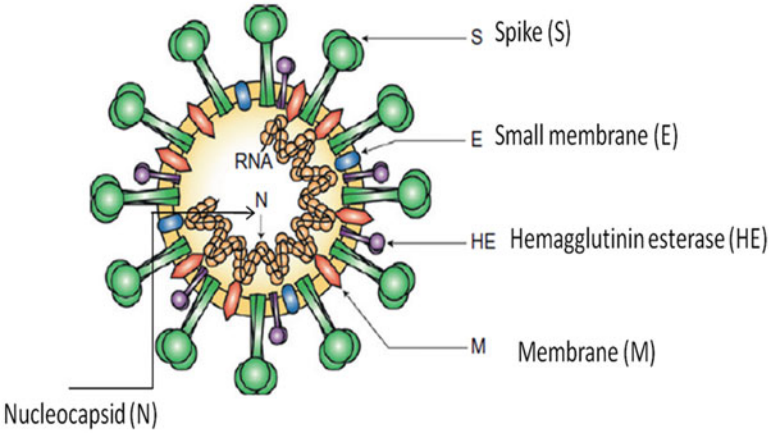

Fig. 12.1 Structure of coronavirus (the RNA genome is complexed with the N protein; HE, hemagglutinin esterase; S, spike; E, small membrane envelope; M, membrane; and transmembrane proteins and forms a spiral enclosure within the viral membrane)

Figure 12.1 is showing the coronavirus structure that has been studied which is known as corona due to its crown-like morphology; the spike protein trimers form peplomers embedded in the envelope, which give the virion its crown-like shape. A few CoVs, such as the HE protein, have smaller membrane spikes; M and E are transmembrane proteins involved in the assembly of CoVs (Kanika et al. 2021; Kumari et al. 2021). According to reports, CoVs have several proteins which perform different tasks, like the S; the glycoprotein is the receptor-binding protein; it can infect and target neutralizing antibodies (Bassetti et al. 2020; Li and De Clercq 2020; Subhash et al. 2021; Kanika et al. 2021; Kumari et al. 2021). An intensive report shows that SARS-CoV-2's only S-protein contains a furin-like cleavage site at the S1–S2 junction and might be responsible for the greater pathogenicity of SARS-CoV-2 (causes high infectious) (Klumperman et al. 1994; Krijnse-Locker et al. 1994; Finlay and Hancock 2004). Similarly, the M glycoprotein involves virus assembly at intracellular membranes in the region of the endoplasmic reticulum Golgi complex (ERGIC), whereas the E protein is a minor component of the viral envelope (Verma et al. 2007; Li and De Clercq 2020; Huang et al. 2020; Subhash et al. 2021; Kanika et al. 2021; Kumari et al. 2021).

12.1.2 Interaction Between the Immune System and COVID-19

As shown in Fig. 12.2, COVID-19 has impacted many countries worldwide and increased the mortality rate. According to clinical studies, these deaths were suspected because of the "cytokine storm" (CS) or cytokine storm syndrome (CSS) or "cytokine release syndrome." It is not included in the International Classification of Diseases (ICD) (Tang et al. 2020). At the same time, Cron and Behrens

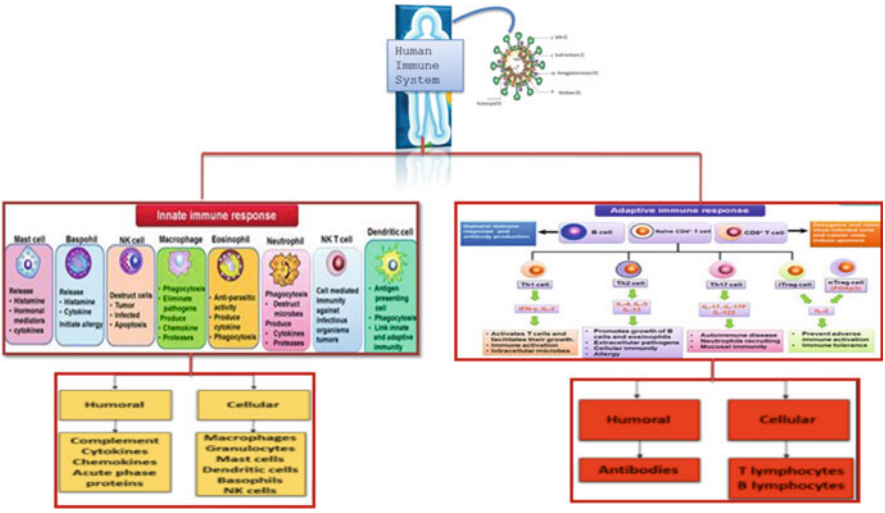

Fig. 12.2 Depicts the human immune response to COVID-19 or SARS-Cov-2, which includes two forms of immune responses: innate (providing early protection against invasive pathogens) and adaptive (providing late protection against invasive pathogens) (later and more effective response against any of infections) (Andhavarapu and Roy 2013; Gautret et al. 2020; Kanika et al. 2021; Kumari et al. 2021)

explained about CS "Activation of auto-amplifying cytokine or proinflammatory synthesis due to unregulated host immune response to various triggers" (Huang et al. 2020).

Clinical research reports have supported that the numbers of WBC (white blood cells), neutrophils, CRP (C-reactive protein), procalcitonin, and various other inflammatory or cytokine secretion (IL-6, IL-7, TNF-alpha) are significantly increased in the severe case of COVID-19 (Ademokun and Dunn-Walters 2001; Patil et al. 2012; Andhavarapu and Roy 2013). The effect of the bronchoalveolar lavage fluid (BALF) cells is the release of excessive chemokines such as CXCL10 and CCL2, which is admitted patients in the intensive care unit (ICU) (Patil et al. 2012; Andhavarapu and Roy 2013).

SARS-CoV-2 viruses are involved in triggering the immune system as well as the innate and adaptive immune systems; lead to the uncontrolled production of cytokines; ultimately cause CS; are responsible for the apoptosis of epithelial and endothelial cells and also vascular leakage, affecting many organs; and, at last, cause acute respiratory distress syndrome (ARDS) (due to an increase in the number of T-helper (Th) 17 cells and the high cytotoxicity of the CD8+ T cells) and even death (Patil et al. 2012; Andhavarapu and Roy 2013; Gautret et al. 2020).

Numerous immune-response studies have supported, similarly, other immune-associated diseases (allergic diseases and cancers, COVID-19 is also associated with inflammation (Gautret et al. 2020; Cynthia et al. 2020) and as like this mechanism

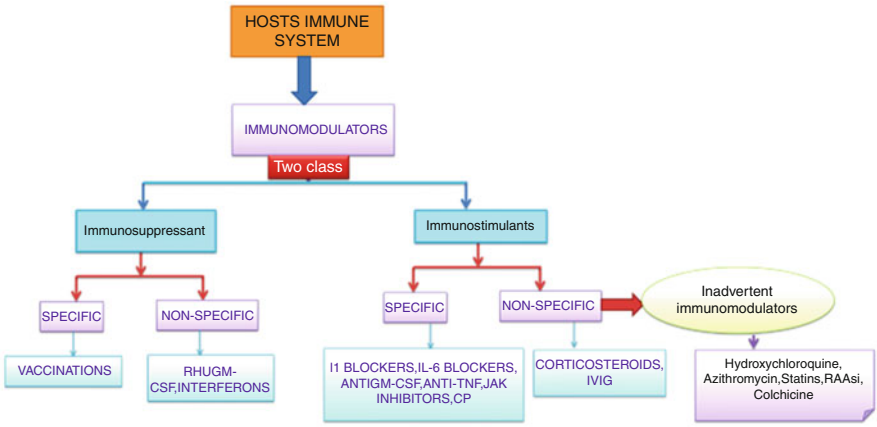

Fig. 12.3 Immunosuppressants and immunostimulants like immunoadjuvants

several other immune machinery processes are utilized to keep the body in homeostasis (in the restoring immune balance of body) (Andhavarapu and Roy 2013).

There are a variety of immunomodulators that are responsible for modulating the immune system (some are naturally occurring, although most are synthetic chemical-based due to high toxicity, low bioavailability, and stability issues) (both innate and adaptive) (Gautret et al. 2020; Cynthia et al. 2020; Kumari et al. 2021). It can be divided into two groups (Fig. 12.3), immunosuppressants and immunostimulants like immunoadjuvants (Gautret et al. 2020) and its growing interest in discovering many natural immunomodulators to boost and prevent resistance to infectious and also non-infectious diseases. It is also to deal with many immune disorders of the host by optimum modulation of both immune responses (Andhavarapu and Roy 2013).

12.2 Traditional Herbal-Based Immunomodulators' Cure for COVID-19: A Possible *Rasayana Chikitsa*

Several vaccines and antiviral drugs such as hydroxychloroquine ($C_{18}H_{26}ClN_3O$), favipiravir, ritonavir, oseltamivir, lopinavir, ganciclovir, remdesivir, and azithromycin have significant therapeutics to control viral infection, and still no approved vaccines with 100% efficacy exist against COVID-19 (Huang et al. 2020; Kumari et al. 2021). Various clinical reports of these chemicals, like lopinavir/ritonavir (400/100 mg 12 hourly), chloroquine (500 mg 12 hourly), and hydroxychloroquine (200 mg 12 hourly), have shown their antiviral activities, and they can be used against COVID-19 (Lai et al. 2020) but associated with minimal effect. According to WHO guidelines, few safeguards from COVID-19 diseases, such as social distancing, quarantine, and isolation of a suspected or infected person,

are the best tools together with maintaining proper hygiene, frequent handwashing with alcohol-based sanitizers, and adequate wear for a mask (Kanika et al. 2021).

According to WHO, various anti-coronavirus vaccines (like Covaxin Vaccine and Covishield) are currently developed after preclinical and human clinical trials (Lai et al. 2020; Shereen et al. 2020; YD et al. 2021). As per the recent report, 330 vaccine candidates are in various stages of development as of July 2021, with 102 in clinical trials, including 30 in Phase I trials, 30 in Phase I–II trials, 25 in Phase III trials, and 8 in Phase IV development (*vac-lshtm.shinyapps.io*. 2021). It could take months or even years to develop these vaccines. Despite international efforts to control coronavirus infection and prevent it from spreading around the world, in the absence of clinically proven prophylaxis and therapeutic strategy, we must look into alternative therapies that should be actively researched without wasting time (Patil et al. 2012; Andhavarapu and Roy 2013; Tillu et al. 2020; Cascella et al. 2021; Kumari et al. 2021; Rastogi et al. 2022).

Clinical studies, traditional evidence, current scientific studies, and our experiential experience have all indicated that herbal drugs or herbal-based immunomodulators play an essential role in preventing various infections (Lai et al. 2020; Shereen et al. 2020; Kumari et al. 2021). Traditional studies, which provide a detailed description of the disease's cause and treatment (Janapadodhwamsa), make herbal interventions more common, if not essential, at this time. The majority of research and development is still focused (Neem turmeric in treatment of COVID-19 2020; Akhtar et al. 2019) on biochemical, biological moieties, or single compounds as lead compounds that target specific COVID-19-related targets in different parts of the world (Moradali et al. 2007; Vesely et al. 2011; Shereen et al. 2020; Rastogi et al. 2022; Gautret et al. 2020; Cynthia et al. 2020; Kumari et al. 2021). Research and development of herbal compounds with high selectivity, ability, and low toxicity for molecular or cellular targets, as well as COVID-19 (Moradali et al. 2007; Vesely et al. 2011; Rastogi et al. 2022; Subhash et al. 2021; Kanika et al. 2021; Kumari et al. 2021).

Ancient reports have been designing and creating herbal drug candidates to gain interest or worldwide recognition from the source of alternative medicines. Several plants or their components have been used as herbal medicinal products for a variety of diseases, (Serafino et al. 2008; Sadlon and Lamson 2010; Nain et al. 2012; Jantan et al. 2014) like *vinblastine*, *vincristine*, and semi-synthetic derivatives isolated from *Madagascar periwinkle* (*Catharanthus roseus*), *capsaicin from chili peppers* (*Capsicum* species), *paclitaxel from Pacific yew* (*Taxus brevifolia*), and g*alantamine* from the *Caucasian snowdrop* (*Galanthus caucasicus*) are examples of medicines based on plant compounds (Vesely et al. 2011; Gautret et al. 2020). WHO has announced that some plant-based immunomodulatory medicine is under preclinical trials and clinical trials (Chakraborty et al. 2020). Simultaneously, the AYUSH Ministry in India has suggested using herbal components as preventative measures for health and immunity enhancement, specially respiratory health or COVID-19 (Guha et al. 1996; Ifeoma et al. 2013; Harikrishnan et al. 2018a; Harikrishnan et al. 2018b; Gautret et al. 2020; Cynthia et al. 2020; Subhash et al. 2021).

In this context, scientists have announced the use of immune-enhancing herbs that might also strengthen the body's defense mechanism against COVID-19

infection or prevent it from reproducing in the host cell. COVID-19 prevention, control, and treatment using plant-based herbal medicines have been recorded throughout human history and ancient cargo space history (Patil et al. 2012; Andhavarapu and Roy 2013). A few other studies show that herbal medicines with immunomodulatory properties stimulate both specific and non-specific immunity (Cynthia et al. 2020; Kumari et al. 2021). More than 55 plants are classified as Rasayanas in the Ancient Indian medicine system, with various pharmacological properties such as immunostimulant, tonic, neurostimulator, antiaging, antibacterial, anti-rheumatic, anticancer, adaptogenic, and anti-stress. Ayurveda's Materia Medica has an entire portion dedicated to herbal medicine called "Rasayana," which enhances body resistance (Pandey 2019; Cynthia et al. 2020; Ranjan et al. 2020; Subhash et al. 2021).

According to Ayurveda, "Vyadhikshamatva" means combating a specific infectious or contagious disease. Such medicine is known as self-explanatory "Vyadhibalavirodhitvam," and "Vyadyutpadapratibandhakatvam" means battling a specific infectious or communicable disease, and such a medicine offers resistance to the loss of dignity, proportion, (Kirtikar and Basu 1984; Mirazimi et al. 1996; Kher and Chaurasia 1997; Kuttan and Harikumar 2011; Li et al. 2020) and the interrelationship between the Dosas and Dhatus Dosas of humans (Pandey 2019; Ranjan et al. 2020). Several plants are documented with their possible immunomodulatory effects, and some of them have already been studied (in vivo and in vitro) (World Health Organization 2020) to determine their pharmacological properties. Some are also in the clinical route approved by the AYUSH Ministry in India (Singh and Verma 2012; Guo et al. 2020).

Scientific evidence have declared that the immune system will be changed with growing age, called immunosenescence, and reported such differences in refractory responses to vaccination or infection, reductions in protective immunity previously developed, and increased morbidity of the disease. B cells play a key role in developing and maintaining protective immunity, including the production of protective antibodies, the presentation of antigens, and, more recently, the recognition of regulatory functions (Guo et al. 2020; Kanika et al. 2021).

This current chapter describes the immune dysfunctions that pose a significant problem associated with severe thermal injuries, trauma, and sepsis and cause severe lethal infections. Consequently, these defects are repaired by the use of herbal immunomodulating agents (Opstelten et al. 1995; Nahar et al. 2011; Plants of the World Online 2017; Dilipkumar and Nayak 2021). We assumed that we could encourage human health benefits by using herbal immunomodulators and restore the body's balance and provide the body with sufficient energy to combat COVID-19. Here we offered an analysis of a few immune-boosting herbs or plant-based immunomodulators (*Phyllanthus emblica*, *Azadirachta indica*, *Swertia chirata*) and their significant characteristics with evidence of their antiviral activities and natural immunomodulatory products or their extracts (Fig. 12.4). Their immunomodulatory bioactive moieties may provide immunomodulatory effect or with applicable entities to develop as novel immunomodulatory agents to treat COVID-19.

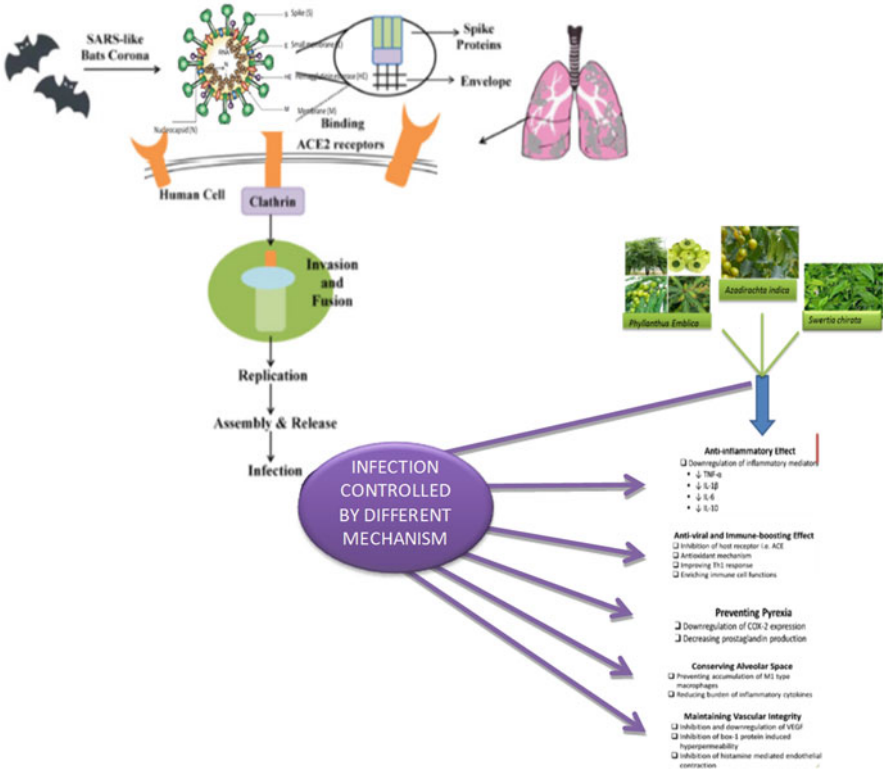

Fig. 12.4 Herbal Immunomodulators with their potential mechanisms (act as antiviral, immune-boosting, vascular integrity) for the treatment of COVID-19

12.2.1 Phyllanthus emblica (Commonly Known as Amla)

Phyllanthus is one of the largest genera and belongs to the family of Phyllanthaceae with 11 sub-genera like *Bortryanthus*, *Cicea*, *Conani*, *Emblica*, *Ericocus*, *Gompidium*, *Isocladus*, *Kirganelia*, *Phyllanthodendron*, *Phyllanthus*, and *Xyllophylla*. *Phyllanthus* have 1270 species and are found in the tropical, subtropical, African, and American tropical regions (Caribbean and Brazil) and also Asia and tropical Oceania (Singhal 2007; Sharma and Bhagwan 2013a, b, c; Subhash et al. 2021).

Phyllanthus emblica (*P. emblica*—synonym: *Emblica officinalis Gaertner*) is scattered all over India's tropical and subtropical regions. *P. emblica* is a deciduous (small to medium in size, its height 1 to 8 meters) tree. Its flowers are green with yellow and have a fleshy spherical shape (1.5 cm diameter-color light greenish-yellow, very smooth with hard) fruit (Kanika et al. 2021).

From the ancient period in India, for the treatment of the different spectra of diseases including infectious disease, *Phyllanthus* species (*P. amarus*, *P. emblica*, *P. niruri*, *P. reticulatus*, *Phyllanthus corcovadensis*, and *P. fraternus*) have been used also utilized for the enhancement of the immune system (Sharma 2013).

Several species of *Phyllanthus* are used to treat and manage various clinical issues like genitourinary, hypertension, different cancers, skin problem, gastric issue, and hepatic and respiratory illnesses and valuable for diuretic. Globally, its species are used as an anti-inflammatory, antiviral, antiseptic, antiplasmodial, anticancer, and antimalaria and to also control body temperature, etc. (Singhal 2007; Michael et al. 2009; Sastry 2010; Sharma and Bhagwan 2013a, b, c). Mainly, its five species are widely used in the treatment of anuria, dropsy (swelling), hypertension, sore throat (painful, dry/scratchy feeling), and hepatitis B, including blood- and bile-related disorders (Ghaisas et al. 2009).

Many studied have suggested that its ethnomedicinal and therapeutic potential properties due to the presence of tremendous bioactive molecules like lignans, triterpenes, sterols, alkaloids, flavonoids, ellagitannins, polyphenols, and other molecules—corilagin, geraniin, gallic acid, phenylpropanoids hypophyllanthin, ellagic acid, phyltetralin, niranthin, catechin, quercetin, astragalin, and chebulagic acid (Unander et al. 1995; Calixto et al. 1998; Patel et al. 2011; Jantan et al. 2019).

In vitro and in vivo experiments have illustrated the immunomodulatory activity of different *Phyllanthus* species, and a few phenolic compounds, able to inhibit MPO, MDA, and NF-κB translocation as well as the development of reactive oxygen species, proinflammatory cytokines, and nitric oxides. Consequently, with the reported possible mechanism, *Phyllanthus* species show an immunomodulatory effect (Adil et al. 2010; Sarin et al. 2014; Subhash et al. 2021).

12.2.2 *Azadirachta indica* (Neem, Nimtree/Indian Lilac)

Azadirachta indica (AI) or neem (Nimba is a Hindi word derived from Sanskrit) is a tree (genus *Azadirachta*) in the Meliaceae family. It can be found in India's tropical and semi-tropical regions, island areas, Pakistan, Bangladesh, and Nepal (Tan and Vanitha 2004; Sadekar et al. 1998; Sultana et al. 2007; Panyod et al. 2020; Mandal et al. 2021). Neem oil can be found in the fruits and seeds of the neem tree. Various of its products play an essential role in disease prevention, control, and treatment by enhancing anti-oxidant activity, inhibiting bacterial expansion, and modulating the expression of genetic pathways, among other mechanisms (Arumugam et al. 2014; Barstow and Deepu 2018).

Azadirachta indica (*AI*) is very similar to Chinaberry or *Melia azedarach*, an evergreen, fast-growing plant, and most have a height of 12–20 m, rarely 35–40 m, and its seed is roundish. The Chinaberry (*Melia azedarach*), a direct descendant of the neem tree, has a similar appearance (Henry and Burnell 1996; Compact Oxford English Dictionary 2013). They are essential in the treatment of infectious diseases. The fruits, which have a smooth or glabrous olive drupe shape and are 1.4–2.8 cm in diameter when ripe, are a good source of antibacterial oil (Govindachari et al. 1998; Encarta World English Dictionary 1999; Brahmachari 2004; Ketkar and Ketkar 2004).

In a traditional medicinal system, like Ayurveda, Unani, and homeopathy, and now also in modern medicine, whole neem and its different parts have been utilized to treat various infectious diseases, metabolic disorders, and cancer. Several studies have supported that its therapeutic role in various disease prevention and treatment;

still, it is an enthusiastic research point due to the presence of tremendous active molecules (Singh and Sastry 1997; Arora et al. 2011; Subhash et al. 2021). Also, it is effective in different preparation which may be single preparation or poly-preparation formulation in different forms—tablet, oil, powder, and liquid—or as pure active molecular formulations (Bandyopadhyay et al. 2004; Subhash et al. 2021).

Nimbin, nimbidin, nimbolide, limonoids, sodium nimbinate, gedunin, salannin, and quercetin are all complex active compounds of *Azadirachta indica* that modulate several metabolic and signaling pathways (Bandyopadhyay et al. 2004; Ebong et al. 2008). Quercetin and ß-sitosterol (polyphenolic flavonoids), as well as nimbin, nimbanene, 6-desacetylnimbinene, nimbandiol, nimbolide, ascorbic acid, n-hexacosanol, and amino acid, 7-desacetyl-7-benzoylgedunin, and 17-hydroxyazadiradione, are found in their leaves and possess antifungal, anti-inflammatory, and antibacterial properties (Mordue and Nisbet 2000; Biswas et al. 2002), as well as antiarthritic, antipyretic, hypoglycemic, and antidiabetic properties (Mordue 2000; Biswas et al. 2002; Mohamad et al. 2020; Subhash et al. 2021).

Many In Vitro and in vivo experiments suggested that active molecules like azadirachtin (a complex tetranortriterpenoid limonoid) possess an antimicrobial effect through the inhibitory effect it breaks down the cell wall via toxic effects the side the microbes and inhibits the growth. Certain studies have described the antibacterial effect on *Staphylococcus aureus* and MRSA with the most distinct zones of inhibition (Mordue and Nisbet 2000; Biswas et al. 2002) and anticancer effect through the regulation of cell signaling pathways and modulation of tumor suppressor genes including p53 and pTEN, angiogenesis (VEGF), transcription factors (e.g., NF-B), and apoptosis (e.g., bcl2, bax). Other results confirmed its anti-inflammatory activity by inhibiting the synthesis of proinflammatory cytokines and regulating cyclooxygenase (COX) and lipoxygenase (LOX) enzyme activities (Sarmiento et al. 2011; Hossain et al. 2013). So, that bioactive molecules and pharmacological molecules are involved in the activation of various anti-oxidative enzymes that break down microbe cell walls, control cell signaling pathways, and play a vital role in the treatment of a variety of diseases (Hossain et al. 2013; Subhash et al. 2021).

Following the announcement of the pandemic, it was suggested that neem leaves might be beneficial in the suppression of COVID-19 infection, and the Malaysian Ministry of Health myths have supported the use of the leaves of neem for the prevention of coronavirus disease (Hossain et al. 2013). According to some experimental studies, neem leaves have growth-promoting and immunomodulatory effects and improved antibody titer, growth performance, gross return, and an immunomodulatory response during both humoral and cell-mediated immune responses (Hossain et al. 2013).

12.2.3 Swertia Chirata Roxb. ex Flem. (Chiretta)

In 1848, Roxburgh was first described as *Swertia chirata*, which is a member of the Gentianaceae family, the second most diverse group of annual and perennial herbs, with 135 species in India (Kumar and Van Staden 2016). It is a vertical, annual,

branched herb with robust and cylindrical stems that grows between Kashmir and Bhutan and is also known as a Himalayan plant. It is found in a cold region of Himalayan altitudes in the sub-temperate parts (between 1200 and 3000 m altitudes) and grows between Kashmir and Bhutan (Clarke 1885).

In India, from ancient times, Chiretta is considering the essential medicinal herb in the traditional therapeutic method of history. Its various spices are commonly used in the indigenous system of medicine for the treatment of multiple diseases. It is also famous by the other name in different religions of the world—in Sanskrit, Anaryatikta, Bhunimba, Chiratitka, and Kairata; in Arab and Farsi, Qasabuzzarirah; in Urdu, Chiaravata; in Burma, Sekhagi; but in Nepal, Chirrato or Chiraita (Joshi and Dhawan 2005).

Chirrato or Chiraita is also an ethnomedicinal herb and contains remarkable pharmacological bioactive molecules like amelogenin, mangiferin (antiviral), swerchirin, sweroside, amaroswerin, gentiopicrin, and swertiamarin (act as anti-hepatitis). Amarogentin is responsible for the bitter taste and acts as anticancerous and antidiabetic (Phoboo et al. 2013). Its bioactive compound, xanthones (first isolated xanthones, chiratanin), and other derivatives, lignans, alkaloids, flavonoids, terpenoids, iridoids, and secoiridoids, and other compounds such as chiratin, ophelic acid, palmitic acid, oleic acid, and stearic acid (Saha et al. 2004).

The experimental reports of both studies—in vivo and in vitro—explain that mangiferin (1,3,6,7-tetrahydroxyxanthone-C2-β-D-glucoside) possesses a variety of biological activities, including anti-oxidative, antiaging, antitumor, antibacterial, antiviral, immunomodulatory, antidiabetic, hepatoprotective, and analgesic effects (Zheng and Lu 1990; Dar et al. 2005).

Mangiferin is a natural miracle pharmacological active molecule to treat the disease better and is involved in the modulation of apoptosis, autophagy induction, cell cycle arrest, and oxidative inhibitor stress (Saha et al. 2006). It has inhibited the LPS-stimulated expression of TNF-α, IL-6, and IL-1β in macrophages and improved immunity response. The miracle compounds of mangiferin indicated the better option to treat as antivirus of SARS-CoV-2 (Duang et al. 2011; Imran et al. 2017).

12.3 Miscellaneous Plant Sources for COVID-19 Management

Scientists from the University of Nottingham recently developed a plant-based antiviral that is effective at low concentrations to activate the innate immune system and suppress the replication/growth of human pulmonary viruses and other viruses such as COVID-19. Plant-based antiviral drugs have also been shown in studies to have the ability to regulate the spread of active infections (Deng et al. 2020).

Wattanathorn Jintanaporn indicated that other plants were also sufficient as an antiviral drug to manage and prevent coronavirus—Clitoria ternatea with metalloproteinase inhibitors inhibiting the expression of S-protein. *Solanum melongena* L (eggplant skin) has antiviral and immunomodulatory effects with anti-inflammatory properties that are considered mmp-2 and mmp-9 protease inhibitors (Deng et al. 2020).

Ginger (*Zingiber officinale*) has many active compounds like zingiberene, gingerol, gingerdione, shogaol, paradol, hexahydrocurcumin, and gingerenone A; garlic (*Allium sativum*) has alliin, allicin, ajoene, vinyldithin, S-allylcycsteine, and diallyl sulfides. Onions (*Allium cepa*), which contain allyl propyl disulfide and diallyl disulfide, are a popular inexpensive home remedy with a wide range of therapeutic properties, including antibacterial, antiviral, anti-inflammatory, anti-oxidant, and antitumor effects (Deng et al. 2020).

These plant-based medicines are also essential for improving immunity, preventing viral or communicable pathogen invasion, and improving clinical signs in COVID-19 patients (Deng et al. 2020). Some herbs have antiviral properties in addition to immunomodulatory effects. *Aloe vera*, *Angelica gigas* (Korean angelica), *Astragalus membranaceus* (Mongolian milkvetch), *Ganoderma lucidum* (lingzhi mushroom), *Panax ginseng* (ginseng), and *Scutellaria baicalensis* (Chinese skullcap), and their activity based on activating lymphocytes, raising the number of natural killer cells, enhancing macrophage activities, and stimulating phagocytosis, could boost the immunity and improve the immune system (Pyankov et al. 2012; Surphan et al. 2020).

Citrus bergamia (bergamot), *Eucalyptus globulus* (eucalyptus), *Pelargonium graveolens* (geranium), *Cinnamomum zeylanicum* leaf oil (cinnamon), and *Cymbopogon flexuosus* have all been documented to have inhibitory mechanisms against many microbes. However, scientific records and other clinical trials are needed to confirm these findings (Luo et al. 2020; Surphan et al. 2020).

12.4 Conclusion and Future Prospects

Like COVID-19, other infectious diseases are also a public health threat and are currently a global problem for medical professionals, scientists, drug manufacturers, and others. There is no specific vaccine or medication with 100% potency against COVID-19 pandemic disease, and the only treatment option is alternative/supportive therapy. As a result, to achieve better therapeutic benefits at a lower cost and with more minor side effects, find alternative medicine from a natural source, such as herbal remedies, which are traditionally used and now have a safety profile. These plant-based medicines can improve infected patients' immunity.

In the modern medical system, knowledge of medicinal plants aids in developing herbal-based medicines that act as antiviral drugs that could be more effective than current medicine-based therapy to treat such patients. Hence, the need to explore medicinal plants globally to improve cognitive function due to their less adverse effects is a must today to overcome viral diseases like COVID-19. The single most active source of producing new herbal medicines has been Ayurvedic medicinal plants; more than a hundred new products are made and clinically used. However, it is essential to validate these findings by scientific or other clinical studies.

This chapter compiles information on the phytochemistry, biological and cellular activities, and clinical applications of different medicinal plants to provide adequate basic drug information for herbal drug development campaigns and development

processes, resulting in new practical leads for different types of infection. Consequently, various antiviral herbal medicines derived from the extract of Ayurvedic medicinal plants were systematically evaluated. Studies have shown that antiviral herbals stimulate free radical scavengers' mechanisms and strengthen infected patients' immune systems. In short, the requirements of natural or alternative derivatives in the prevention of clinical problems are increasing day by day globally since they are associated with fewer side effects. Ayurvedic herbal medicine has therapeutic implications and plays a pivotal role in preventing infectious diseases. Its impact has been found by modulating the pathways of cell signaling and by inhibiting the virus.

Competing Interest Nil.

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Part IV

Enzyme, Hormone, and Biomolecules

Role of Cytokines as Immunomodulators

13

Hardeep Kaur and Soma Mondal Ghorai

Abstract

The resilience of our immune system is remarkable. It is always on guard against various pathogens that we encounter whether we eat, work, or sleep. A healthy body needs a balanced immune system to combat a variety of disease-causing agents. More importantly, the harmonious cross-talk between the immune system and other systems of our body is the key to good health. When this multi-level system fails us, we become prone not only to germs and diseases but also to many autoimmune disorders and cancers. Immunomodulators are substances that assist in the proper functioning of the immune system directly or indirectly by turning down some proteins (immune suppressors) or turning up others (immune enhancers). Among different classes of immunomodulators, cytokines play an important role as messenger molecules that effectively connect different processes and regulate immune cell growth and their maturation and responsiveness. They are the group of small glycosylated polypeptides with molecular mass less than 30 kDa and are key mediators in innate and adaptive immunity, hematopoiesis, inflammation, tumorigenesis, viral pathogenesis, etc. The chapter here deals with cytokines and their action as immunomodulators at different levels of the immune system of our body and their role in immunotherapies.

Keywords

Interleukins · Interferon · Lymphokines · Chemokines

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13.1 Introduction

Immunology is one of the most rapidly developing research fields in the area of medical science and holds a great promise in the prevention and treatment of various diseases and immunological disorders. The function and activity of immune system are determined by the exogenous and endogenous factors resulting in either immunosuppression or immunostimulation (Jantan et al. 2015). It also includes the correction of immune defects by the suppression of hyperactive immune responses or enhancement of depressed immune responses by immunomodulators. These molecules also have the ability to produce alterations in the host response (Dham 1995). Immunomodulators, therefore, may be defined as the substance that has the ability to stimulate or suppress the component of an immune system including both innate and adaptive systems of the immune response. Immunomodulators are generally used to correct the immune system that has gone out of balance and hence called immunorestoratives, immunoaugmentors, or biological response modifiers (Patil et al. 2012). A large number of immunomodulators have been identified which include cytokines, checkpoint inhibitors, agonists, adjuvants, etc. These can be natural or synthetic in nature and play a key role in different immunotherapies. Among these, cytokines play a very important role as regulatory molecules of innate and adaptive immune responses. They are the low-molecular-weight glycoproteins (about 30–40 kDa) and act on their target cells to induce systemic or localized immune responses. Cytokines were originally considered as biologically active factors in the supernatants of in vitro cultured leukocytes and were so named because of their specific activities on other leukocytes. Nearly 200 cytokines have been identified (Nain and Gemsa 2005) and are found to be extremely important in maintaining a delicate and intricate balance in the immune system.

13.2 Immune System in the Vertebrates

The immune system has evolved to protect the host from a variety of pathogenic microbes. Immunity is defined as the body's own natural defense system against various infectious diseases. Various factors like immunization, previous infection, and external stimuli trigger immune response to generate immunity. This system is able to discriminate between the body's own cells and foreign non-self particles. As soon as a foreign particle is identified in the body, an immune response is generated which is a collective effect of various immune cells and mediators formed in the body against the foreign particle. It is categorized into two types: innate immune system and adaptive immune system (acquired immune system) (Chaplin 2010). The innate immune system represents the first line of host defense system, while the adaptive immune system defines the second line of defense system. The innate system includes the physical and chemical barrier. The physical barrier includes the cell-cell contacts like tight junctions and cadherin-mediated interactions between the cells. The chemical barrier includes the production of saliva in the mouth and acid in the stomach which has the ability to kill the pathogens. The innate immunity

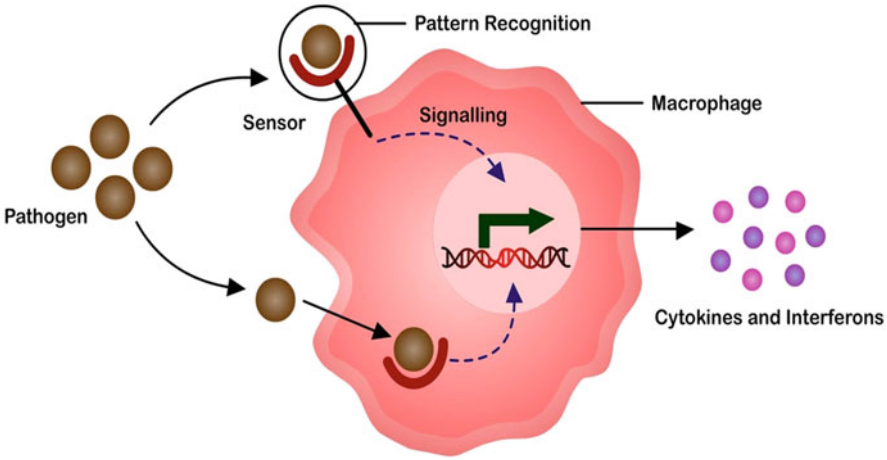

Fig. 13.1 Pathogen recognition in innate immune system

also includes the soluble proteins (complement proteins) and certain bioactive molecules (defensins and ficolins) which are generally present in the biological fluids or can be secreted from the cells, for example, cytokines and chemokines, which attract the lymphocytes at the site of inflammation. The innate immune system also includes various kinds of membrane-bound receptors and cytoplasmic proteins. These membrane-bound receptors are called pattern recognition receptors (PRRs) which play an important role in host defense against various microbes (Nicholson 2016). These PRRs have the ability to recognize different types of the molecules expressed by the microbe called pathogen-associated molecular pattern (PAMP). Once the PRRs recognize the microbial PAMP, immune response is generated through the induction of different chemokines and cytokines (Fig. 13.1).

Cytokines form an essential part of extracellular signaling network that eventually links innate and adaptive immune systems. The adaptive immunity is composed of specialized cells and processes that removes pathogens and produces an exaggerated response if the same pathogen is again encountered. The specificity and memory associated with adaptive immunity are due to certain specialized cells called lymphocytes. In humoral adaptive immunity, B lymphocytes are activated to produce antibodies specific to the antigen, while T lymphocytes produce cell-mediated adaptive immunity against the exogenous or endogenous antigens. Cytokines are not only the critical mediators of communication in both the systems, but they also mediate the cross-talk between the specialized cells of almost all biological processes be it embryonic development, disease pathogenesis, aging, etc. (Silva-Barrios and Stäger 2017).

13.3 History of Cytokines

The story of cytokines started back in 1957, with the discovery of interferons by Alick Isaacs and Jean Lindenmann (Isaacs and Lindenmann 1957). It was from 1957 to 1960 interferon had been firmly established as the mediator of virus interference, as a protein which was purifiable, and as an important new lead in dealing with virus infections. Interferon was heralded as the new, exciting molecule that had clear medical applications. The next big milestone was deciphering the structure of immunoglobulins in 1962, the groundbreaking work of Rodney Porter (Porter 1962). This was followed by pioneering research by Jacques Miller in 1967, who studied thymus and established the crucial role of T cells in immunity. Miller studied lymphocyte population dynamics and the mechanisms of the human immune response. Along with Graham Mitchell, he demonstrated that mammalian lymphocytes can be divided into what became known as T cells and B cells (Watts 2011). Almost a decade later in 1976, the first cytokine that was discovered to have therapeutic properties was interleukin-2 (IL-2). The team of Robert Gallo and Francis Ruscetti demonstrated that this cytokine has the ability to influence the growth of T cells and natural killer (NK) cells; thus, it was later even came to be known as a T-cell growth factor (Morgan et al. 1976). The discovery and purification of IL-2 allowed researchers to grow T cells and study their immunology, which led to the unearthing of human T-cell leukemia virus (HTLV), the first retrovirus documented in humans. Further on, the discovery and function of cytokines in 1983 showed that the cloned alpha subunit of IL-2 acted as a growth factor not only for T cells but also for B cells (Zubler et al. 1984). And in the year 1985, IL-2 was used as the first effective cytokine for immunotherapy. Brunet and coworker in 1987 worked on for the isolation and characterization of certain peptides expressed mostly on cytotoxic T lymphocytes (CTLs). This group of molecules were discovered as cytotoxic T-lymphocyte-associated protein-1 (CTLA-1) and CTLA-3 (serine-proteases), CTLA-2 alpha and beta (homologs to the pro-region of cysteine-proteases), and CTLA-4 (a member of the immunoglobulin superfamily). CTLA-4 was shown to not only activate T cells, but after T-cell activation, CTLA-4 is stored in the intracellular vesicles and recruited to the immunological synapse formed between T cells and APCs and inhibits the further activation of T cells by blocking signals initiated by T-cell receptors and CD28. Thus, CTLA-4-positive cells are considered to provide an essential regulatory mechanism for FoxP3+ regulatory T cells and cause cell-extrinsic regulation on other autoreactive T cells. Thus, CTLA-4 was the first immune-checkpoint molecule and was considered for therapeutic approaches and an effective immunotherapy by reversing T-cell tolerance against tumors (Brunet et al. 1987).

In August of 1987, a collaborative study between Yoshimura and Matsushima showed that highly purified or rIL-1 had no chemotactic activity for neutrophils, but was due to a 10 kDa protein which initially they named as CXC conserved cysteine residue motif in the N-terminal region of the molecule. This was the first report on identification of the chemotactic cytokine (or chemokine) that would eventually be known as neutrophil-activating protein and later interleukin-8 (IL-8). IL-8 is an

autocrine factor, produced by normal hematopoietic progenitors, mature blood cells, and leukemic cells, that promotes cell survival and proliferation in response to hematopoietic cytokines, functions as a chemo-attractant, and activates neutrophils (Yoshimura et al. 1987).

In 1989, Pastan and FitzGerald created single chain recombinant immunotoxin called chimeric toxins. They were made originally by using chemical cross-linking reagents to couple *Pseudomonas* exotoxin (or other toxins) to cell-binding proteins and opened new avenues to understand receptor function in treating various diseases. In 1991, the United States Food and Drug Administration (US FDA) finally approved the use of IL-2 as an immunotherapeutic treatment in metastatic kidney cancer.

Next, a cDNA clone of a cytokine which is also a T-cell growth factor was identified by Grabstein et al. in 1994. This cytokine was named IL-15. It was originally discovered as a T-cell stimulatory agent that shares important functional attributes with IL-2, including enhanced proliferation, survival, and differentiation of natural killer cells, T cells, and B cells. In 2011, Thomas Waldmann and his team initiated the first IL-15 clinical trial. The results of the trial published in 2015 showed IL-15 dramatically increases growth and activity of T and NK cells (Conlon et al. 2015). IL-15 are now being investigated for their potential to act as vaccines against those viruses that promote autoimmune diseases and cancer.

Johnston et al. in 1994 reported that a new member of the Janus family of kinases (JAK-3) shares similarity with other receptor components of the hematopoietic receptor superfamily IL-2 receptor and activates JAK kinases. Later, pioneering study by Melero et al. in 1997 demonstrates that in vivo administration of agonistic anti-4-1BB monoclonal antibody (mAb) has potent anti-tumor properties. The 4-1BB glycoprotein is a member of the tumor necrosis factor (TNF) receptor superfamily and binds to a high-affinity ligand (4-1BBL) expressed on several antigen-presenting cells (APCs) such as macrophages and activated B cells. This molecule was the first evidence for T-cell anti-tumor response.

Mackall and coworkers in 2011 characterized another cytokine, IL-7, as a master regulator of T-cell homeostasis or equilibrium. In the first human clinical trial with IL-7, they found that the cytokine drives regeneration of T cells that are critical to the immune system but become depleted during chemotherapy. IL-7-based therapies also might restore immune function in other immunocompromised individuals, such as those with HIV and the aged, and might enhance the activity of vaccines and other cancer immunotherapies (Mackall et al. 2011). Other cytokines also affect certain cell types, for instance, tumor necrosis factor alpha (TNF-α) induces cancer cell death. It also plays a role in eliminating pathogen-infected cells. Though already discovered in the 1960s, its true identity was unraveled by Aggarwal and coworkers in 1984 after they successfully isolated two cytotoxic factors: one, derived from macrophages (molecular mass 17 kDa), and the second, derived from lymphocytes (20 kDa). Both exhibited 50% amino acid sequence homology and bound to the same receptor and thus were named TNF-α and TNF-β.

Another cytokine that is capable of targeting tumors with incredible precision is interferon gamma (IFN-γ). Robert D. Schreiber had worked tediously to understand

IFN-γ as an immuno-surveillance molecule and characterized the receptor to which IFN-γ binds as well as the signaling pathways that it activates within cells. Schreiber made the world's first antibodies to IFN-γ (Dunn et al. 2002).

13.4 General Properties of Cytokines

Cytokines are the messenger molecules secreted by white blood cells and other cells in the body (with the exception of red blood cells) in response to some stimuli. The word comes from Greek words *cyto*, from *kytos* meaning cell, and *kines*, from *kinēsis* meaning movement. Just like endocrine hormones, they bind to specific receptors on the target cell membrane and induce signal transduction that ultimately triggers gene expression in the target cell. These cytokines can function in an *autocrine* (binds to the specific receptor of the same cell that secretes it); *paracrine* (binds to the target cell in immediate vicinity to the cell that secretes it); or *endocrine* manner (exerts an effect on distant cell) (Fig. 13.2). There is high affinity between cytokines and their receptors, so minute concentration (in picomolars) is sufficient to trigger a specific biological effect.

Cytokines secreted by lymphocytes are called *lymphokines*; those secreted by some leukocytes and acting on others are called *interleukins (IL)*; while those produced by monocytes and macrophages are called *monokines*. *Chemokines* are the cytokines that affect chemotactic behavior of leukocytes and play a significant role in inflammatory response. Almost all cytokines have α-helical structure with

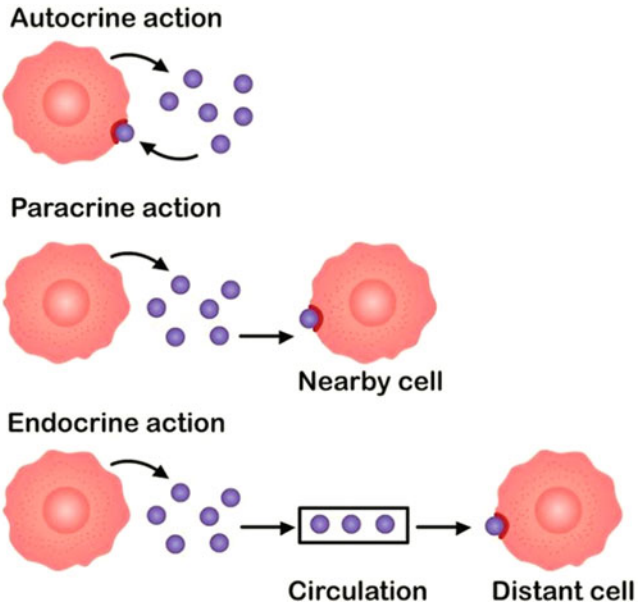

Fig. 13.2 Mode of action of cytokines

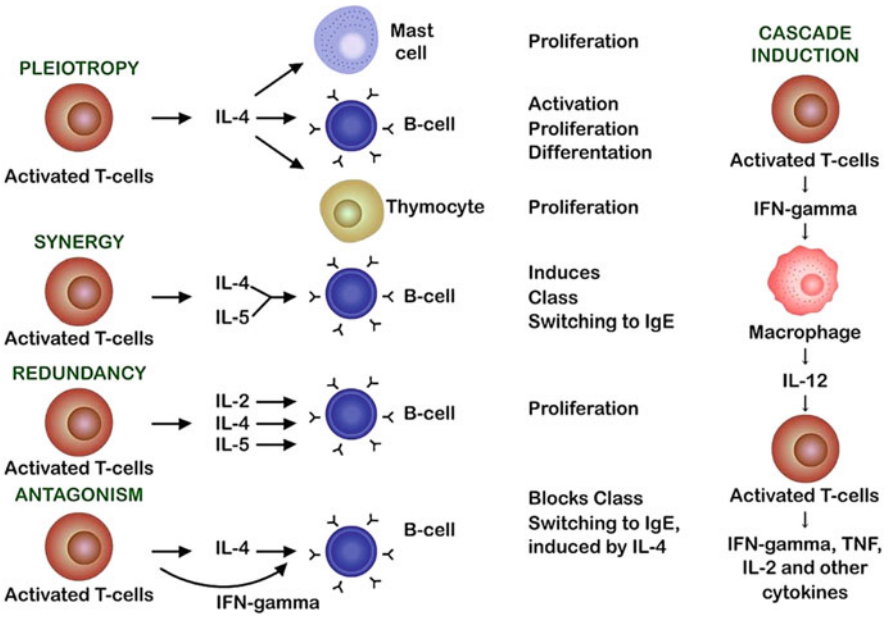

Fig. 13.3 General properties of cytokines

little or no β sheets. Some of the other important properties of cytokines are shown in Fig. 13.3 and are as follows:

1. ***Pleiotropy***: Certain cytokines may act upon different cell types bringing about different biological effects, e.g., IL-4 secreted by active T_H cells can cause proliferation of thymocytes; activation, proliferation, and differentiation of B cell; and proliferation of mast cells.
2. ***Redundancy***: Some cytokines can have redundant action, i.e., different cytokines may mediate similar functions, e.g., IL-2, IL-4, and IL-5 all can lead to B-cell proliferation.
3. ***Cascade effect***: Cytokines secreted by one cell may influence the synthesis of another cytokine by other cell which in turn may induce other target cell to produce another cytokine, e.g., activated T_H cell produces cytokine IFN-γ that acts on macrophage that starts producing cytokine IL-12, which acts on T_H cell that further secretes IFN-γ, TNF, IL-2, etc. (Chokkalingam et al. 2013; Carpenter et al. 2002; Tian et al. 2005).
4. ***Synergistic effect***: In this case, two or more cytokines interact to produce much greater cellular activity, and their combined effect is much greater than the additive effect of individual cytokines, e.g., concerted action of cytokines IL-4 and IL-5 on B cell is much greater than their individual action on B cells (Grignani and Maiolo 2000; Carson and Kunkel 2017).

5. ***Antagonism***: Certain cytokines are antagonistic to each other, i.e., they inhibit the effect of each other, e.g., IL-4 induces immunoglobulin class switching in B cells which is inhibited by IFN-γ.
6. Certain cytokines act as ***mitogens***, i.e., they stimulate mitosis in target cells.
7. Cytokine secretion is transient and is initiated each time by new gene transcription. There is no preformed or precursor or inactive cytokines. They also have a very short half-life in blood and extracellular fluids.

13.5 Cytokine Families

Based on their function in the body, cytokines are classified into the following cytokine families:

1. ***Interleukins (IL)****:* These cytokines have different structures and functions. They are mainly produced by T_H cells, monocytes, macrophages, and endothelial cells (Dinarello et al. 2010). They have been named as IL-1, IL-2, and so on. They belong to a family of diverse genes involved in immune cell differentiation and activation and mediate the host response to infection through both direct and indirect mechanisms. The pro-inflammatory interleukins help in viral clearance and mediate complement-mediated lysis (Gabay and Kushner 1999). In acute influenza, these are responsible for increasing IgM antibodies and recruit $CD4^+$ T cells to the site of infection (Schmitz et al. 2005).
2. ***Interferons (IFN):*** There are three types of IFN named as type I IFN, type II IFN, and type III IFN. *Type I IFN* includes IFN-α (IFN-α1, IFN-α2, IFN-α4, IFN-α5, IFN-α6, IFN-α7, IFN-α8, IFN-α10, IFN-α13, IFN-α14, IFN-α16, IFN-α17, and IFN-α21) and IFN-β. *Type II IFN* has only one candidate, IFN-γ. *Type III IFN* has IL-28/29. All IFNs are functionally similar as they act via the JAK/STAT signaling pathway. The signaling cascades activate the transcription factors of innumerable IFN-related genes, thus coding for protein products with antiviral, antiproliferative, or immunomodulatory properties.
3. ***Tumor Necrosis Factor (TNF):*** There are more than 40 types of TNF molecules, most prominent being TNF-α (commonly named as TNF) and TNF-β (also named lymphotoxin alpha). Best known for their tumor regression properties in mice (Carswell et al. 1975), tumor necrosis factors are the most potent pro-inflammatory cytokines and play a prominent role in the "cytokine storm." Apart from inducing pathogenesis in malaria and sepsis (Beutler et al. 1985; Clark 2007; Clark et al. 1981), they are the main cytokines that are released during viral infection. TNFs belong to a superfamily of proteins with 19 members that can signal through 29 varied receptors (Aggarwal 2003). These act in a pleiotropic manner and are expressed on a variety of immune cells along with primary receptor TNFR1 that also appears in almost all cell types, confirming pervasive effects of TNFs. Excessive TNF production is associated with chronic inflammatory and autoimmune diseases, like the bowel disease, psoriasis, and rheumatoid arthritis (RA) (Kopf et al. 2010; Sethi et al. 2009). TNF inhibitors are

largely being considered as therapeutics in treating patients with sepsis or autoimmune disorders (Fowler et al. 1984), though not much success is achieved in this field (Clark 2007).

4. ***Growth Factors (GF):*** Transforming growth factor-beta (TGF-β) is produced by platelets, macrophages, lymphocytes, and mast cells. Epidermal growth factor (EGF) and nerve growth factor (NGF) are also examples of GFs.
5. ***Colony-Stimulating Factors (CSF):*** Granulocyte macrophage colony-stimulating factors (GM-CSF), macrophage colony-stimulating factors (M-CSF), and granulocyte colony-stimulating factor (G-CSF) are the main cytokines of this family and are involved in the proliferation and differentiation of hematopoietic cells.
6. ***Chemokines***: These are produced by many cell types in response to infection or injury, e.g., MCP-1 (monocyte chemo-attractant protein), MIP-1α (macrophage inflammatory proteins), etc. They are the largest family of chemo-attractants that control the migration of immune cells and contribute to the process of embryogenesis, innate and adaptive immune development, and even cancer metastasis (Raman et al. 2011).
7. The cytokines of interleukin family and those from colony-stimulating factor family are also collectively called **hematopoietin cytokine family**.

13.6 Function of Cytokines in the Immune System

Cytokines are known to have three main functions in the immune system. They are as follows:

13.6.1 Stimulation of Hematopoiesis

Hematopoiesis is the process of formation of blood cells. The process takes place in the bone marrow in an adult vertebrate. The pluripotent hematopoietic stem cells (HSC) undergo division and differentiation and form mature leukocytes. The cytokines that are involved in the process are mainly CSFs. Two other cytokines IL-3 and IL-7 are also involved in the growth of lymphocyte progenitor cells (Kumar and Tiku 2016) (Fig. 13.4).

13.6.1.1 Colony-Stimulating Factors (CSF)

Present within the microenvironment of the hematopoietic tissue, CSF mainly is involved in the proliferation and differentiation of hematopoietic cells of the myeloid lineage in the bone marrow. GM-CSF, M-CSF, and G-CSF also function to increase the number of cytokine-producing macrophages at the site of inflammation, thus serving to perpetuate inflammatory reactions. These form a part of the pro-inflammatory network along with IL-1 and TNF molecules and play a prominent role in inflammation (Hamilton 2008).

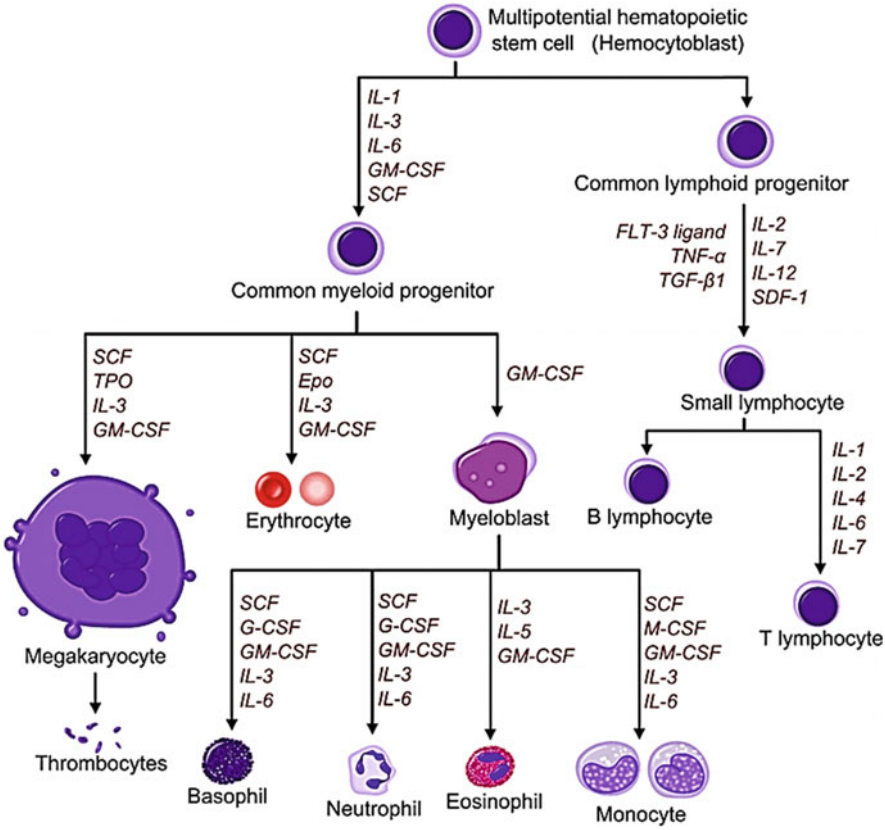

Fig. 13.4 Role of cytokines in hematopoiesis. (Source: By A. Rad and M. Häggström. CC-BY-SA 3.0 license; Adapted from Lodish et al. 2000) (https://commons.wikimedia.org/wiki/File:Hematopoietic_growth_factors.png)

13.6.1.2 Stem Cell Factors

These factors are produced by stromal cells in the bone marrow, and they increase the responsiveness of stem cells to CSFs.

13.6.1.3 Interleukin-3 (IL-3)

These cytokines are also called multi-colony-stimulating factor (multi-CSF). IL-3 has a molecular weight of about 20–26 kDa and is produced by T cells. It acts on immature bone marrow progenitor cells and leads to the differentiation of mast cells, macrophages, and other cell types.

13.6.1.4 Interleukin-7 (IL-7)

These interleukins are mainly produced by stromal cells in the bone marrow and by fibroblasts. IL-7 increases the proliferation of early B and T cells.

13.6.2 Mediator of Innate Immunity

The cytokines that play a primary role in innate or natural immunity are TNF-α, IL-1, IL-6, IL-10, IL-12, type I interferons (IFN-α and IFN-β), IFN-γ, and chemokines. These cytokines are primarily produced by macrophages and dendritic cells and provide protection against viral infections and initiate inflammatory reactions.

13.6.2.1 Tumor Necrosis Factor-Alpha (TNF-α)

TNF-α is the key mediator of inflammatory response and if produced in excess can lead to shock. It plays an important role in producing host response to gram-negative bacterial LPS (lipopolysaccharide) or endotoxin. TNF-α is produced by activated macrophages, monocytes, and dendritic cells in response to LPS of gram-negative bacteria. It acts on endothelial cells that produce adhesion molecules and chemokines that initiate the migration of neutrophils and macrophages to the site of infection (*diapedesis*, *chemotaxis*). It also promotes the secretion of IL-1 by activated macrophages and acts on the hypothalamus to induce fever. Besides TNF also acts on neutrophils and promotes extracellular killing by these cells. It stimulates the secretion of acute-phase proteins by the liver and is also considered to be cytotoxic for tumor cells.

13.6.2.2 Interleukin-1 (IL-1)

IL-1 is mostly produced by activated macrophages and monocytes, and its main function is to activate T cells. Like TNF-α, it plays a very important role in host inflammatory response in innate immunity. At low concentration, IL-1 induces the synthesis of IL-6 and IL-1 from vascular endothelial cells and phagocytes, promotes local inflammation and coagulation, and activates the release of adhesion molecules and chemokines. At high concentration, IL-1 exerts endocrine effect causing fever and induces the production of amyloid A protein from hepatocytes leading to metabolic wasting.

13.6.2.3 Interleukin-6 (IL-6)

IL-6 is mainly synthesized by phagocytes, fibroblasts, and vascular endothelial cells in response to induction by IL-1 and is mostly detected in blood following infection by gram-negative bacteria. It induces hepatocytes to produce acute-phase proteins. It also plays a role in B-cell differentiation. IL-6 has also been found to act as a growth factor for hematopoietic stem cells (HSCs) in the bone marrow.

13.6.2.4 Interleukin-10 (IL-10)

IL-10 is mainly produced by activated macrophages and T_H2 cells. It inhibits the activity of macrophages and T_H cells by preventing the production of cytokines by these cells. It also inhibits the activity of APCs. Since IL-10 decreases the ability of macrophages to remove microbes, therefore it can lead to the chronic accumulation of microbes in the form of granuloma.

13.6.2.5 Interleukin-12 (IL-12)

Mainly produced by macrophages and dendritic cells, IL-12 plays a key role in early innate immune response. It increases the synthesis of interferon gamma (IFN-γ) by T cells and NK cells. It also increases the activity of CTLs and NK cells.

13.6.2.6 Type I Interferons

British bacteriologist Alick Isaacs and Swiss microbiologist Jean Lindenmann discovered interferons in 1957. Later much research was conducted on interferons and it was found that they play an important role in preventing viral infections and affect the growth of cancer in some cases (Fensterl and Sen 2009; Katze et al. 2008). Interferons are classified into three types depending upon their receptor specificity:

- Type I interferons include IFN-α and IFN-β. They are induced by viral pathogens or artificially by double-stranded RNA and signals via heterodimer interferon-α/β receptor complex (IFNAR1/IFNAR2).
- Type II interferons (IFN-γ) are produced in response to foreign antigen or mitogen and signals through IFN-γR1/IFN-γR2.
- Type III interferon (IFN-λ or IL-28/29) that has a similar antiviral role as type I interferons.

Type I interferons include IFN-α (IFN-α1, IFN-α2, IFN-α4, IFN-α5, IFN-α6, IFN-α7, IFN-α8, IFN-α10, IFN-α13, IFN-α14, IFN-α16, IFN-α17, and IFN-α21) and IFN-β. They inhibit viral replication and also enhance the lytic ability of NK cells. Type I interferons also increase the expression of major histocompatibility complex (MHC) class I molecules on virally infected cells. These molecules also stimulate the development of T_H1 cells. The number of these interferons increases during an infectious process and can be easily detected in the blood. IFN-α is produced in leukocytes, while IFN-β is produced in fibroblast cells. Interferons are detected in cerebrospinal fluid in the case of viral meningitis. Patients who are on immunosuppressive drugs or those having malignant lesions have low levels of IFN-α and IFN-β in their blood.

Interferon therapy is used to treat cancers and various other diseases (Borden et al. 2007; Friedman 2008). In fact, IFN-α2b injections are used in the treatment of hepatitis B and C, malignant lymphomas, genital warts, Kaposi sarcoma, etc.; however, there are some serious long-term side effects of interferon therapy, and therefore alternate therapies are considered before using interferons (Goodsell 2001; Heim 2012; Theofilopoulos 2012).

13.6.2.7 Chemokines

Large numbers of chemo-attractant cytokines (chemokines) have been found to play an important role in early response to infections. These are present in fish, birds, and mammals and are responsible for directing the movement of certain immune effector cells like neutrophils, monocytes, and lymphocytes to the site of pathogen invasion (cell/lymphocyte trafficking) (Zlotnik and Yoshie 2012; Murphy 2002). These molecules bind to transmembrane G-protein-coupled receptors and lead to

intracellular signaling causing cell polarization, migration, and adhesion (Griffith et al. 2014; Nagarsheth et al. 2017). These molecules are small-sized (approximately 8–10 kDa in size) proteins and have four cysteine residues in conserved locations that are key to forming their three-dimensional shape.

As of now, there are 44 members of chemokines, and they are grouped into 4 types: CXC, CC, CX_3C, and C. These molecules are classified based on space between cysteine moieties. They bind to more than 21 G-protein-coupled receptors demonstrating differential expression with different ligands (Comerford and McColl 2011). The members of CXC family are responsible for the chemotaxis of neutrophils, while the members of CX_3C family are responsible for the chemotaxis of monocytes, dendritic cells, T cells, and NK cells. The CC chemokines are chemo-attractant for monocytes and lymphocytes, while C chemokines recruit lymphocytes (Garin and Proudfoot 2011).

Besides their role as chemo-attractants, chemokines also play an important part in angiogenesis, tumorigenesis, wound healing, and inflammatory response.

13.6.3 Mediators of Adaptive Immunity

The cytokines that play an important role in the adaptive immune system include IL-2, IL-4, IL-5, TGF-β, IL-13, and IFN-γ. These cytokines are responsible for the proliferation and differentiation of B lymphocytes and T lymphocytes after antigenic stimulation.

13.6.3.1 Interleukin-2 (IL-2)

IL-2 is mainly produced by T_H and T_C cells. It is a primary growth factor for T-cell proliferation and was originally called T-cell growth factor (TCGF). It can act on T_H cells in an autocrine manner. It is synthesized by T cells only upon stimulation by an antigen. It also activates the synthesis of other cytokines like IFN-γ and lymphotoxins from T cells.

13.6.3.2 Interleukin-4 (IL-4)

IL-4 is also called "prototypic immunoregulatory cytokine" and is produced by macrophages and T_H2 cells. It plays a pivotal role in antibody production, hematopoiesis, inflammation, and effector T-cell development. It is also involved in allergic reactions and provides effective response in helminthic and arthropod infections. It produces a counter effect to IFN-γ, thus inhibiting cell-mediated immunity.

13.6.3.3 Interleukin-5 (IL-5)

IL-5 is produced by T_H2 cells and functions as a growth factor for the differentiation of B cells and eosinophils, especially in response to infections by helminths and arthropods.

13.6.3.4 Transforming Growth Factor-Beta (TGF-β)

TGF-β is chiefly produced by T cells, B-cell-activated macrophages, and platelets. It has inhibitory activity against the proliferation of endothelial cells, macrophages, T cells, and B cells. It also inhibits immune response in chronic inflammatory reactions, production of NK cells, and TNF-α production. However, it can activate fibroblast, monocyte, and neutrophil chemotaxis.

13.6.3.5 Interleukin-13 (IL-13)

IL-13 is primarily produced by T_H2 cells. It increases the production of IgE by B cells, inhibits macrophages, and increases the production of mucus.

13.6.3.6 Interferon Gamma (IFN-γ)

IFN-γ is produced by naïve and mature T_H cells and T_C cells. It is also produced by NK cells in immune-deficient mice. IFN-γ is antiviral and antiproliferative and has a primary role in immunoregulation. It activates macrophage activity, increases MHC-I expression, promotes the differentiation of naïve T cells to mature cells, and enhances cytolysis by NK cells. It also plays an important role in tumor immunotherapy.

13.7 Cytokine Receptors

Cytokines act on certain cells by binding to their specific receptors and initiate downstream signaling leading to certain response by the cell. These receptors are multi-subunit transmembrane glycoproteins which can be monomeric or multimeric (Fig. 13.5). In the past few years, the study of cytokine receptors has gained immense prominence as it has been seen that deficiency of certain cytokine receptors can lead to certain debilitating immunodeficiency states. Hence, the study of cytokine receptors is now being considered clinically and experimentally very important.

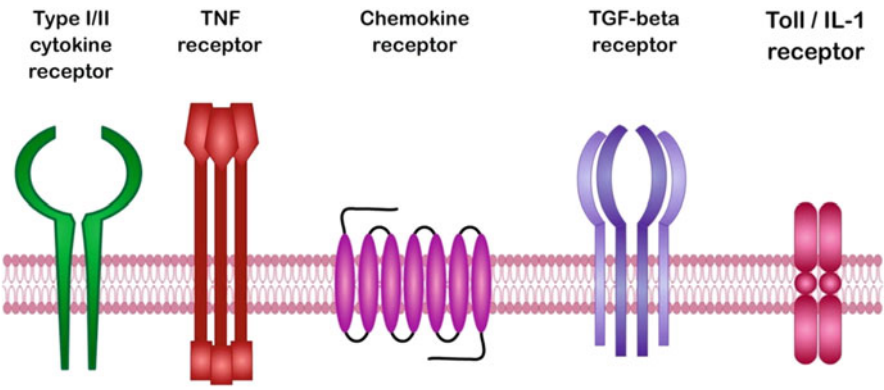

Fig. 13.5 Cytokine receptor families

The receptors have been divided into several families based on their structure and the activities they perform:

13.7.1 Immunoglobulin Superfamily Receptors

These are localized on the surfaces of various cell types, e.g., IL-1 receptor and CSF-1 receptor.

13.7.2 Type I Cytokine Receptor Family/Hematopoietin Receptor Family

These receptors are generally in the form of dimers or trimers and possess certain conserved amino acids in extracellular domains, e.g., GM-CSF receptor, G-CSF receptor, and type I IL (IL-2, 3, 4, 5, 6, 7, 9, 11, 12, 13, 15) receptors.

13.7.3 Type II Cytokine Receptor Family/Interferon Receptor Family

These receptors possess two extracellular conserved cysteine domains, e.g., IFN-β and IFN-γ.

13.7.4 Chemokine Receptors

These receptors are G-protein-coupled receptors and possess seven transmembrane helices. They acts as binding protein for HIV, e.g., CC chemokine receptor and CXC chemokine receptors.

13.7.5 TNF Receptor Family

These receptors are in the form of trimers and have cysteine-rich extracellular binding region, e.g., TNF receptors, CD40 receptors, and CD30 receptors.

13.7.6 TGF-Beta Receptors

These receptors are serine/threonine kinase receptors.

The differential expression of the cytokine receptor on the membrane of target cell can further regulate the activities of various cytokines.

13.8 Signal Transduction by Cytokine Receptors

Cytokines bind to their specific cell surface receptor in order to initiate intracellular signaling to alter the cell function. The altered cell function may be the upregulation and/or downregulation of certain genes in the nucleus of the cell eventually leading to the production of other cytokines (cascade induction) or an increase in the number of cell surface receptors or inhibition of its own activity (feedback inhibition). Many factors are involved in this process that include the type of cytokine binding to the receptor, the number of receptors on the cell membrane, and the downstream signaling after binding of cytokine to its receptor. A large number of cytokine receptors function by JAK/STAT pathway (e.g., receptors for IL-2, IL-3, IL-4, IFN-γ, etc.). In fact, the unifying model of cytokine receptor-mediated signaling has emerged from studies of the molecular events that were triggered after binding of IFN-γ to its type II receptor molecule. The cytokine receptors are generally heterodimers with one cytokine receptor subunit and another signal transducing subunit. The fact that many of these receptors share same signal transducing subunits leads to redundancy of action by different cytokines. These receptors also lack tyrosine kinase activity; therefore, they get associated with cytoplasmic tyrosine kinases known as Janus kinases (JAKs) to facilitate changes in gene expression. Binding of cytokine to the cytokine receptors leads to the dimerization of the receptors. The associated JAKs then cross-phosphorylate and get activated. The activated JAKs then phosphorylate the receptor sites, and this creates a docking site for signal transducers and activators of transcription (STAT) family of transcription factors. These STAT proteins get phosphorylated by the JAKs, after which they dissociate from the receptor, undergo dimerization, and translocate to the nucleus where they regulate transcription of target genes (Fig. 13.6). It should be noted that only those target genes whose expression is permitted by a specific cell type will be activated within that variety of cell, e.g., IL-4 activates a specific set of genes in T cells and different set of genes in B cells.

(https://en.wikipedia.org/wiki/Janus_kinase_3#/media/File:JAK3_signal_transduction.jpg)

13.9 Role of Cytokines in Various Diseases

Considered as key mediators for inflammatory responses, cytokines are now known to play an important role in many autoimmune disorders like multiple sclerosis, rheumatoid arthritis, osteoarthritis, and systemic lupus erythematosus. Cytokines and their role in cancer have also dominated much recent research. In recent times, the focus of the general public and the scientists has shifted to understand the role of cytokines in pathogenic diseases like viral infections especially respiratory disorders like influenza, and more recently SARSCoV2. Cytokines also do play critical roles in other non-pathogenic diseases like bronchial and allergic asthma, psoriasis vulgaris and psoriatic arthritis, and fibrosis.

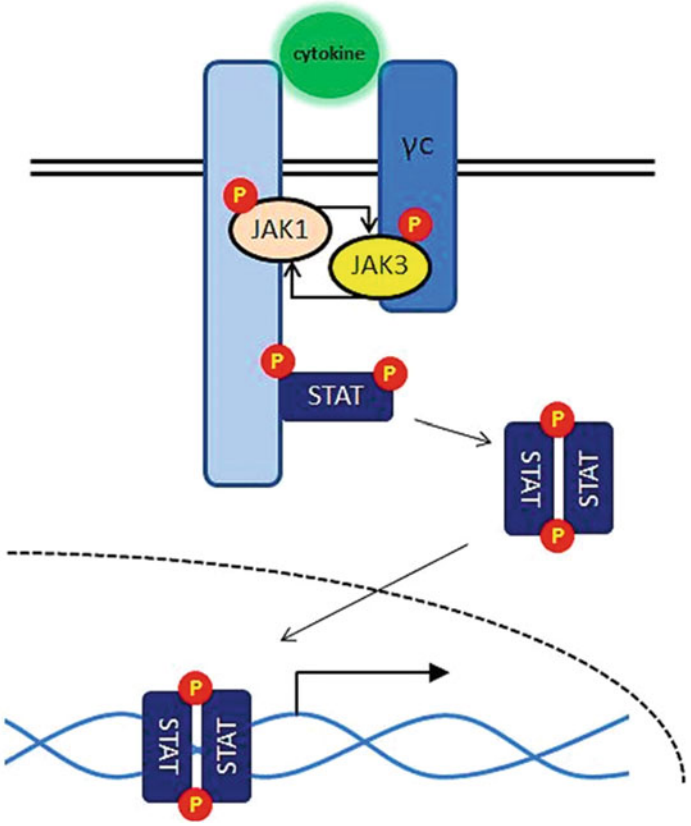

Fig. 13.6 Signal transduction by cytokine receptors (Source: Rayray7, CC BY-SA 3.0 <https://creativecommons.org/licenses/by-sa/3.0>, via Wikimedia Commons. Adapted from Leonard and O'Shea 1998)

13.9.1 In Autoimmune Disorders

Before studying any disease pathogenesis related to the immune system, an understanding of the various immune players involved in managing a diseased condition within the host is important. Majorly, T-cell subsets (T_H1/T_H2) mutually cross-regulate each other to maintain T_H cell-driven cytokine balance in any disease pathogenesis (Romagnani 1997; Coffman 2006). To be precise, T_H1-driven responses are arbitrated by cytokines produced by T_H1 cells (e.g., IL-2, IFN-γ, and TNF-α) and macrophages (e.g., IL-1, IL-6, IL-12, and TNF-α), whereas T_H2-driven responses are mediated by cytokines such as IL-4, 1L-5, and IL-13 (Coffman 2006). The immune system tries to reach homeostasis by deliberating various immunomodulatory regimes to restore the cytokine balance during the cross-regulation between T_H1 and T_H2. For instance, in a T_H1-mediated disease, the immune system employs many strategies to deviate immune responses to a suppressed T_H2-type cytokine

response (Romagnani 1997). Over the past two decades, research on mechanistic and therapeutic aspects of autoimmune diseases was based on the T_H1/T_H2 regulation. Almost all autoimmune diseases could be categorized as predominantly T_H1-driven if the major events were cell-mediated in nature or predominantly T_H2-driven if antibodies and/or immune complexes served as the main mediators.

Interestingly, a twist in the understanding of autoimmunity arose with the advent of new research findings which had helped resolved many critical gaps and contradictions in understanding the mechanisms underlying the pathogenesis of autoimmunity. A paradigm shift in the TH1/ TH2-centric view of autoimmunity came with the understanding that majority of immune effector responses that were previously attributed to IL-12 and IFN-α were actually mediated in vivo by IL-23 and IL-17 (IL-17/IL-23 axis) (Langrish et al. 2004). Subsequently, the role of IL-23 in eliciting IL-17 response was discovered (Khader et al. 2007), and a new subset of T cells (T_H17) that formed IL-17 was recognized discrete from T_H1 subset (Stockinger and Veldhoen 2007). IL-17 was finally appreciated for its contribution to autoimmune diseases after much spearheading research in human patients as well as in animal models (Baldeviano et al. 2010; Rajaiah et al. 2011). IL-17 was shown to play a critical role in disease pathogenesis of multiple sclerosis (MS) (Komiyama et al. 2006) and rheumatoid arthritis (RA) (Murphy et al. 2003).

Presently, intense research in autoimmunity has been undertaken to answer certain critical questions related to the roles of IL-12/IFN-γ versus IL-23/IL-17 in the induction, progression, and regression of autoimmunity. Both IFN-γ and IL-27 either mediate distinct clinical/histopathological phenotypes of the autoimmune disease or show sequential pro-inflammatory activity (Rajaiah et al. 2011). Further, it was noted that T_H1 lineage is antagonistic for both T_H2 and T_H17. while T_H17 in turn can control the activity of T_H1 (Bettelli et al. 2007; Steinman 2008; Basso et al. 2009; Peck and Mellins 2010). Besides the paradigm shift in the cytokine field, various other cytokines display characteristics that further compound the analysis and interpretation of their role in autoimmune diseases. There are certain additional T-cell subsets like T_H22, T_H9, and T-follicular helper cells (Tfh) that display specific cytokine secretion profiles and other unique attributes. As already mentioned in the chapter, cytokines can modulate any disease pathogenesis via pleiotropy, redundancy, duality of action, and plasticity (the capability of being molded or being made to assume a desired form) (Annunziato and Romagnani 2009; Moudgil and Choubey 2011). T regulatory cells (Treg) present within the inflammasome change their phenotype to T_H17-like cells, and under other set of conditions, T_H17 can adopt a T_H1-like or a Treg-type phenotype (Feng et al. 2011). Seemingly, these observations reflect the biological system's economy and optimal use of the available, functionally relevant T-cell subsets depending on the need and local milieu.

13.9.1.1 Rheumatoid Arthritis (RA)

RA is a systemic autoimmune disorder and is characterized by chronic inflammation of the synovium, particularly of small joints, which often leads to the destruction of articular cartilage and juxtaarticular bone (Harris Jr 1990). RA pathogenesis seems multifactorial and is still not fully understood. Over the past two decades,

understanding the basic biology of RA has tremendously helped in its therapeutics. Targeted therapies which selectively inhibit the progression of destructive arthritis, yet leaving host-defense mechanisms intact, should be of prime concern. Thus, targeting the cytokine misbalance might represent an avenue to future clinical therapies for RA. The cytokine network in RA is quite complex and involves two groups, the pro-inflammatory and anti-inflammatory cytokines. Regulation of two key pro-inflammatory cytokines IL-1 and TNFα is of crucial importance in the RA disease. Recent work also showed that blocking these cytokines had counter effect on RA pathogenesis. Novel cytokines such as IL-17, IL-18, and RANK ligand (RANKL (receptor activator of nuclear factor kappa-B ligand), also known as tumor necrosis factor ligand superfamily member 11) are paving way to understand the disease etiology and may contribute to the improvement of current therapies. Pleiotropic cytokines such as IL-4 and IL-10 can also be considered as promising modulators in the control of RA (Lubberts and van den Berg 2013).

13.9.1.2 Multiple Sclerosis (MS)

MS is one of the most common neurological autoimmune diseases characterized by plaque formation in the central nervous system (CNS). It is characterized by inflammation and demyelination resulting in clinical manifestations such as weakness and fatigue, numbness, depression, vision impairment, cognitive problems, loss of muscle coordination, bowel changes, and bladder dysfunction (Goldenberg 2012; Loma and Heyman 2011; Kister et al. 2013). The key cytokine players in MS are secreted from various cell types, and their concentrations vary according to the severity of the diseased condition in patients. In this disease, T cells and its subsets (T_H17 cells, γδ T cells, NKT cells) produce high levels of GM-CSF and IL-17, and elevated levels of IFN-γ are produced from T_H1 cells, NK cells, and NKT cells, while production of IL-10 is suppressed by Treg cells. Innate immune cells like monocytes, macrophages, and dendritic cells (DCs) secrete elevated levels of IL-1β, IL-12, IL-23, and TNF-α (Palle et al. 2017).

13.9.1.3 Systemic Lupus Erythematosus (SLE)

SLE is an outcome of both environmental and genetic factors which commences due to the loss of tolerance and the presence of autoreactive lymphocytes in the periphery. The defect in immune tolerance which gets exacerbated by both the innate and adaptive arms of the immune response results in skin rashes, arthritis, leukopenia, nephritis, and inflammation of the nervous system (Kumar et al. 2006; Crow 2008). SLE pathogenesis is clearly due to increased levels of cytokines and their receptors in hematopoietic cells as well as in target organs. Moreover, high levels of anti-DNA and related antinuclear autoantibodies are also seen, suggesting that those cytokines that activate B and T cells constitute important disease drivers. In SLE, myeloid DC (mDC) engulfs apoptotic cells and nucleosomes and presents autoantigens to $CD4^+$ T cells, thus activating B lymphocytes to undergo proliferation and clonal expansion for the production of autoantibodies (Blanco et al. 2001). The autoantibodies, in turn, form immune complexes with neutrophil products and nucleosomes and directly stimulate Toll-like receptors (TLR) on plasmacytoid dendritic cells (pDC), which

then secrete more IFN-α, thereby propagating the inflammatory response (Niewold et al. 2007; Kariuki and Niewold 2010). High serum levels of TNF and the soluble TNFRs have been reported in SLE patients with active disease (Studnicka-Benke et al. 1996). IL-6 as well as IL-10 is elevated in the serum of lupus patients (Malide et al. 1995; Park et al. 1998). Similarly, IFN-γ is increased in the serum of some SLE patients, and its levels have been shown to correlate with disease activity (al-Janadi et al. 1993; Akahoshi et al. 1999). Elevated serum levels of IL-17A and increased numbers of T cells secreting IL-17 have been detected in SLE patients and in murine lupus models (Ouyang et al. 2008; Yang et al. 2009). In accordance to other autoimmune diseases, T_H17 cells too increase in SLE, promoting cytokine production and inflammation (Yang et al. 2009; Zhang et al. 2009). Upregulation of several cytokine genes, including IL-10, IFNG, and IL-21, and the receptor for IL-21 (IL-21R) have also been directly associated with SLE susceptibility (Sawalha et al. 2008; Gateva et al. 2009; Webb et al. 2009; Kim et al. 2010).

13.10 In Cancer

Cancer pathogenesis depends largely on the assortment of varied cytokines released within the cancer microenvironment. Many times cancers get inhibited to the cytokines produced in response to inflammation and infection; and at times cancer can get exaggerated to the host-induced cytokines which promote cell proliferation, weaken apoptosis, and facilitate invasion and metastasis. Cytokine release must tread a fine balance to either control or overwhelm cancer conditions. Carcinogen-induced injury, infection, cellular stress, or even inflammation causes a diverse range of cytokine release. These cytokines may either control cellular stress or contain the damage promoting tissue repair, but failure to resolve the injury may lead to continuous cytokine production that can cause excessive tissue damage and affect cancer progression. Cytokines and chemokines during inflammatory conditions increase the risk of cancer by promoting angiogenesis, metastasis, subversion of adaptive immunity, and changing response to hormones. Thus, cytokines can impact cancer at several stages of their progression (Table 13.1).

TNF was demonstrated to involve different pathways depending upon the stage of cancer (Waters et al. 2013). Low concentration of TNF has a pro-tumor function and in combination to chemokine system induces CXCR4 causing stimulation of epithelial to mesenchymal transition (Wilson and Balkwill 2002). Similarly, IL-1 was shown to cause a first wave of myc-driven angiogenesis in pancreatic islet tumor model (Germano et al. 2008). Recently, IL-1α was also shown to play a pivotal role in the pathogenesis of liver cancer (Sultan et al. 2017). The inflammatory cytokine IL-1 produced by macrophages in the tumor microenvironment converts selective androgen receptor modulators (SARM) from inhibitors to stimulators (Germano et al. 2008). IL-6 also promotes tumor growth in multiple myeloma (MM) by acting as a key growth-promoting and anti-apoptotic inflammatory cytokine (Urashima et al. 1996). IL-6 serum levels are usually high in patients with colon cancer (Gasche et al. 2011), breast cancer (Yadav et al. 2011), or hepatocellular carcinoma (HCC)

Table 13.1 Endogenous cytokines involved in cancer pathogenesis

Cytokines	Role in cancer development
TNF	Multi-functional: as pro-inflammatory cytokine, it can promote inflammation-associated carcinogenesis; as an acute-phase protein, it is cytotoxic for tumor cells
IL-1	Angiogenesis and tumor invasion
IL-6	Exacerbates chemically modulated lymphomas
IL-12	Antagonist to chemical carcinogens
IL-15	Promotes leukemia of NK cells
IFN-γ	Inhibits STAT1 and Rag2 carcinomas and lymphomas
M-CSF	Promotes cancer invasion in the breast
GM-CSF	Attenuates carcinomas and lymphomas
MIF	Inhibits p53 tumor-suppressor functions
TGF-β	Modulates various cancers
Fas-Fas ligand	Inhibits cancers of lymphoid tissues

Source: https://www.sinobiological.com/resource/cytokines/role-of-cytokines-in-cancer

(Wu et al. 2015), substantiating the fact that presence of specific polymorphism in IL-6 promoter region promotes cancer growth.

IL-12 is usually associated with anti-cancer activity and promotes the conventional T_H1 immunity (Song et al. 2000); but low concentration or defective IL-12 gene may promote differentiation toward the T_H17-type cells causing release of immunosuppressive cytokines like IL-10 and TGF-β (Gagliani et al. 2015). Deficiency in the type II TGF-β receptor is associated with metastasis and recruitment of MDSC (myeloid-derived suppressor cells) (Bierie and Moses 2010). TGF-β receptor promotes chemokine-mediated cytokine release like CXCL5 and CXCL12 (Agostini and Gurrieri 2006) facilitating metastasis via metalloproteinase activity (Wick et al. 2001). CC chemokines have a tremendous role in carcinogenesis and have been associated with the recruitment of leukocytes in tumors (Mantovani et al. 2004). Chemokines have been researched extensively to understand their role in angiogenesis and tumor promotion. Many chemokines, including CCL2, CXCL12, CXCL8, CXCL1, CXCL13, CCL5, CCL17, and CCL22, are co-produced in the tumor microenvironment. CXCL1 and related molecules (CXCL2, CXCL3, CXCL8, or IL-8) cause melanoma progression by promoting inflammation and inducing angiogenesis and neoplastic growth. Also, CCL2 was reported to play an important role in the regulation of angiogenesis. Upregulation of certain chemokine receptor also contributes to angiogenesis (Mantovani 2010). CXCR4 receptor is the most frequently upregulated chemokine receptor in cancer cells, and it is associated with advanced stages and metastasis. CXCR4 is upregulated in the downstream pathway, thus activating the von Hippel-Lindau syndrome and tyrosine kinase for the expression of oncogenes and TNF (Fanelli et al. 2012).

13.10.1 In Infectious Diseases

13.10.1.1 Human Immunodeficiency Virus (HIV)-Mediated Infection

In HIV infection, the dysfunction of immune homeostasis occurs in the overall cytokine profile of the body. There is upregulation of T_H2 cytokines like IL-4 and IL-10 and pro-inflammatory cytokines like IL-1, IL-6, IL-8, and TNF-α, while T_H1 cytokines such as IL-2 and IFN-γ are observed to be downregulated during infection pathogenesis. Moreover, an abnormal cytokine production of TNF-α, TNF-β, IL-1, and IL-6 stimulates HIV replication in T cells and monocyte-derived macrophages. Certain other cytokines which promote HIV growth like IL-2, IL-7, and IL-5 enhance HIV-1 in T cells; and M-CSF augments HIV in monocyte-derived macrophages. Nonetheless, IFN-α, IFN-γ, and IL-16 are important cytokines that suppress HIV-1 replication in T cells and in macrophages, whereas IL-10 and IL-13 inhibit it in macrophages only. Thus, IFN-γ, IL-4, and GM-CSF are bi-functional cytokines with both inhibitory and stimulatory effects on HIV infection (Kedzierska and Crowe 2001).

13.10.1.2 Influenza

Infectious influenza in humans as well as in other vertebrates is a cause of concern as now this viral disease is being heralded as *zoonoses* (jumping from animals to humans). This disease is manifested by the extensive replication of viruses in respiratory epithelial cells causing inflammation and an abrupt onset of severe pathogenesis. Growing evidences have proven that the cytokines released at the site of infection mediate many of the clinical and pathological indexes. The major pro-inflammatory cytokines that are released are interferon gamma-induced protein 10 (IP-10), IL-8, IL-1α, IL-1b, IL-6, IL-18, and TNF-α and antiviral interferons IFN-α/β and monocyte-attracting chemokines (Sladkova and Kostolansky 2006). During the viral infection, the production of many chemokines is documented; among them are normal T-cell-expressed and T-cell-secreted cytokine RANTES (Regulated upon Activation, Normal T Cell Expressed and Presumably Secreted), monocyte chemo-attractant proteins (MCP) MCP-1 and MCP-3, and macrophage inflammatory protein-1 alpha (MIP-1 alpha). Diseased humans and animals encounter concurrent rise in several of the cytokines which shows typical clinical symptoms like rise in body temperature, anorexia, and lung inflammation (Van Reeth 2000).

13.10.1.3 COVID-19

Coronavirus disease 2019 (COVID-19) is a highly infectious and pathogenic strain of beta coronavirus that causes acute respiratory distress syndrome (ARDS), often leading to lung dysfunction, arrhythmia, and even death (Wu et al. 2020). Cytokines play a key role in the development of inflammation that drives the clinical features distinct of SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2-virus that causes COVID-19) (Peiris et al. 2003; Nassar et al. 2018). During SARS-CoV-2 infection, the body responds to inflammation for an antiviral role, but a strong cytokine storm due to an unbalanced response results in lung damage and multiple organ failure (Fig. 13.7). Patients with severe COVID-19 showed significant

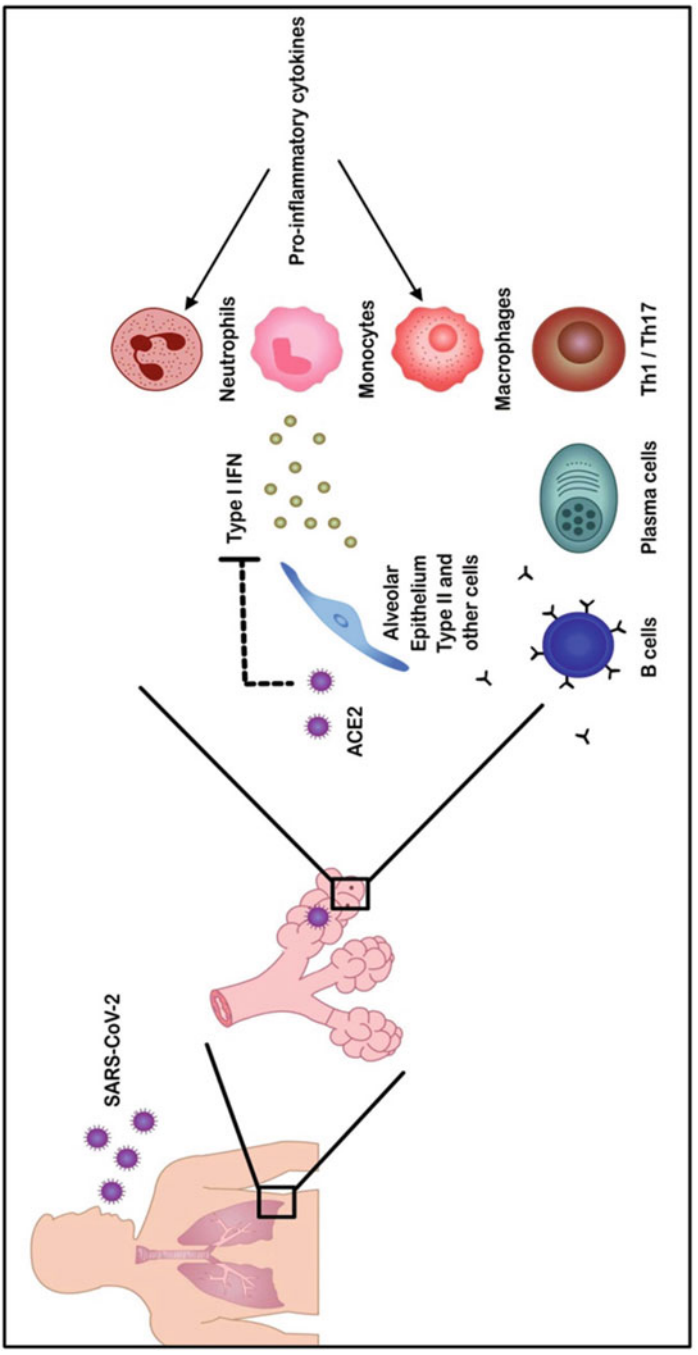

Fig. 13.7 Role of cytokines in SARS-CoV-2 infection (Source: Adapted from Prompetchara et al. 2020)

increases in cytokines such as IL-2, IL-7, IL-10, GSCF, IP10, MCP-1, MIP-1A, and TNF-α, with the characteristics of a cytokine storm (Huang et al. 2020).

13.10.2 In Other Diseases

13.10.2.1 Bronchial and Allergic Asthma

Allergic or bronchial asthma is an inflammatory disease associated with bronchial hyper-reactivity and reversible airway obstruction. T-cell-derived cytokine predominantly of the T_H2 subtype is responsible for the release of pro-inflammatory cytokines like TNF-α, IL-4, IL-5, IL-9, and IL-13 (Barnes 2008). In the asthmatic lung, IL-4 stimulates cellular inflammation by the induction of vascular cell adhesion molecule (VCAM-1) on the vascular endothelium (Steinke and Borish 2001). Thus, T_H2 cell differentiation and IgE-dependent mast cell activation are due to IL-4 production. This induces airway eosinophilia and causes bronchial hyperresponsiveness, stimulating the mucus-producing cells and fibroblasts of bronchial airways. Similar biological activities as of IL-4 are shown by the presence of increased amounts of IL-13 in asthmatic airways (Kips 2001). Another cytokine IL-5 is also responsible for the activation of eosinophils and its infiltration in the airways. Bronchial biopsy samples of asthmatics in human patients also showed enhanced expression of IL-9. IL-9 is known to stimulate the proliferation of activated T cells, promote proliferation and differentiation of mast cells, as well as direct the production of IgE from B cells. TNF-α was also noted to upregulate adhesion molecules causing leukocyte recruitment, thereby inducing cytokine and chemokine synthesis (Kips 2001). IL-12 is the essential cofactor for T_H1 development, and it primarily regulates T_H1 cell differentiation while suppressing the expansion of T_H2 cells. IFN-γ, a T_H1 marker, exerts inhibitory effects on T_H2 cell differentiation (Kips 2001). In allergic and bronchial asthma, few cytokines act in a pleiotropic manner. IL-10 is a pleiotropic cytokine that upregulates the expression of anti-inflammatory cytokine IL-1ra and suppresses the pro-inflammatory functions of cytokines like TNF-α, IL-1, IL-6, and IL-8 (Chung 2001). IL-5 in combination with IL-9 leads to excessive activation, maturation, and differentiation of eosinophils causing tissue damage (Mahajan and Mehta 2006). Among T_H2 cytokines, IL-4, IL-9, and IL-13 bind to high-affinity IgE receptors on B lymphocytes and stimulate the expression of IgE antibodies. Binding of T_H2 cytokines with FcεRI on target mast cells leads to degranulation with the release of inflammatory mediators like histamine, prostaglandin D2, and leukotrienes which in turn acts on smooth muscle cells to induce bronchoconstriction.

13.10.2.2 Fibrosis

Interstitial fibrosis is characterized by the accumulation of excessive functionally irrelevant collagenous extracellular matrix synthesized by the fibroblastic cells. The epithelial cells of fibroblast cells are hyper-activated by cytokines which stimulate the expression of multiple genes involved in extracellular matrix production and deposition of collagen and proteoglycans (Sivakumar and Das 2008). Similarly,

TGF-β contributes to fibroblast activation, collagen overproduction, and tissue fibrosis (Varga 2008). TGF-β has anti-proliferative effects in most epithelial and endothelial cells resulting in the apoptosis of epithelial cells contributing to the profibrotic growth factor that is regulated by extracellular matrix (Sivakumar and Das 2008).

13.10.2.3 Psoriasis Vulgaris and Psoriatic Arthritis

The pathogenesis of psoriasis vulgaris and psoriatic arthritis is marked by the regulation of IL-23 pathway and T_H cell induction leading to severe inflammation and hyperproliferative, poorly differentiated keratinocytes. Moreover, IL-17 and IL-22 also cause hyperproliferative keratinocytes and synoviocytes leading to cellular proliferation and inflammation in both skin and joints. Further, cytokines in synovial tissue promote the formation of osteoclast that results in bone erosion (Nograles et al. 2009).

13.11 Cytokines as Therapeutic Agents

The prospect of using purified cytokines in specific clinical therapies has been found to be very intriguing; however, there are a number of factors that make this process difficult. One of the main problems that one faces is to maintain the right or optimum dosage of the cytokine. During an immune response, the cells producing a particular cytokine are in vicinity to the target cell; hence, the concentration of cytokine is high in the region. The systemic introduction of the cytokine in the body of the patient may not ensure such a situation. Besides the half-life of most cytokines is very short (recombinant IL-2 has a half-life of 7–10 min); hence, continuous administration may be required. Furthermore, the cytokine effect is very unpredictable, and slight dosage variation can lead to undesirable side effects. However, despite all these problems, the idea of using cytokines as effective immunomodulators and therapeutic agents is gaining momentum. Interferons, interleukins, and colony-stimulating factors are now increasingly used to combat different diseases. Most of these cytokines have been developed by recombinant DNA technology (Dimitrov 2012).

13.11.1 Interleukin Therapy

IL-2 has been found to have tremendous potential to combat AIDS and certain cancers. Aldesleukin, a recombinant interleukin-2 (rIL-2), is marketed as a protein therapeutic and has a brand name as Proleukin. This recombinant IL-2 targets the IL-2/IL-2R pathway and has been approved by the US FDA for the treatment of cancers like malignant melanoma and renal cell cancer (Noble and Goa 1997; Bhatia et al. 2009; Pollack 1990). Interking is another rIL-2 and has serine at position 125 and marketed by Shenzhen Neptunus. Neoleukin 2/15 is a computationally designed mimic of IL-2 and was designed to have minimum side effects (Silva et al. 2019). It is currently being commercialized into a therapeutic. Similar effects were

also projected for TNF-α for cancer patients. However, disturbing inflammatory response produced by these cytokines was not well tolerated by patients leading to abandoning of such therapies. IL-10 was also thought to be a good option to combat autoimmune diseases, since it suppressed IFN-γ, IL-1, TNF-α, and IL-6 production as well as possessed other anti-inflammatory activities. Several trials of recombinant human IL-10 showed limited efficacy in psoriasis, RA, and Crohn's disease, but the cytokine has never been approved for therapeutic use.

13.11.2 Interferon Therapy

Interferons have been a subject of extreme research in the last 50 years due to their antiviral potential. They were later found to have immunoregulatory effects as well. They increase the cellular expression of MHC-I and MHC-II molecules and also increase the activity of NK cells. The increased expression of MHC on APCs increases their efficacy in antigen presentation in cell-mediated immunity. IFN-α has been effectively used to treat melanoma. It helps in antigen presentation and re-polarizes the immune response toward T_H1 response. It also promotes activity of T cells and NK cells against tumor cells and survival of memory T cells. IFN-α also inhibits the proliferation and angiogenesis of melanoma. IFN-α adjuvant treatment has been found to be very effective with high-risk cutaneous melanoma patients. IFN-α-**2a is** a cytokine that targets the IFNAR1/2 pathway and has been approved by FDA for subsets of patients with leukemia and sarcoma, while IFN-α-2b **(Intron A) also** targets the IFNAR1/2 pathway and has been approved by the US FDA for subsets of patients with leukemia, lymphoma, melanoma, and sarcoma. Another cytokine p**eginterferon alfa-2b (Sylatron/PEG-Intron) t**hat targets the IFNAR1 pathway has been approved by FDA for subsets of patients with melanoma. Interferons have also been found to play a very important role in combating hepatitis C virus infections (Ishikawa 2008). In fact, use of recombinant IFN-α (rIFN-α) is the first line of treatment against chronic hepatitis C. Lately, it has been found that PEGylated IFN-α (with covalently attached polyethylene glycol) and ribavirin are more effective in the treatment of hepatitis C virus. IFN-β has also been found to be useful for treating patients of MS (Rice et al. 2001), while IFN-γ has found its use in treating chronic granulomatous disease (CGD) patients (Gallin et al. 1995). Interferon therapy is generally administered for a long duration (treatment for hepatitis C generally last for 6–12 months) and leads to long-term side effects. They might also induce autoimmune disorders in the recipients. Therefore, tremendous care is taken in prescribing interferon therapy in patients.

13.11.3 CSFs

G-CSF has been widely used to overcome neutropenia in patients who have been administered chemotherapy that causes extreme low levels of WBCs and myelosuppression. Neutropenia can also be the result of some drugs (e.g., clozapine

used in schizophrenia) (Myles 2017) or could be an effect of stem cell transplant in a donor. However, side effects are usually common after treatment with GM-CSF and G-CSF. The recombinant human G-CSF (rhG-CSF) is called filgrastim (brand name Neupogen) and treats low neutrophil count. Lenograstim (brand name Granocyte) is another recombinant granulocyte colony-stimulating factor that functions as an immunostimulator. GM-CSF has also been found to be useful in the treatment of Crohn's disease.

13.11.4 Other Cytokines

Erythropoietin (EPO) is also used in patients with anemia and bone marrow failure. Bone morphogenetic protein (BMP), an important group of growth factors, is used to treat bone-related conditions.

13.12 Cytokine Antagonist Therapy

Cytokine antagonists are the proteins that inhibit the activity of cytokines. They are mostly found in the bloodstream or in extracellular fluids. They either bind to the cytokine receptors (Fig. 13.8) or bind directly with the cytokines and obstruct the activation process, e.g., IL-1Ra (IL-1 receptor antagonist) binds to IL-1 receptor and

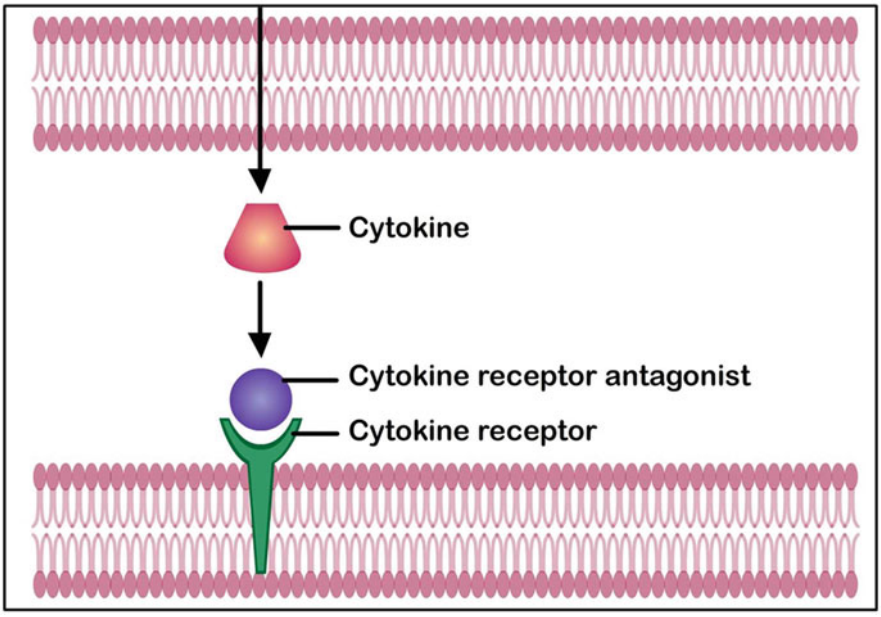

Fig. 13.8 Action of cytokine antagonist

inhibits binding of IL-1 cytokine. Certain viruses like EBV (Epstein-Barr virus), cytomegalovirus, etc. imitate these cytokines and manipulate the immune system. EBV produces an IL-10-like molecule that gets bound to IL-10 receptor and suppresses T_H cell-mediated response that is generally produced when the body is invaded by intracellular parasites like viruses. Pox viruses produce IL-1 binding protein and TNF binding protein and thus hinder cytokine activation process. Human herpes virus produces IL-6 homolog that binds to IL-6 receptor protein. It also produces homologs of chemokines MIP-I and MIP-II. The presence of cytokine antagonists is a very important modification in viruses to overcome the barrier of host immunity. Of late, these antagonists have found a great use in clinical practice with positive results. Use of etanercept, a TNF-α antagonist, in refractory asthma patients for 12 weeks has led to great improvement in asthma control and systemic inflammation (Morjaria et al. 2008).

13.13 Adverse Effects of Cytokine Therapy

13.13.1 Cytokine Storm

A storm in literal term indicates danger or change or foreshadows something terrible on the horizon. It symbolizes an interpretation that is based on the imagery and description the author uses for the storm event and the reaction of the people who endure the storm. In scientific literature, the word "cytokine storm" has captured the attention of both scientific community and the media, though the actual meaning is somewhat misleading. In general, cytokine storm means uncontrolled and excessive release of pro-inflammatory cytokines. Proper understanding of what tips the storm to precipitate as pathogenic or the molecular events that lead to such an event is still lacking. Of late, much focus is being laid on the anti-cytokine therapies to quell or prevent this storm from causing mortality in patients especially with respiratory infections (Fig. 13.9).

The term "cytokine storm" gained precedence when it was first applied for H5N1 influenza virus infection in 2005 (Yuen and Wong 2005). Though the term first appeared in an article on host-graft rejection in 1993 (Ferrara et al. 1993), it was associated with many infectious and non-infectious diseases as well as some failed attempts of cytokine therapy (Suntharalingam et al. 2006). Before the early 2000s, scarce reports were available which mentioned about cytokine storm in diseases mainly pertaining to autoimmune disorders like MS (Link 1998), pancreatitis (Makhija and Kingsnorth 2002), or multiple organ dysfunction syndromes (Wang and Ma 2008). Occasional reviews were written on the cellular and molecular mechanisms assisting to the cytokine storm in viral disease (Hussell and Goulding 2010; La Gruta et al. 2007), some of which specifically focused on influenza (Peiris et al. 2009, 2010). It later found more usage in reports of many diseases, namely, *Cytomegalovirus* (Barry et al. 2000), *Epstein-Barr virus*-associated hemophagocytic lymphohistiocytosis (Imashuku 2002), group A *Streptococcus* (Bisno et al. 2003), influenza virus (Yokota 2003), *Variola* virus (Jahrling et al. 2004), and severe acute

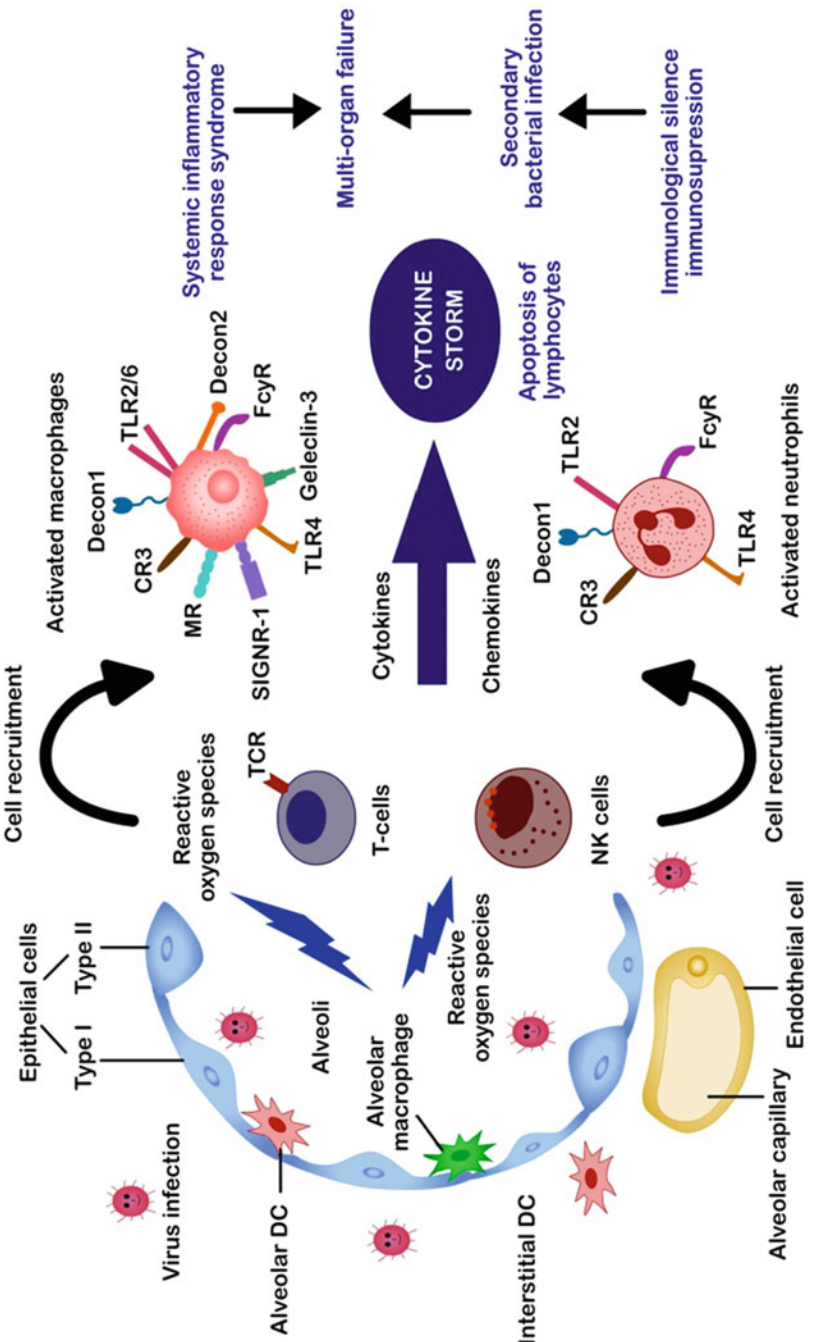

Fig. 13.9 General representation of "cytokine storm"

respiratory syndrome *Coronavirus* (*SARS-CoV*) (Huang et al. 2005). There is a strong belief that cytokine storm was the main cause of mortality in "Spanish flu" that was due to H1N1 influenza A virus and lasted more than 2 years from February 1918 till April 1920 and almost killed one-third of world's population. More recently, it drew enormous public interest worldwide on the much famous pandemic of COVID-19 due to *SAR-CoV-2* (Ragab et al. 2020).

13.13.2 Cytokines Involved in "Cytokine Storm"

The phenomenon of cytokine storm is quite an enigma to understand as the major groups of cytokines fall within a complex network of pathways with considerable degree of redundancy and overlap. This makes it complicating to identify the key steps in the cytokine response to infection and in targeting specific cytokines for therapeutic intercession. Moreover, most of the infections follow broadly the same cytokines but differ in their clinical presentations. Broadly, the cytokines that are involved in the cytokine storm are categorized into groups as given in Table 13.2.

13.13.3 Dynamics of Cytokine Storm

Immunology as a branch of science has come a long way; yet, we have not understood its complex nature and probably have underestimated its dynamic nature during acute infection. A normal viral infection usually activates the inflammatory pathways in the host immune system, though an anomalous or embellished response may lead to severe uncontrolled immunological response (Braciale and Hahn 2013). Innate immune response is characterized by the recognition of special molecular patterns, referred to as PAMPs, present on the invading pathogens by the PRRs. Specifically among the PRRs, Toll-like receptors (TLR) and intracellular receptors for DNA and RNA (NOD-like and RIG-I-like receptors) bind to microbial products and elicit innate immune response (Imai et al. 2008; Jiang et al. 2005). Subsequently, this results in the activation of several signaling pathways and transcription and expression of those genes involved in the coding of innumerable pro-inflammatory cytokines, chemokines, and adhesion molecules. The major pathways that are

Table 13.2 Cytokines involved in cytokine storm along with their mode of action

Type of cytokines	Actions
Interferons	Regulation of innate immunity, activation of antiviral properties, anti-proliferative effects
Interleukins	Growth and differentiation of leukocytes; pro-inflammatory
Chemokines	Control of chemotaxis, leukocyte recruitment; pro-inflammatory
Colony-stimulating factors	Stimulation of hematopoietic progenitor cell, proliferation, and differentiation
Tumor necrosis factor	Pro-inflammatory, activates cytotoxic T lymphocytes

activated by PRRs are interferon response factors 3 and 7, protein activation 1, and nuclear factor-Kβ. The end event is finally to recruit plasma proteins, APCs, and leukocytes to the site of infection to combat the eliciting infection (Thompson et al. 2011). Endogenous inflammatory responses are also related to mitochondrial membrane proteins and cellular ATP in releasing massive amount of cytokines (Zhang et al. 2010).

Most evident fallacy of the scientific and the medicine community is to characterize any pathogenesis based on direct measurements of a few cytokines and chemokines in the peripheral blood compartment. Record studies have failed to understand the entire immune cascade in the context of the infecting pathogen and the rapidly changing immune environment in localized tissues, where the infection has led to the severity of local and systemic cytokine storms. For example, the basic difference between a normal influenza virus and that of SAR-CoV is that influenza virus infects the epithelial cells of the conducting airways, whereas SARS-CoV infects type II pneumocytes in the alveolar walls; yet both cause acute lung injury (ALI) due to sepsis, respiratory failure, and heightened cytokine storm (Shimizu 2019; Ishikawa 2012). Similarly, some cytokines act in a pleiotropic fashion; TNF promotes IL-1 production within the endothelial cells of local microenvironment (Nawroth et al. 1986), but also can spill over into the systemic circulation, providing direct communication between the lungs and the bloodstream (Kurahashi et al. 1999). So, it is imperative to comprehend immunopathology of the infection within the deep tissues rather than rely on intermittent sampling from one compartment (typically the peripheral blood) (Cillóniz et al. 2009; Lee et al. 2010; Shinya et al. 2006). Likewise, in infections of the central nervous system (i.e., bacterial and tuberculous meningitis, encephalitis, and fungal infections) and in infections such as dengue, in which the clinical syndrome is dominated by capillary permeability and plasma leakage (Simmons et al. 2006, 2007), studying the compartmentalization of the tissues becomes more important.

Thus, regulation of both pro- and anti-inflammatory cytokines (e.g., TNF and IL-1α) and their receptors (TNFR1, TNFR2, and IL-1R) should be balanced to bring down the cytokine storm (Park et al. 2001). Some specific cell types may be involved in dampening the inflammation, e.g., alveolar macrophages express CD200R that helps restrain macrophage activity and decrease lung inflammation during influenza virus infection (Snelgrove et al. 2008). Aberrant TLR expression can be controlled by negative regulators such as Toll-interacting protein (TOLLIP), phosphoinositide-3-OH kinase (PI3K), IL-1-receptor-associated kinase (IRAK-M), suppressor of cytokine signaling 1 (SOCS1), and zinc finger protein A20 (Coornaert et al. 2009). Another way to check pro-inflammatory responses is to overexpress anti-inflammatory cytokines like IL-10 by macrophages (Snelgrove et al. 2011) and certain types of T cells (T_H2 and regulatory T cells) and B cells (Moore et al. 2001). Thus, a balance between pro- and anti-inflammatory mechanisms is critical in maintaining immune homeostasis, and it has been noted that if either one or more of these regulatory mechanisms are absent or aberrantly regulated, it contributes toward cytokine storm (Fig. 13.10).

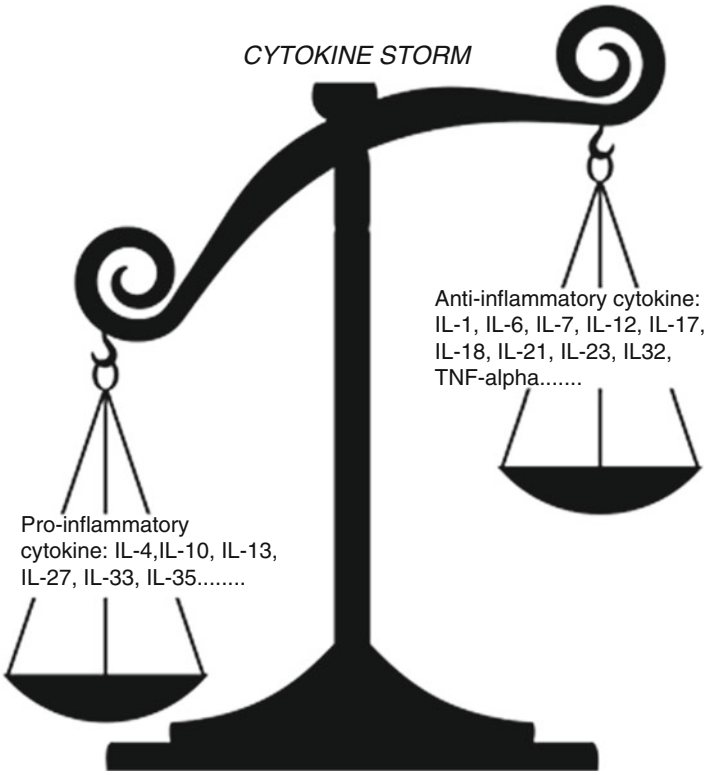

Fig. 13.10 Cytokine storm-disturbed balance between pro- and anti-inflammatory regulators

13.13.4 Challenges for Current Immunotherapies

The past few decades have witnessed certain emerging pathogenic infections; be it the H5N1 flu, dengue, or the recent SARS-CoV-2 (COVID 19), all are measured by a powerful and potentially damaging immune response that self-inflicts injury to the host in response to these infections (Simmons and Farrar 2008). In view of this, it will be more prudent to target the excessive immune responses during acute infections that have proved to be extraordinarily difficult and largely unsuccessful. In simple terms, during any infection, certain elements of the immune response enhance at times and get suppressed at other times. The immune system may behave differently at an early stage of infection when the pathogen is dividing rapidly and reaching high infectious loads, than possibly at a later stage when the pathogen either has reached a steady-state or the pathogen load has fallen due to some therapies or even cleared from the host's system by the immune response. Knowing this complex system is more essential in treating patients with the "right" immunomodulating agent at the "right time," or else it could worsen the clinical effect. We are still too far to understand these intricate details of the rapidly changing immune response, and

until we do, it is doubtful that any rational therapies that target the exact phase of the immune cascade are developed. Moreover, individuals respond differently to infections, and individual responses to immunomodulators and adjunct therapies also vary (Tobin et al. 2010). It might be that within the long list of adjunct interventions that have been tried in acute severe infections, therapies exist that would be successful if only the immune system dynamics were understood so that they could be used at the right time and their pharmacological profile fits the need for a potentially short-acting drug.

A variety of anti-inflammatory drugs and adjunct approaches have been devoted to the treatment of many severe infections using anti-cytokines and anti-chemokine agents, corticosteroids, aspirin, statins, and the celebrated plasma therapy and mAbs (Meijvis et al. 2011). Many immunomodulatory drugs that diminish inflammation during infection show therapeutic benefits. Notwithstanding these efforts, none has been proven to be completely operative and in some cases was seen to adverse the outcome, for reasons that remain elusive (Brun-Buisson et al. 2011). Malaria too evokes a powerful immunological cascade, and many interventions targeting the host immune response have been tried over the past 20 years. But both the artemisinin drugs and other treatments with chloroquine have failed owing to drug-resistant malarial parasites (Bate et al. 2008; Dondorp et al. 2009). Similarly, drugs for dengue with anti-cytokine therapies and corticosteroids did not show any benefit. Sphingosine receptors play an important role in innate immune responses by activating the production of cytokines like IFN-α, CCL2, IL-6, TNF-α, and IFN-γ (Teijaro et al. 2011); thus, sphingosine analogs are being considered as a potential therapy to control the cytokine storm caused by influenza virus (Marsolais et al. 2009). Though these sphingosine analogs (S1P1-selective agonists, CYM-5442, and RP-002) inhibited the influenza virus in the epithelial lining, it has also been noted that they increased susceptibility to *Cryptococcus neoformans* influenza virus and respiratory syncytial virus (Williams et al. 2005). Sometimes, it was shown that an inhibitor alone may not be effective, but in combination with another agonist may decrease the mortality of the infected individual (agonists are generally small molecules included in the immunotherapy and produce a synergistic effect and reduce cytokine dysfunction and eventual mortality). Cytokine storm appears to be one of the main consequences of mortality in the recently declared pandemic of COVID-19. Therapeutic approaches to managing the COVID-19 cytokine storm might provide an avenue to decrease the COVID-19-associated morbidity and mortality and are the focus of upcoming studies.

13.14 Cytokines as "Targets" for Immunomodulation by Medicinal Plants

Cytokines are the chief mediators of cell differentiation, inflammatory response, immune pathology, and regulation of immune response. It is very important that a perfect balance is struck between pro-inflammatory and anti-inflammatory cytokines to develop a well-regulated effector immune response. Immunomodulators, either

natural or artificial or synthetic, are generally used when either the immune system is inadequate to reduce the infection or the immune system is showing highly exaggerated response to various diseases like autoimmune disease (Bascones-Martinez et al. 2014). Many of these immunomodulators have the ability to affect the cells that are mainly involved in the synthesis of soluble extracellular mediators like cytokines. Certain medicinal plants have been found to have the ability to modify the concentration of different cytokines in the body and therefore help in treating diverse diseases.

A plant named as *Astragalus membranaceus* also called as spleen chi tonic is used in various diseases. The root extract of this plant has the ability to lower the level of IL-6 in the human body. Similarly, *Allium sativum* (garlic) is also found to lower IL-1 and IL-6. IL-6 is mainly secreted from the immune cells during the inflammation. These plants have anti-inflammatory and anti-oxidant activities (Spelman et al. 2006). *Aloe vera* is a popular plant grown in arid climatic region and has anti-inflammatory activities. It mainly reduces the level of TNF-α and IL-6 in the human body and is mainly used in wound and burn healing. Acemannan (polyacetylated mannan) is the major carbohydrate obtained from the gel of *Aloe vera* (Agarwal and Singh 1999) that enhances the production of IL-1 and TNF-α from the macrophages. Curcumin is the most important medicinal plant and is studied for its immunomodulatory properties. It functions by reducing inflammatory responses by inhibiting the nitric oxide (NO) production, cyclooxygenase-2 (COX-2), nuclear factor-kappa β (NF-Kβ), IFN-γ production, and TNF–αactivated macrophages. The polysaccharide of *Juniperus scopulorum* had potent immunomodulatory activity. This polysaccharide leads to the activation of macrophages for an enhanced respiratory burst, stimulates NO production via the induction of nitric oxide synthase, and induces the macrophage to secrete both inflammatory (IL-1, IL-6, TNF-α) and anti-inflammatory (IL-10) cytokines. The genus *Juniperus* belongs to the family Cupressaceae. The crude extracts of *Juniperus* exhibit anti-inflammatory, anti-bacterial, and anti-tumor activities. The polysaccharide consists of arabinogalactan type 2 structure which is highly branched and composed of 3- and 3,6-linked galactose (Schepetkin et al. 2005). *Allium sativum* contains the organosulfur compounds, and it inhibits the growth of tumors and helps in modulating the various kinds of chemical carcinogens. It also helps in the activation of NK cells and T lymphocytes and enhances the production of IL-2. *Azadirachta indica* (neem) possesses nonspecific immunostimulatory properties. It stimulates the production of IL-1, IFN-γ, and TNF-α and also activates T_H1 type of response (Agarwal and Singh 1999). *Tinospora cordifolia* (giloy) possess immunomodulatory activity. The (1,4)-alpha-d-glucan derived from giloy activates human lymphocytes with downstream synthesis of pro- and anti-inflammatory cytokines (Saha and Ghosh 2012).

13.15 Conclusion

Cytokines are miniscule biomolecules, mainly proteins that communicate between cells and play a broad role in the body's response to inflammation and immune attack as well as to certain drugs. Strangely, cytokines may be "good," "bad," and even "ugly." They are "good" when they fight against foreign pathogen or cancerous cells and even to bring down overwhelmed immune responses like reducing IFN-β during neuron inflammation in patients with MS. They are "bad" if their overexpression causes inflammatory diseases, for instance, increased TNF-α in RA or asthma and Crohn's disease. Recently, much research is focused on the "cytokine storm" that had caused irreparable harm and mortality in patients infected with COVID-19. Cytokines can even turn "ugly," for example, if certain therapeutics go haywire, they cause more harm than good by releasing harmful and unnecessary cytokines. Therefore, therapeutic modulation of cytokine expression should be carefully assessed before they are considered effective therapies to treat unmet needs. Cytokines are also called immunomodulators, as these are involved in immunotherapy by inducing, enhancing, or suppressing an immune response to treat diseases. These molecules have come a long way, and now there are a diverse array of recombinant, synthetic, and natural preparations of G-CSF, interferons, and cellular membrane fractions from bacteria that are used to treat patients. Innumerable clinical and preclinical studies have already established certain cytokines like IL-2, IL-10, chemokines, and interferons as future therapeutics. Better understanding of the dynamics of the immune system vis-à-vis cytokine production and their role at different concentrations will help in producing effective treatment of different diseases in times to come.

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Immunomodulatory Properties of Proteins and Peptides: Food Derivatives Approach

14

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Abstract

Food represents a millennial source of multiple molecules with potential as health enhancers, not only from a nutritional point of view, and proteins are described as one of them. Proteins and their derived peptides could interact in a wide range of biological levels but claim attention as immunomodulating agents. The immunomodulatory system represents a key component to maintain human health, and peptides and protein from exogenous sources could intervene in different immune response stages. This chapter aims to analyze the food-derived proteins' and peptides' role in the immune response with an emphasis on their employment as health promoters, the involved mechanisms, and their potential incorporation in products.

Keywords

Food proteins · Food peptides · Immunomodulatory action

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14.1 Introduction

Proteins constitute one of most abundant molecules; being part of at least a 50% of cell weight (Onwulata and Qi 2004), these macromolecules are described as a biopolymer, integrated by simplex nitrogenated compounds named amino acids. The main structure is displayed in Fig. 14.1, composed of a central carbon atom joined to an amino group (N-terminus) and a carboxylic group (C-terminus), both present in amino acidic unit, and substituted with a radical (R*), being substituted in each amino acid (Emery 2013).

Multiple amino acids are linked by peptide bonds to form protein structures (Fig. 14.1), and the quantity, variety, and extension of amino acids form the high variety of proteins; and establishing their primary structure (Emery 2013). This linear amino acidic sequence generate a group of intra-molecular forces that lead to create spatial conformations, denominated as secondary and tertiary structures, while when alternative polypeptide sequences interact is denominated as quaternary structure (Ustunol 2015a).

Proteins represent ubiquitous molecules, and shows diverse functionalities, acting as molecular transporters, in the case of myoglobin; generating support in the case of skin and muscle proteins, acting as key molecules in the immune system like antibodies, likewise in multiple cell receptors, being a critical step in biological reactions, or well as essential biological catalyzer like enzymes (Ustunol 2015b); but further, represent a vital ingredient in the human diet.

Proteins consume, provide amino acids, which supply the nitrogen needed for protein synthesis, promote growth and esnure biological maintenance; where human body specially needs the named essential amino acids (nine amino acids), and in consequence, is forced to supply it through food consume (Onwulata and Qi 2004), the recommended protein intake varies in accordance with parameters as age, gender, or situations as pregnancy or lactation stage, body weight, physical activity, lifestyle or health conditions, being from 0.8 to 1.0 protein g per kg in healthy adults.

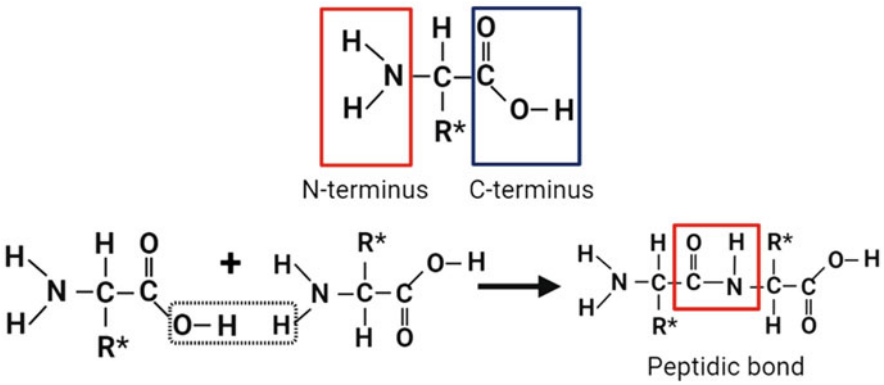

Fig. 14.1 Protein's basic structure

However, the efficiency in protein intake is related to other kinds of protein parameters as bioavailability, digestibility, amino acid profile, purity, antinutritional factors' presence, and processing (Gomes et al. 2020).

Proteins present in food, are not only important since a nutritional approach, also posses many techno-functional and organoleptic features (Foegeding 2015), this attributed mainly to physicochemical properties derived from protein composition and their interactions, and traduced in characteristics as solubility, water holding capacity, oil or fat binding, emulsifying capacity, stabilizing or gelling properties, which could intervene during preparation, processing, storage and consumption of proteins and foods prepared with it (Haque et al. 2016), making proteins a suitable and highly important ingredient in food processing and food develop; nevertheless, the proteins, also could be carriers of sequences, which exhibit important biological functionality; denominated as bioactive peptides.

Bioactive peptides, are defined as amino acidic sequences generally with extension from 2 to 20 amino acids, normally trapped in parental proteins wich could be released by proteins cleavage, using diverse methodologies as simple gastrointestinal digestion, or by exogenous or endogenous enzymes, chemical substances, microbial action or emergent technologies (Hernández-Almanza et al. 2017; Martínez-Medina et al. 2018), these sequences could play antimicrobial, antihypertensive, antioxidant, anticoagulant, hypocholesterolemic, opioid, mineral, antiappetizing or immunomodulatory effects (Korhonen and Pihlanto 2006), and could be adquired trough ingestion of traditional, functional or fermented food, or by new food-derived compounds as nutraceuticals consumption.

Recently, has been emerged the concern for elucidating how food, or their ingredients, could modulate health state after consume. Health promotion by food is normally, attributed to a wide type of molecules, which specially target to multiple body illness or tissues. However, just a few of studies have been focused in a very special area which contributes to the improvement and maintenance of human health: The immune system.

The immune system comprises a very complex group of cells, molecules, tissues, and mechanisms, which main role consists, in the differentitation between self-components and non-self-components, also in the identification and attack of hazardous potential components as bacteria, fungi, viruses, parasites or tumor cells (Descotes 2014), proportionating protection to the host; and which homeostasis and correct functionalization depends in several factors including nutrition, where their efficiency could be affected by food composition, variety and quality, and where components as micronutrients, carbohydrates, fat and proteins consume, maintain a close relationship with diverse components in immune system (Redondo et al. 2018); nevertheless, this key part of defense system in the body, is not exempt of failures, which could result in serious illness as cancer, abnormal progression of other ailments derived from diverse etiologic agents, or even an adverse response against self-components (autoimmunity); or increase the risk of various cardiovascular diseases or cerebrovascular disorders, (Chalamaiah et al. 2014; Harun et al. 2020; Yuan et al. 2019).

The immune system could be negatively affected by oxidative stress, malnutrition, infections and immunosuppressive agents (chemotherapy drugs); also diverse immunomodulating agents are applied at clinical levels, such as actinomycin, vincristine, dexamethasone, levamisole and Thymosin α1, however, they are related to important adverse effects, being a priority the employment and discover of immunomodulating agents from natural sources (Chalamaiah et al. 2014; Gao et al. 2019; Harun et al. 2020; Yu et al. 2020).

Proteins and their derivates, has been reported as potential immune modulators (Chalamaiah et al. 2014). One of the primary examples is the breast milk, recognized as the first food consumed; and where bioactive peptide chains with immunostimulant activities, has been identified (Wada and Lönnerdal 2014), anyhow, also has been detected in animal milks (Cai et al. 2020), diverse marine animals (Kang et al. 2019; Li et al. 2019), egg (Lozano-Ojalvo et al. 2016), soy (Ashaolu et al. 2017), rice, wheat and amaranth (Chalamaiah et al. 2018), among others (Kiewiet et al. 2018) and thus make food, and specially food protein, a remarkable field of study for novel immune modulators obtention.

The aim of this chapter is to analyze and expose how food proteins and their derivates could participate in the modulation of the immune system, for health improvement, and how they could be employed as a smart alternative as co-adjuvant treatment, improve or prevent immune irregularities.

14.2 Immune System

The immune response is the mechanism used by body as defensive reaction against foreign pathogens such bacteria, viruses, fungi, protozoa, and others, or even against cancer cells. This system is driven by specialized cells like macrophages, dendritic cells (DC), natural killer cells (NK) and T γ δ cells, tissues, and organs, and supported by various barriers such as anatomical mediators (skin, mucosa), physiological (low pH, temperature and chemical mediators) and inflammatory components (cytokines, interferons, complement, defensins, leukotrienes, acute phase proteins, and prostaglandins) (Moriber 2014) that eliminate or avoid the survival of exogenous or altered self-components.

The immunomodulatory effect of food derived peptides, has been evaluated using in vitro (cell lines) and in vivo (animal models) techniques, among these tests the mouse macrophage cell line (RAW 264.7) THP-1 (human monocytic cell line) and Jurkat T cells (model of human T lymphocytes) are commonly used (Ahn et al. 2015; Cai et al. 2013; He et al. 2015; Masotti et al. 2011; McCarthy et al. 2013; del Carmen Millán-Linares et al. 2014).

These assays have resulted in increased immune response, stimulating signaling molecules such as cytokines, including tumor necrosis factor-alpha (TNF-α), IL-1β, and IL-6, and nitric oxide (NO), which have important functions in the modulation of the immune response (Ahn et al. 2012, 2015; Chalamaiah et al. 2018).

The immune system is mainly divided into two main categories: innate or no specific and adaptive or specific immunity (Chalamaiah et al. 2018; Parnham and

Nijkamp 2019), despite both branches of immunity response are different, this mechanism act in total synergism; also, numerous immune response mediators possess proteinaceous nature, supplying the opportunity to proteins and their derivates to impact on the quality, efficiency, and direction of immune responses (Acosta et al. 2019; Mao et al. 2020).

14.2.1 Innate Response

Innate immunity is the first defense step in immune process; it also uses indirect mechanisms like mucus, antimicrobial agents, epithelial cells, microbiome, low pH, enzymes, and antimicrobial peptides as primary barrier; nevertheless, if the hazardous agent trespass it, the phagocytic cells go into action with a simultaneous production of diverse cytokines, chemokines and proteins providing an inflammatory response, calling other kind of immune cells (T cells), and activating or giving rise to the adaptive immune response (Chalamaiah et al. 2018; Chase and Lunney 2019).

Innate immunity is performed by cells as phagocytes, which main labor is to absorb, kill and dissolve bacteria, control viruses, fungi and carcinogenic cells presence, classified in: The granular leukocytes, (granulocytes) characterized by a group of granulomas charged with diverse substances, (neutrophils, basophils, mast cells and eosinophils), and mononuclear phagocytes (blood monocyte tissue macrophages, histocytes or migrating macrophages and dendritic cells); (Chase and Lunney 2019).

The neutrophils, are normally present in blood stream and represent the 90% of total granulocytes, being the phagocytosis of foreign microorganisms their main function, for posterior enzymatic lysis (Levy 2004); other granulocyte type, are the eosinophils, constituting among 4–7% of circulant granulocytes, and involved in the immunity against parasites, their granulomas content proteins and peroxidase; while basophiles correspond to near of 0.2% of circulating granulocytes, with granulomas filled of heparin, histamine, neutral proteases and Tumoral Necrosis Factor- α (TNF-α), and finally the mast cells, their granuloma's also are charged with tryptase and this cells remains protecting mucosal surfaces or in connective tissues, both important effector in allergenic responses (Levy 2004).

The other class of innate response cells: Monocytes, constitues cells produced in bone marrow, and released to blood stream, which migrate to diverse tissues, becoming in to tissue macrophages or dendritic cells, and swallowing and digesting foreign large particles, debris and dead cells, and connecting the other part of immune system, by "antigen" presentation (AP) procedure which mainly consists in the degradation of foreign particle in to a proteinaceous signal which could be interpreted and processed by lymphocytes T (A key component in adaptative response) (Chase and Lunney 2019; Levy 2004); other class of lymphocytes are the Natural Killer, cells specialized in to eliminate tumor cells and viral-infected cells, without the demand of an antigen, nevertheless posses a specialized receptor that allows the recognition of sugars which act as markers in this pathogenic scenarios,

and finally recruting the adaptative response (Chase and Lunney 2019; Davies 2013).

Jørgensen et al. (2010), explore the effect of intact and enzymaticaly hydrolyzed bovine colostrum, focusing in the low molecular weight fraction. They observe that in murine intestinal cells (mIC_{12}), both type of proteins, could stimulate the immune response in a dose-dependent behavior at the presence of bacterial ligands (peptidoglycan and lipopolysaccharides). Nevertheless they remark that, the behavior is not only related to the type of immunostimulant protein, also is mediated by the class of stimulant ligand which emulate the class of pathogen that immune system comabts. This studies demonstrate, that the employment of this class of immunomodulators need to be extensively tested, but would be adjuvant in diverse infection treatments.

Hydrolyzed proteins also, could contribute to cease inflammatory response started by macrophages, normally exacerbated in pathologies or amplified by high stress environments. (Qian et al. 2020) use soft oyster muscles hidrolyzed with pepsin, trypsin and Maxipro™, for peptide obtention. The peptides inhibits the inflammatory mediators production in RAW264.7 cells. Peptides not present cytotoxicity and shows antioxidant properties, highlighting the fact the peptides could present multi-functionality, and contribute to decrease other pathologic states, that synergically contribute to inflammatory procedures.

Also has been reported the employment of food by-products for peptide production, making suitable the production of food derived immune-modulators, as Ren et al. 2015, which probe the in vivo efficiency of hazelnut dregs (hazelnut oil extraction by product) derived peptides, produced using Alcalase. They incorporate in long term (30 days) mice feeding, and observe that promote in a general way an improvement in diverse immunological indicatives, but remarking the potentiation of macrophage activity after 10 days of treatments.

14.2.2 Adaptative Response

Adaptive immunity, also called specific immunity or acquired immunity, is prominently specific for highly dangerous foreign antigens. The adaptive response is divided into two types, cell-mediated or cellular immunity and antibody-regulated or humoral immunity. The lymphocytes (T cells) and B lymphocytes (B cells) represent the main elements of the adaptive immune system. In humoral immunity, the B lymphocytes in interaction with specific antigens respond to the production of antibodies (Chalamaiah et al. 2018), which constitute a group of key molecules, identified as soluble proteins that are joined explicitly to antigenic materials and promote their neutralization, inactivation, or removal (Booth 2007).

The adaptative immunity, starts with the previosly presented antigen by innate immunity cells. Antigen is regularly a part of invader organism, with variable nature (carbohydrate, lipids, proteins, or nucleic acids) (Brenner and Miller 2001; Stewart 2004) which could stimulate the adaptative response components, which generate memory and amplifies the responses when the individual is exposed to the same

antigen. Likewise, this immunity can generate optimal responses for defense against the presence of different microorganisms (Toche 2012).

For adaptative response, the effector cells are lymphocytes, classified as T and B. Lymphocytes T are developed in thymus; and are classified as cytotoxic T lymphocytes, that lead to eliminate tumor and virus-infected cells; the T helper lymphocytes, which produce diverse cytokines, sub-divided in TH1 that promote the inflammatory and immune response against intracellular pathogens; TH17 produce inflammatory and immune response against extracellular microbes, TH2 promote the B-cells stimulation/activation, TH9 recognized as proinflammatory and T regulatory cell which are immune response suppresser, but also exist a T follicular helper and repressor cells which contribute with particular help for B cells in lymph nodes (Davies 2013; Tang 2016).

Otherwise, lymphocytes B, are developed in bone marrow. The differentiation in this class of cells, are related to the type of transmembranal receptor named immunoglobulin (Ig) present, this receptor has protein nature, and are classified as IgG, IgA, IgM, IgD, and IgE; which serve as antigen receptor (Buttriss 2002; Tang 2016). Mature lymphocyte possess IgM and IgD on their surface, and the other isotypes are produced after activation by antigen (Rieber 2011). When B cells confronts an antigen, they clone their self, and the cloned cells are recognized as memory cells, with a longer survival rate; and the parental B cells (Plasma B-cells) generate soluble immunoglobulins (antibodies) which survive, until pathogen was eliminated (Frenette and Dixon 2019). The Ig's generated by B-cells are produced in different stages, Ig M is produced as general primary response, while IgG is produced in secondary response, but during bacterial and viral infections, the IgA is produced specifically in mucosal membranes, in addition IgE is produced specifically in parasitic infections and finally IgD is produced in allergy reactions (Stewart 2004).

This short-explained route demonstrates that the adaptative immunity is a highly specific and structured response against resistant pathogens, which could be improved or assisted by food-derived protein molecules.

The immunomodulatory work of bioactive peptides, is mainly based, in the regulation of chemokines and cytokines, enhancement of antibodies synthesis, stimulation of immunoglobulins and the reactive oxygen species mediation, also, promote proliferation and maturation of immune cells, such lymphocytes, likewise activate NK cells and phagocytosis of macrophages, and immobilize inflammatory compounds (Görgüç et al. 2020; Maestri et al. 2016; Rodríguez Hernández et al. 2014).

Also, bioactive peptides have shown inhibitory effects in food allergy processes, modulating local immune responses of T and B cells, stimulating regulatory cell subpopulations and cytokines, and interrupting the gene expression of intestinal pro-inflammatory mediators (Fernández-Tomé et al. 2019), and indirectly the antioxidant properties of biopeptides have been shown to contribute positively to immunological function. These peptides stand out for their ability to reduce oxidative stress, related to aging and cell damage (Herrera-Ponce et al. 2019).

During early studies, Boon et al. (2002) analyzed at least 25 compounds and their effect in in vitro immune response against influenza virus. They includes N-acetyl-cysteine (NAC) and Acetyl-L-carnitine (both amino acids) and their effects in involved immune procces. NAC increase the specific lymphoproliferation, promote the production of inflammatory cytokines, and improve the cytotoxic effect of T-cells; while, other authors as De Rosa et al. 2000, analyze NAC consume effects in HIV-infected patients, NAC improve their immune response. The authors belive that the dipeptide could be the base of Glutathione (GSH) production, which is a tripeptide (γ-glutamyl-cysteinyl-glycine) and potent antioxidant; in other works, has been reviewed that lymphocytes, and their key reactions as DNA synthesis, are high sensitive to oxidative stress, constituting the consume and presence of cysteine crucial fact in their development.

The use of foods as generators of immunomodulant agents represent an attractive alternative, as An et al. 2020 demonstrate, using *Spirulina platensis* as a matrix for fermentative proccedure, using the probiotic strains *Lactobacillus plantarum* B7 and *Bacillus subtills* 168. Where the low molecular weight protein fraction, could promote in vitro cell differentiation of T lymphocyts TH1 and TH2 cells in murine splenocytes, and their proper cytokine production, which present a dose-dependent behavior and possible synergistic effects with immune stimulants as Concanavalin A.

14.3 Gastrointestinal Tract Protein Interactions

The immune system possess multiple working mechanisms, as the mucosal and epithelial barriers, both present in pulmonary, genitourinary, and gastrointestinal (GI) systems. This is referred as a mucosal immunologic system, and integrated by a specialized network of cells, tissues, and signaling components. Nevertheless, GI system generates the largest interaction within the exogenous components; normally underestimated and only focusing in their nutritional importance; but is a key component during individual defense (Jakaitis and Denning 2015).

The GI system, possess direct and indirect defensive instruments as Fig. 14.2 shows, since stomach acidity, mucus over the epithelial barrier, antimicrobial peptides, peroxidase to peristaltic movement. However, the epithelial barrier is recognized for trigger an important immune response, due to the presence of cell receptors, that could identify pathogens and commensal communities presence, and further activate the signaling process through pro-inflammatory cytokine production, (Chang and Shanahan 2018), if well the entire immune response, is affected by multiple factors, the consumed food is one of them, the diversity and extensive checkpoints that are part of the GI system, and their intimate correlation, make them, the first step in employ natural derived-molecules for enhancing health.

One of first interactions from individuals, with food components and with proteins as immune system effectors, is during breast feeding, providing passive protection and help to develop a health immune system in newborns, making less

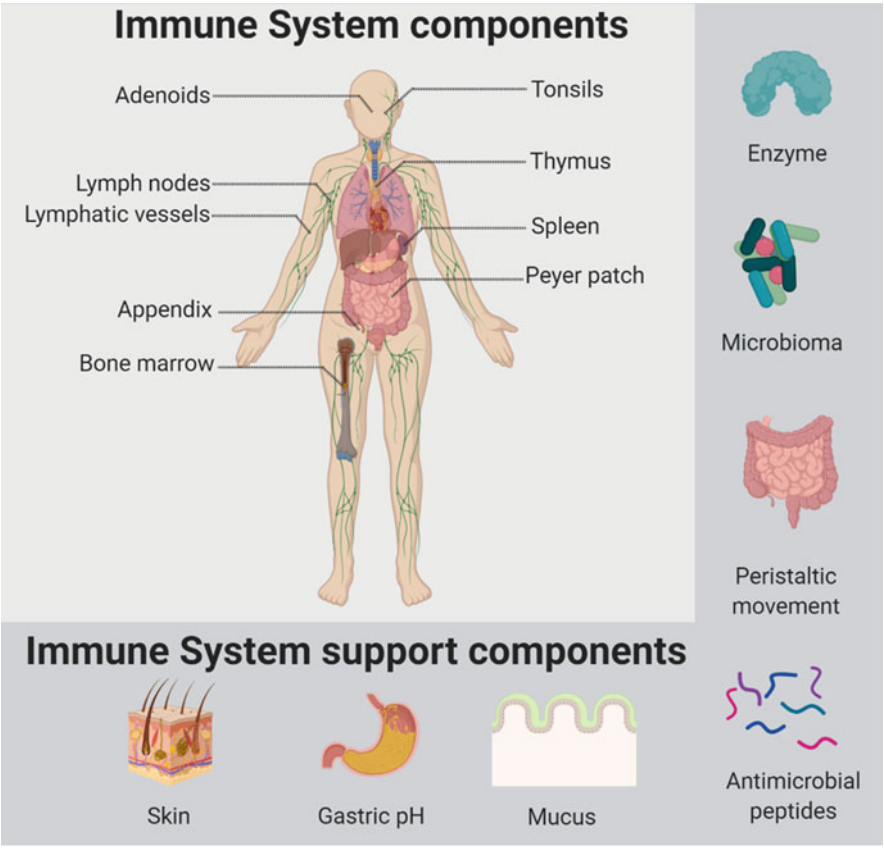

Fig. 14.2 Immune system components

suitable to develop illness as allergies, immune diseases, and diabetes mellitus (Jakaitis and Denning 2015).

GI tract possess a varied cell composition, where 90% are enterocytes, which main function is to absorb nutrients, their hair-type-shape allows to maximize the uptake area, are able to produce diverse hydrolases and are covered by a mucus layer; the mucus it is composs by glyco-proteins secreted by the goblet cells; additionally, the M-cells, represents less than 1% of cell composition, and are present in a region named Peyer patche, with lymphoid follicles and lack of villi structures, instead substituted by microfold structure; the M-cells tend to absorb non-self colloidal particles, from antigenic and proteinaceous nature, being an active part of immune system, and finally the Paneth cells, which release bactericidal lysozymes (Pawar et al. 2014).

GI tract represents the start point during in vivo food-derived bioactive compounds performance; in the case of protein and peptides, firstly, after their consume, and posterior to breakdown by upper-GI tract including acid media and

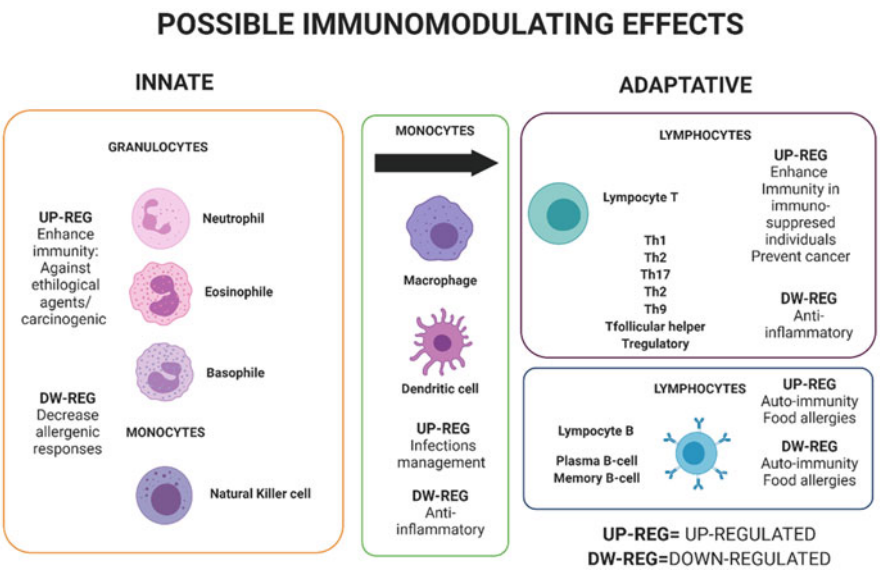

Fig. 14.3 Possible immune system targets and benefits

pepsin effect, the proteinaceous molecules arrives to small intestine, where interact with brush border enzymes as trypsin, pepsin and chymotrypsin, that could modify the structure of proteins (Pawar et al. 2014), and where also interacting with microbiome, at this intestinal poitn, normally the major nitrogen content present is derived from: peptides near to 20–30%, also by urea, ammonia and free amino acids with 10–15%, and finally between 48 and 51% in form of proteins (Xu et al. 2019). The group of molecules which could be transported to blood stream are constituted by low molecular weight peptides or free amino acids, transported by enterocytes, specifically, trough side spaces existent between enterocytes (paracellular); by transcellular mechanism, employing concentration gradient (M-cell); and finally, by carrier and receptor transport mechanisms, where typically use protein components to transport the molecules of interest, but also, intervene specialized ligands and vesicle transportation. The transport mechanisms depends on physicochemical characteristics of peptides as spatial orientation, hydrophilic and lipophilic proportion, pK and pH; and finally enter into individual system, trough portal vasculature or gut associated lymphatic network (Pawar et al. 2014).

Has been known that food components, could exhibit multiple benefits to enhance immune acvtivities and Fig. 14.3 summarizes the possible immunomodulating effects of bioactive peptides.

Yu et al. 2020, employs an immunosuppressed murine model, and fed it with low molecular fraction of previously hydrolyzed *Nivea japonica* skin proteins, and observe a big enhancement in multiple mouse immunity paramaters. Also, Tong et al. 2019, identify a peptide sequence present in rice, and after that, synthetic homologuesshows antiapoptotic, antioxidant and anti-inflammatory effects, using an

atherosclerosis prone- mice model; while He et al. 2019, use a group of rapeseed derived-peptides with antihypertensive activity in spontaneous hypertensive rats, which also exhibit anti-inflammatory effect.

This group of experiments demonstrate that, the food derived peptides boost the immunity at diverse system levels, and they could be used in complicated and multifactorial diseases treatments. Moronta et al. 2016, which studies the effect of an amaranth encrypted peptide, in mouse allergenyc response to food components, specifically milk, obtaining that the presence of this peptides, could down regulate this class of immune-regulated illness.

14.4 Health Effects

14.4.1 Anti-inflammatory Effects

Inflammation in the body is a complex process. It is a natural defense mechanism originated by the immune system in which pro-inflammatory mediators like tumor necrosis factor-α (TNF-α), interleukin (IL)-1, IL-6, IL-8, and interferon-y (INF-y) are activated to restore the affected body site. These mediators can amplify the response to inflammation through interaction with other cellular components (Guha and Majumder 2018).

At the systemic level, the activity of the immune system is evident. Nonetheless, involves the activation of mechanisms at the molecular and cellular levels; and consequently other systems that collaborate to achieve body self-regulation. The joint participation of the central nervous system, peripheral, endocrine, cardiovascular, together with the aforementioned immune system, are key to counteract the inflammatory effect (Rojas et al. 2007).

So, effective therapy addresses the balance of all these systems. However, to determine adequate treatment, it is essential to know the etiological origin of the effective process. One of the causes of inflammation is attributed to infectious situations where viruses, bacteria, and other microorganisms are responsible for activating the body's defense mechanisms. On the other hand, there are non-infectious situations that involve physical (trauma, burn, physical damage, etc.), chemical (toxins, alcohol, chemical irritants, etc.), biological (damage cells), and psychological (excitement) damage of origin (Chen et al. 2018).

Regardless of the injury that initiates the inflammatory process, the first and brief physiological changes involve events that affect cells and vascularized tissue causing a phenomenon of vasocontraction and vasodilatation. Consequently, there are alterations in blood flow that cause an increase in permeability in the blood vessels, and with it, an increase in the exchange of other components in the medium. Signals emitted from damaged tissue are detected by chemotaxis by the white blood cells. In addition to recognition, the speed and effectiveness of healing processing depend on such chemotactic action. An important part of the adequate response process to inflammation originates from the chemical activity emitted by structural compounds, such as endothelial cells, together with elements of the blood plasma, platelets, and

mast cells (Nunes 2020; Derek et al. 2019). Continuing with the reaction phases, inhibitory mechanisms are generated that aim to balance the inflammatory process and, consequently, repair all or part of the damaged tissues.

The description of the previous process is the ideal response of the functional organism to acute inflammation, which is characterized by being decisive against the etiology of the inflammation in short periods, that is, minutes, hours, and may last for a few days (Pahwa et al. 2020).

When the inflammation persists for weeks, months, even years, chronic inflammation develops. It can appear as a result of an acute infection inadequately treated or not treated at all, disorders in the immune system, prolonged and constant exposure to toxic substances, among other causes (Ferrucci and Fabbri 2018; Pahwa et al. 2020). Chronic inflammation is highly damaging, because various systems involved in the search for the homeostatic disease remain alert, and in constant attack, being one of the causes in prevalence of chronic non-communicable diseases mediated by inflammation, including hypertension, arthritis, atherosclerosis, type-2 diabetes, inflammatory bowel disease and other cardiovascular diseases (Chauhan and Kanwar 2019; Eberhardson et al. 2020; Furman et al. 2019).

The scientific research that has deepened and correlated current lifestyle with many affections that devalue human health is not very recent. Although, that industrial development bring us are innumerable, nevertheles also generates changes in the way of life, provoking different psychological status and therefore physical alterations including chronic inflammation.

Without a doubt, diet plays a crucial role in maintaining or losing health. A diet high in fats, refined carbohydrates, processed food products rich in sugar, and salt ensures obesity, type 2 diabetes, and cardiovascular disease (Shah et al. 2020). There is sufficient scientific evidence about the relationship between poor diet and obesity with pro-inflammatory changes in several organ systems including pancreas, adipose tissue, liver, gut, in addition to pro-inflammatory changes generated in the central nervous system, specifically observed in the hypothalamus, in addition to other brain regions (Leigh and Morris 2020).

The literature, through numerous studies, exposes the benefits of the Mediterranean diet as a promoter of cardiovascular health (Sánchez-Sánchez et al. 2020; Schwingshackl et al. 2020; Spadaro and Provident 2020). The characteristic diet of this dietary model is based on products rich in polyphenols, antioxidants, and micro- and macronutrients that have been shown to combat inflammation, oxidative stress, endothelial function, and with it, the reduction of cardiovascular risk (Shah et al. 2020).

Anti-inflammatory peptides present in animal- and plant-based nutrients have been studied due to the ability they have shown to reduce inflammation directly or indirect way, after denaturation during digestive process, or in fermented or maturated food (Dadar et al. 2019; Guha and Majumder 2018). Among the immunomodulatory properties attributed to anti-inflammatory peptides stand out, the modulation of differentiation of immune cells, as well the prevention of exclusive

pro-inflammatory responses, stimulation of wound healing, angiogenesis, and microbial clearance is remarkable too(Dadar et al. 2019).

Multiple reports have demonstrated the anti-inflammatory activity of bioactive peptides present in different food sources.

Milk is one of the foods that contain a lot of protein like whey and casein. Peptides such as VPP (Val-Pro-Pro) and IPP (Ile-Pro-Pro) have been obtained by bacterial fermentation of casein; they demonstrate anti-inflammatory activity, inhibiting the activation of the NF-kB (nuclear transcription factor-kappa B) pathway that plays a key role in regulating the immune response to infections (Chakrabarti and Wu 2015). Likewise, a peptide derived from ß-lactoglobulin hydrolyzed by pronase has been identified with anti-inflammatory effects in Caco-2 cells activated by TNF-α that decreases the production of IL-8 (Oyama et al. 2017).

Honey contains different bioactive compounds with possible benefits in pro- and anti-inflammatory pathways, in addition to its effects against cellular oxidative stress. In vivo studies show its ability to decrease the levels of MDA, TNF-α, IL-1B, and IL-6 in the gastrointestinal mucosa been proven as effective natural product against specific pathologies of inflammatory origin (Hosseini et al. 2020).

Soy is one of the foods that stands out for its high nutritional content, as well being a good protein source of plant origin. Due to its accessibility in costs, soy has been used as a means of extracting bioactive peptides for its application as additives that improve the quality, solubility, and emulsification of food products, highlighting the biological benefits that they also provide to the consumer. The anti-inflammatory activity of peptides derived from soy protein has been demonstrated by proinflammatory cytokines (TNF-α, IL-6, and IL-1B) inhibition induced by lipopolysaccharide (LPS) in RAW24.7 (Yi et al. 2020). Therefore, soy protein-peptide derivatives could contribute to the development of foods with anti-inflammatory properties, specifically of immune origin.

14.4.2 Anticancer Effects

Cancer remains among the most devastating diseases, with near eight million people deaths from this lethal disease at worldwide, turning in to the second leading cause of decease (Ferlay et al. 2008; Fitzmaurice et al. 2017). Carcinogenic cells, are affected in key process as proliferation, differentiation, metabolism, and survival rate; presenting an excessive growth and with the possibility of infect other organs (Grazioso et al. 2019; Seung Rak et al. 2018).

Conventional cancer treatment via methods such as chemotherapy, hormonal therapy, radiation therapy, and targeted therapy has not presented expected success, due to the high cost and its harmful impacts, because the treatment kills and affects diseased cells and normal cells (Harris et al. 2009; Thundimadathil 2012). Therefore research efforts are focusing on developing new treatments for cancer (Lohmueller and Finn 2017; Gohary 2017).

Over the last few decades, anticancer peptides (ACPs) were discovered, and their anticarcinogenic effects were proved and considered a promising alternative and

novel treatment for cancer, because it did not affect normal body physiological functions (Akbar et al. 2020; Yi et al. 2019). Where Pre-clinical and clinical trials have also demonstrated ACP's action against different tumor types (Yi et al. 2019).

ACPs have different mechanisms of cancer prevention or treatment; as cancer is characterized by uncontrolled cell proliferation and metastasis there are several protection mechanisms to counteract cancer developing and adverse effects among them by killing cancer cells by perturbing the permeability of their cell membranes and/or apoptosis induction (Deslouches and Di 2017; Hoskin and Ramamoorthy 2008; Schweizer 2009). This mechanism works selectively as the outer leaflet of the cell is neutral when healthy, so a negatively charged cell is indicative of an altered cell since negatively charged lipids remain in the intracellular leaflet. Therefore exposing negatively charged lipids such as phosphatidylserine (PS), negatively charged O-glycosylated mucins, on the membrane surface is an indicator of a cancer cell (Vaezi et al. 2020). In addition, cancerous cells have higher amounts of surface-exposed negatively charged O- glycosylated mucins, sialylated gangliosides, and heparin sulfate (Arias et al. 2020; Vaezi et al. 2020).

That selective toxicity represents almost no harmful effect on normal cells.

Data suggests that aggregation is a very useful tool for peptides as it strongly increases peptide selectivity, by reducing the effective peptide hydrophobicity and thus the affinity towards membranes composed of neutral lipids (Vaezi et al. 2020).

Epidemiological evidence, in vitro and in vivo tests in humans and animals have demonstrated that bioactive peptides present in the diet can reduce cancer risk and even sensitize tumor cells in anticancer therapies (De Kok et al. 2008).

Egg proteins such as ovomucin, ovotransferrin, cystatin, lysozyme, and phosvitin exert anticancer activity by a variety of mechanisms, including protecting DNA from damage, apoptosis, decreasing the invasion ability of cancer cells, and enhancing the antimutagenic and cytotoxic and activity of the cells (Lee and Paik 2019).

This carcinogenic cells are not recognized by the immune system as self-components, and during regular homeostasis, the immune system usually remove them, this fact bring us the possibility to employs a treatment or a preventive strategy for this class of illness driven by immune system. The main players in regulating the immune response during cancer develope are NK cells, T cytotoxic, T helper lymphocytes and specialized antigen-presenting cells as macrophages and Dendritic Cells, where therapies that enhance their proliferation could help.

Also, another target that could be treated is the chronic inflammation proccess which achieve cancer development, also, molecules as Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-kB), or another intracellular components essential for the Janus kinase (JAK)/signal transducer could be controlled to down-regulate inflammation (Florean et al. 2020).

Horiguchi et al. 2005, probe that after healthy individuals consume wheat gluten-hydrolysates, the NK-cell activity significantly increases. Also, Wong et al. 2014, analyzes the enhancement of the NK-cell in influenza-infected mouse, after bovine colostrum consume, and they noticed an enhancement in NK-cell viability, putting on perspective, the proteins employment as preventive treatment in deasseases where NK-cells faillure are a triggering factor. In the other hand Wang et al. 2017,

employs dipeptides derived from ovotrasnferrin as anti-inflammatory agents in Caco-2 cells (enterocytes), and demonstrate that the peptide Histidine-Carnosine, inhibits the Interleukin-8 production (IL-8), by NF-kB blocking. Also, Sun et al. 2016, use ovomucin an egg prottein to generate hydrolysates and analyze the anti-inflammatory effect, in human dermal fibroblasts after radiation exposition, and the results shows the inhibition of two pro-inflammatory factors: NF-kB and tumor necrosis factor-α (TNF-α), demonstrating the potential that peptides, specially with low molecular weight, could protect against, one of many factors that could trigger a multifactorial illness such cancer.

14.5 Proteins Composition and Mechanisms Relationship

The immunomodulatory action of bioactive peptides obtained from hydrolysates of food proteins is related to the amino acid composition, sequence, length, charge, hydrophobicity, and structure of the peptide molecule (Görgüç et al. 2020).

Proteins and peptides are bioactive compounds that can interact with specific receptors to produce the stimulation or inhibition of certain effects in the immune system, causing a positive impact on health, which is why they are considered biomolecules with immunomodulatory activity (O'Keeffe and Fitzgerald 2015).

According to different investigations, proteins and peptides have shown evidence of possessing this activity, for example, they induce or modulate the production of cytokines and antibodies, stimulate the proliferation of lymphocytes, increase the phagocytic capacity of macrophages, increase the activity of natural killer cells, improve the body's defensive capacity against invading pathogens and inhibit the pro-inflammatory responses of host cells to bacterial components such as lipopolysaccharides (Chalamaiah et al. 2014; Hou et al. 2012; Wu et al. 2016).

The length of the hydrolyzed peptides with effect on the immune system must be short (2–10 residues) (Ahn et al. 2015; He et al. 2015), and also there are several reports where they agree that proteins or peptides with high immunomodulatory potential are constituted by hydrophobic amino acid residues such as glycine, valine, leucine, proline, phenylalanine and also by negatively charged amino acids such as glutamic acid and aromatic amino acids such as tyrosine. In addition, certain amino acids have been identified to facilitate immunomodulatory activity in peptides obtained from food products, such as hydrophobic amino acids and residues of glutamine, glutamic acid, tyrosine, tryptophan, cysteine, asparagine and aspartic acid (He et al. 2015; Hou et al. 2012; Eun-Kyung et al. 2013; Vo et al. 2013), while most of the bioactive peptides with immunomodulatory activity present in plants have been shown to have low molecular weight with sequences of Met-Met-Leu-Asp-Phe and Leu-Asp-Ala-Val-Asn-Arg (Görgüç et al. 2020).

The mechanism by which proteins and peptides exert immunomodulatory effects is still unclear; however, some important aspects are known. Through several studies it has been determined that depending on the composition, sequence, length, charge, and hydrophobicity of the amino acids that integrate these biomolecules, they will be directed to specific receptors and will trigger cellular signaling processes

(Chalamaiah et al. 2018; Haney and Hancock 2013; He et al. 2015). The amino acids present on the protein surface are responsible for ensuring that the bond has high specificity and affinity (Gokhale and Satyanarayanajois 2014).

It has been possible to identify some specific mechanisms, such as in the case of hydrolyzed whey proteins, where these are capable of activating Toll-like receptors (TLRs), which are one of the most studied receptors in immune signaling since they belong to the family of pathogen recognition receptors. These hydrolysates activate multiple TLRs, such as TLR2, 3, 4, 6, 7, 8 and 9; this causes the production of TNFα, IL-10, and IL-8 (Kiewiet et al. 2017). On the other hand, studies show that if the protein source of the hydrolysate is casein, the TLRs are inhibited, and in addition, the inhibited TLRs are different in each hydrolysate, being among the most inhibited the TLR5 and 9 (Kiewiet et al. 2018). As proteins and peptides vary so much, it is complicated to have universal mechanisms that explain the immunomodulatory effects they have, and even the specific mechanisms already identified are motives for discussion.

In experiments with mice after administration of proteins from dairy products, oysters, salmon, and fish, positive results have been generated by increasing ex vivo the phagocytic capacity of macrophages isolated from the peritoneal cavity (Cai et al. 2013). Also, hydrolysates of oyster, salmon, and carp egg proteins have been found to increase the activity of NK cells in the spleen (Chalamaiah et al. 2015).

14.6 Protein Derived Products with Immunomodulatory Effects

Various bioactive peptides have been highlighted for their humoral and cellular immune functions, so that they have the possibility of being incorporated as ingredients in functional foods, nutraceuticals, and pharmaceuticals, whose biological properties contribute to control and prevention of diseases (Agyei and Danquah 2012); these products are bioactive peptides derived from plants such as, soybean, oat, flaxseed, and wheat or animal-based derived had been more studied such as egg, fish, milk, oyster, sheep, among others (Mao et al. 2020).

The immunomodulatory effects of bioactive peptides, protein hydrolysates or protein fractions present in existing or new food products on the market, are a source of study and great interest due to the potential to regulate diseases related to the immune system (Kiewiet et al. 2018). There are a large number of studies regarding fractions of peptides obtained from proteins in food with immunomodulatory activity. In a recent study, a selenium-containing peptide derived from a hydrolyzed rice protein was identified, stimulating the proliferation and phagocytosis of RAW 264.7 cells from mouse macrophages (Fang et al. 2019). Also, some immunomodulatory peptides derived from hydrolysates proteins of soybean or rice had the ability to stimulate reactive oxygen species (ROS), which activates the non-specific immune system (Kitts and Weiler 2003). Li et al. (2018) propose the use of a protein fraction isolated from the Inca peanut seed as an ingredient in functional foods with

immunomodulatory activity. The polypeptide they isolated is an albumin fraction capable of stimulating splenic lymphocyte proliferation and promoting the production of H_2O_2 and NO in RAW264.7 cells. Udenigwe et al. (2009) analyzed the inhibition of lipopolysaccharide-induced nitric oxide (NO) production and the antioxidant properties of a peptide fraction derived from flaxseed in RAW 264.7. In this experiment, flaxseed was hydrolyzed with seven types of proteases, where the thermolysin and pancreatin obtained fractions act as anti-inflammatory agents due to high scavenging capacity. Mao et al. (2020) described the immune activity of walnut peptides in BALB/c mice with the perspective of be a promising dietary product. By other hand, protein hydrolyzed was obtained from microalgae *Chlorella vulgaris* 87/1 with immunostimulatory activity in malnourished mice. The treatment with Chlorella protein hydrolysate had positive results at the level of hematopoiesis due to a recovery of leukocytes and cell count in the bone marrow (Morris et al. 2009).

Other researches demonstrate that the use of protein hydrolysates have potential applications in the pharmaceutical and food industries. Egg proteins and peptides are of great interest due to their immunomodulatory and anticancer activity (Lee and Paik 2019). The hydrolyzed ovomucin fraction inhibits the TNF-mediated by NF-κB pathway (Sun et al. 2016), also, Chalamaiah et al. 2014 reported protein hydrolysates derived from *Labeo rohita* fish egg and generated with three different enzymes, that show an increase in T lymphocyte production, as well as phagocytic activity and NK cell activity in BALB/c mice.

Fermented milk proteins are another potential regulator in different processes of the body and are a great alternative to be a nutraceutical and functional food. Vinderola et al. (2007) demonstrated the immunomodulatory capacity of a fraction of milk, fermented by *Lactobacillus helveticus* R389 in mice, showing a modulating response in the intestine mucos, where an increase of IgA cells in the intestinal wall was observed. Furthermore, Beaulieu et al. (2007) reported the use of a whey-fermented product with higher immunomodulatory properties than other commercial whey or milk products. This whey-fermented product promotes an increase in the white blood cells, specifically monocyte, lymphocyte, and polymorphonuclear cells in rats. For last, peptides obtained from meat derivatives have also been studied for their immunomodulatory effect (Chalamaiah et al. 2018), and example is the carnosine, which is a dipeptide with multifunctional properties as an antioxidant, anti-inflammatory and immunomodulatory, that it is also used in food as an additive and flavoring for meat. This beneficial peptide can be obtained from a natural source such as skeletal muscle (Waldron 2009).

Food products added with bioactive peptides and diverse proteins are still under investigation, some being in development proccess or currently on the market. The addition of these compounds bring an added value to the product, as well, shows potential to treat diseases and benefit health. However, some products have the challenge to improve the bioavailability, and meet the requirements of toxicity or allergenicity (Hartmann and Meisel 2007).

14.7 Conclusion

The immune system constitutes a key component that helps to maintain the correct functionality in the organism, being the system effector against diverse exogenous and endogenous components, and which failure could generate important ailments. The immune system not always generate an adequate response, attributed to multiple factors as, nutritional conditions, elderly, chronic disease presence, pharmacological treatments, among others; nevertheless, is founded in food, and food components, especially in proteins, molecules which could up- or down-regulate the immune response, and which correctly application, could bring extensive health benefits, if well, not all the question in this topic are answered, they give to food- and pharmaceutical industries and reasearch fields, a novel and interesting issue to explore, being suggested as adjuvants, enhancers and modulators of immunity response and which could support therapies or contribute to preventing multiple health disorders.

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Fatty Acids and Immunomodulation

15

H. Shahrul and M. Tasyriq

Abstract

Cells require energy as source for survival. One of the major sources of energy is derived from lipids which are obtained from either dietary sources or de novo synthesis. The chapter highlights on different types of fatty acids and their pivotal roles in cellular structure and metabolic processes. It is followed by an overview of the immune response. Further explanation is provided on different types of fatty acids, their lipid mediators, and complex lipids that contain fatty acids, with emphasis on their roles in modulating immune response. The chapter provides description of the underlying molecular mechanisms in relation to health and disease pathogenesis. It addresses on the importance of dietary intervention as mode of therapeutic lifestyle modification for chronic diseases such as metabolic syndrome, cardiovascular disease, and several other diseases. Additionally, clinical strategies of targeted therapy to modifying fatty acids to overcome the increasing burden of chronic diseases as well as emerging disease are also presented.

Keywords

Fatty acids · Monounsaturated fatty acids · Polyunsaturated fatty acids · Immune response · Cholesterol · Triglycerides · Diseases

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15.1 Introduction

Cell metabolism requires energy sources which are derived from carbohydrates, lipids, and proteins. Dietary fatty acid composition may induce the development of chronic diseases which are regulated by the inflammation (Khadge et al. 2018). Diet plays a crucial role on health and immune system (Khadge et al. 2018). Lipids comprise diverse groups that have been categorized as simple lipids, complex lipids, precursor, and derived lipids. According to the Lipid Metabolites and Pathways Strategy (LIPID MAPS) Consortium, lipids can be classified into eight categories (Fahy et al. 2009). They are among the major source of energy in metabolic process. Among lipids, fatty acids occur mostly as esters in natural fats and oils. It is also found in unesterified form as free fatty acids (Murrey et al. 2006).

Fatty acids are a class of carboxylic acid with an aliphatic chain, linear or branched, composed from 2 to 36 carbon atoms. It is present as either saturated (without double bonds) or unsaturated (containing one or more double bonds). In general, there are three main classes of fatty acid according to the number of carbons. They are the short-chain fatty acid (C2–C4), medium-chain fatty acid (C5–C10), and long-chain fatty acid ($C > 10$). Examples of short-chain acids are acetic acid, propionic acid, and butyric acid, while examples of medium-chain acids are valeric (C5), hexanoic or caproic (C6), caprylic (C8), and capric acid (C10). The source of short-chain fatty acids is from anaerobic metabolism of dietary fiber and undigested saccharides by intestinal microbiota. Similarly, diet contributes as the source of medium-chain fatty acids, while the human liver also produces fatty acids from peroxisomal beta-oxidation of long-chain fatty acids. Fatty acids modulate multiple mechanisms such as lipoprotein metabolism and cholesterol homeostasis (Fernandez and West 2005; Ulug and Nergiz-Unal 2021), source of energy through beta-oxidation, and ATP generation (Lee et al. 2019) and produce double of ATP compared to carbohydrates (Carracedo et al. 2013), angiogenesis (Duttaroy and Basak 2020), and inflammatory process (Jezernik and Potočnik 2018). It is reported that the composition of microbiota and production of fatty acids can regulate immune responses. Fatty acids could be targeted to improve the outcomes of different diseases (Dei Cas et al. 2020; Saresella et al. 2017). Unsaturated fatty acids are divided into three different groups that comprise monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), and eicosanoids. The monounsaturated groups contain one double bond, while the polyunsaturated group has two or more double bonds. The third group, eicosanoids, are 20-carbon polyenoic fatty acids with involvement in multiple physiological and pathological processes (Duttaroy and Basak 2020; Galet et al. 2014).

Dietary fat plays a pivotal function in health and disease. Cholesterol is the precursor for steroid hormones and bile acids (Prabhu et al. 2016). Phospholipids are among the major composition of plasma membrane. At the non-cytosolic side of plasma membrane, sphingomyelin and glycosphingolipids are the major phospholipids, whereas at the cytosolic leaflet, phosphatidylserine (PS) and phosphatidylethanolamine (PE) are the major composition of plasma membrane (Feigenson et al. 2008). Phospholipid is composed of a glycerol backbone and

fatty acids with ester linkage. Nevertheless, excess fat such as cholesterol or fatty acids may cause adverse effects on health. Imbalance in the ratio of saturated/unsaturated fat may modulate the immune system (Alshatwi and Subash-Babu 2018). High intake of saturated fat (SFA) causes lipid deposition and inflammation that are associated with higher risk of developing inflammatory cardiovascular disease (Alshatwi and Subash-Babu 2018). On the contrary, PUFA and MUFA exhibited protective effect through control in the synthesis and oxidation of SFA, liver fat content, and hypercholesterolemia (Alshatwi and Subash-Babu 2018).

15.2 The Immune System

Immune responses depend on the exposure of foreign substances toward the body systems. The basic concept of the immune system is the capability of the body to differentiate between self and non-self. The system is essential for protecting the organs, such as skin, intestinal tract, respiratory passage, reproductive tract, and other body parts, from foreign particles such as aggressive pathogens, microbes, viruses, tumors, and toxins. The responses mount by the contacts lead to the clearance of the foreign particles from the body circulation. In the body, this role plays by a variety of myeloid and lymphoid cells located in the different tissues and organs. In addition to the role played by the structural and chemical barriers as a shield of infection, the immune system recognizes the non-self through the so-called innate and adaptive immune response.

Innate immune response (non-specific) is well-known as the frontline of host defense against an intruding pathogen. The host defense mechanism is triggered quickly or minutes to hours of encountering foreign substances, even the host never exposed to the particular pathogens. Adaptive immune response (specific) represents the antigen-dependent and antigen-specific response to the particular invading pathogen. The mechanism develops more slowly compared with innate immunity, hours to days between the exposure and maximal response. The main feature of adaptive immunity is the ability to generate immunological memory, which allows the host to mount a faster and intense response in repeated exposure of the same antigen. Both immune responses complement each other in the protection of the host, with defects in one of the responses causing host susceptibility and insensitive system.

15.2.1 Innate Immune Response

15.2.1.1 Role of Innate System

The innate immune response relies on receptor-mediated detection of molecular pattern to identify the non-self. The recognition of foreign substances that usually share common structures in many pathogens, called pathogen-associated molecular patterns (PAMPs) or danger-associated molecular pattern (DAMPs) through the germline-encoded receptor, called pattern recognition receptor (PRR), is the

hallmark of the innate immune response. Beside, pathogen-associated molecules commonly known as pathogen-associated immunostimulants include the outer surface of microorganisms. For example, the peptidoglycan cell wall, flagella of bacteria, lipopolysaccharide (LPS), teichoic acids, and CpG motif absent in the mammalian host stimulate two types of the innate immune response which are called inflammatory response (secreted factors) and cell-dependent mechanisms (phagocytosis and cytotoxicity) (Raff et al. n.d.). The innate immune response takes place mainly at sites of infection in order to eliminate invading pathogens.

15.2.1.2 Types of Cells and Cell Products

Multiple types of cells are engaged in the innate immune response such as macrophages, neutrophils, natural killer (NK) cells, dendritic cells (DCs), and innate lymphoid cells (ILCs). Professional phagocytic cells such as macrophages (long-lived cells) and neutrophils (short-lived effector cells) have a related function. Both cells engulf or phagocytose the pathogens or foreign substances and degrade them through various mechanisms. Formylated methionine peptide was identified as one of the chemoattractants for neutrophils. The exposure of this substance to neutrophils results in the killing of the bacteria by phagocytosis via releasing the lytic enzyme and reactive oxygen species (ROS). In addition to its phagocytic properties, neutrophils produce cytokines (TNF-a and IL-8) and chemokines through the releasing of their cytosolic granules (neutrophil elastase (NE), myeloperoxidase (MPO), and defensins) that help in eliminating the pathogenic microbes. Similar to neutrophils, macrophages also secrete various cytokines (TNF, IL-1, IL-6, IL-8, and IL-12) and chemokines but with additional secreted factors such as leukotrienes, prostaglandins, and complement which are crucial for the recruitment of inflammatory cells. Furthermore, the cells are also involved in presenting the antigen to the T cell during the adaptive immune response (Arango Duque and Descoteaux 2014).

Dendritic cells (DCs) are the innate specific antigen-presenting cells that also play a role in phagocytic processing. The cells capture and engulf the pathogens and present the antigen to T cells to execute an adaptive immune response. Due to this nature, the cells are regarded as a messenger between innate and adaptive immunity. Two main subtypes of DCs are called plasmacytoid (pDC) and myeloid (mDC) DCs which can recognize different pathogen-associated molecular patterns (PAMPs) through pattern recognition receptors (PRR) such as toll-like receptors (TLR), C-type lectins, and intracellular nucleic acid sensors. Activation of these receptors also mediated the production of various cytokines and chemokines, mainly interleukin (IL)-12 and type I interferons. Besides, both subtypes can activate the natural killer (NK) cells during viral infection (Castell-Rodríguez et al. 2017).

NK cells and innate lymphoid cells (ILCs) are the innate lymphocyte cells which are part of the innate immune response. Unlike T cells that express the antigen-specific receptor on its surface, NK cells do not have this receptor and selectively kill virally infected cells expressing a low level of major histocompatibility complex (MHC) class 1 protein. The damaging of virus-infected cells is carried out via the release of perforins and granzymes, followed by promoting the infected cells to undergo apoptosis process. Similar to the way of monitoring the level of MHC class

1 protein in virally infected cells, cancer cells expressing a low level of MHC class 1 proteins are killed by NK cells via the activation of antibody-dependent cell cytotoxicity (ADCC) (Vivier et al. 2011). ILCs, on the other hand, lack the antigen-specific receptor and are mainly used receptor for cytokines to respond to the changes in the microenvironment. Based on their groups (ILC-1, ILC-2, and ILC-3), the cells selectively produce cytokines such as IFN-γ, IL-5, IL-9, IL-13, IL-22, and IL-17 in response to specific pathogens (Panda and Colonna 2019). Consistent with other innate immune cells, NK cells also produce various cytokines and chemokines such as interferon-gamma (IFN-γ) and IL-10 for mobilizing the APCs and enhanced anti-viral immunity (Vivier et al. 2011).

15.2.2 Adaptive Immune Response

15.2.2.1 Roles of Adaptive Immune Response

The adaptive immune response is activated upon pre-processing of the antigen by innate immune cells. The mechanism is specific and characterized by acquired immunity, which is a function of lymphocytes. This immunity system is vital when the innate immune response is unsuccessful in getting rid of harmful pathogens. The generation of memory cells provides long-lasting defense against the re-exposure of foreign substances derived from particular pathogens. The presence of unique antigen-binding receptors on the surface of lymphocytes allows the cells to bind the antigenic peptide. However, the binding process alone is not adequate to promote the generation of effector cells which are responsible for eliminating the pathogens.

Additional signals, such as costimulatory signals provided by specific cells, are needed to stimulate the effector cells (differentiated lymphocytes). In adaptive immunity, this can be accomplished by helper T cells that provide such signals (secreted cytokines) for B cells, while innate specific antigen-presenting dendritic cells provide the costimulatory signal (MHC II – epitope) for T cells (Jain and Pasare 2017). Thus, adaptive immunity is engaged with a firmly controlled interchange between APCs and T and B lymphocytes (den Haan et al. 2014). Two mechanisms of adaptive immune response have been documented, which are known as cellular immunity (cell-mediated response) and humoral immunity (antibody-mediated response) that are carried out by T lymphocytes and B lymphocytes, respectively (Cooper and Miller 2019).

15.2.2.2 Types of Cells and Cell Products

Unlike the innate immune system comprising numerous types of cells, the adaptive immune system consists of only T lymphocytes and B lymphocytes. T lymphocytes or commonly known as T cells are originated from hematopoietic stem cells in the bone marrow before undergoing the maturation process in the thymus. Similarly, B lymphocytes or so-called B cells are also derived from blood stem cells in the bone marrow but stay at the same site to experience the process of maturation. Unlike B cells that are expressing B-cell receptor (BCR) on their surface, T cells express a

collection of distinctive antigen-binding receptors on their surface membrane, known as T-cell receptor (TCR). Both T-cell and B-cell receptors are essential in adaptive immune response since they are used for the binding of biomolecular complex (MHC I/II – antigen's epitope) displayed at the surface of antigen-presenting cells (APCs) and soluble antigen, respectively. After the binding and receiving the appropriate signals, both lymphocytes rapidly proliferate and differentiate to become the effector cells for further killing the pathogens (Bonilla and Oettgen 2010).

Differentiated T cells can be categorized into two types: the first is called helper T (TH) lymphocytes which result from the engagement of naïve CD4+ cells with antigen-fixed MHC I molecules on APC surface (Tubo and Jenkins 2014) and the second is known as cytotoxic T lymphocytes (CTLs) that outcome from the interaction between naïve CD8+ cells and antigen-fixed MHC II molecules on the surface of APCs (Gaudino and Kumar 2019). These effector cells are involved in the cell-mediated and antibody-mediated response of adaptive immunity by two distinctive pathways. CTLs directly kill infected cells via perforin and granzymes before undergoing the lysis process followed by releasing of cytokines such as IFN-γ, TNF-α, and TNF-β to enhance the immune response (Černý and Stříž 2019). TH lymphocytes, on the other hand, bind APCs via MHC class II molecules and stimulate B cells by secreting cytokines such as IL-4, IL-5, and IL-6 and membrane-bound stimulatory molecules such as CD40 ligand to maximize the immune response (Takatsu 1997). Meanwhile, differentiated B cells are known as antibody-secreting plasma cells (short-lived cells), and memory B cells (long-lived cells) are the outputs from the action of TH lymphocytes. The cells engaged with humoral immunity (antibody-mediated response) to interact with antigens from pathogens that are freely circulating or outside the infected cells. Antibodies produced by the B cells bind to antigens, neutralizing them or causing lysis (dissolution or destruction of cells by a lysin) or opsonin promotion of phagocytosis for effective defense against pathogens. The involvement of complement cascade aids in clearing the antibody-antigen complexes from the body systems (Merlo and Mandik-Nayak 2013).

15.3 Association of Inflammation and Fatty Acids

Inflammation is characterized by an increased level of cytokines due to physiological and environmental factors (Soysal et al. 2020). Chronic inflammation has been linked with metabolic syndrome, cardiovascular disease, rheumatic disease, kidney disease, and cancer (Chan et al. 2019; Gasparyan et al. 2019; Kosmas et al. 2019; Prevete et al. 2018). In cancer, the distinct immunological process starts from the release of inflammatory soluble factors. Subsequently, it leads to the mobilization of myeloid cells and differentiation of myeloid cells within the tumor site. They will then orchestrate an immunosuppressive tumor microenvironment and sustain the inflammatory response. This interaction between cancer and inflammatory process

would cause disruption of the T-cell immunosurveillance and prevent anti-tumor immunity (Wattenberg and Beatty 2020).

A better outcome in anti-tumor immune response could be achieved by the coordinated response of both the innate and adaptive immune cells (Khadge et al. 2018). Emerging studies show the association between inflammation and fatty acids. Saturated fatty acids could trigger the initiation of inflammatory response by TLR4 signaling pathway. Further investigation on the interaction between dietary SFAs and TLR4 signaling is needed to facilitate the design of better pharmacological strategies for chronic inflammatory diseases elicited in part by fatty acid overload (Li et al. 2020).

15.4 Essential Fatty Acids

The human body does not synthesize essential fatty acids (EFAs). They are obtained from the dietary source. They are the n-6 (omega-6) and n-3 (omega-3) families. The precursors of essential fatty acids are linoleic acid, 18:2n-6 (LA), and alpha-linolenic acid, 18:3n-3 (ALA). They are necessary for various biological processes such as formation of structure and function of cell membranes. Other functions are to provide substrates for the production of eicosanoids and modulation of gene expression particularly those involved in cell homeostasis (Chen et al. 2013; Szefel et al. 2015). Enzymes that are involved in their metabolism are desaturases, elongases, cyclooxygenases (COXs), lipoxygenases (LOXs), and cytochrome P450 system (Duttaroy and Basak 2020).

15.5 Short-Chain Fatty Acids

The major substrates for SCFA (short-chain fatty acid) generation are derived from carbohydrates (both diet-derived and host-derived) and fermentation of amino acids. This leads to the production of formate, valerate, and caproate (Macfarlane and Macfarlane 2003). They act as a source of fuel for intestinal epithelial cells and modulate electrolytes and absorption of water (Vinolo et al. 2011). SCFAs are the most studied microbial metabolites in inflammatory bowel diseases (IBD). This disease is associated with a multifaceted interaction between various factors such as immunological response, microbes, genetic polymorphism, and environment. SCFAs are secondary metabolites fermentation of proteins, peptides, resistant starches, and undigested fiber by the microbes. An animal model study indicated that both SCFAs and tryptophan had prominent immunomodulation effects. They regulated the innate and adaptive immune cell generation. Another study provided evidence that impaired SCFAs-fermentative pathways are implicated in the pathogenesis of IBD (Huda-Faujan et al. 2010). Composition and diversity of gut microbiota have been changed among patients with IBD (Machiels et al. 2014). They involve fatty acids with less than six carbons (i.e., acetic, formic, propionic, butyric, and valeric acid). Synthesis of these fatty acids is regulated by diet and gut

microbiota species. The role of gut microbiota is to ferment incompletely hydrolyzed diet sources, which generates acetate, butyrate, and propionate (Topping and Clifton 2001).

The types of fatty acids that are produced differ, for example, *Bacteroidetes* members are involved in the production of propionate and acetate, and *Firmicutes* phylum causes the formation of butyrate. SCFAs provide source of energy to colonocytes, or they are circulated to other tissue sites. At the large and small intestines, common SCFAs are propionate and acetate, while butyrate is present in the cecum and colon (Vogt et al. 2015). Previous study reported that GM could regulate the mucosa immune system through T-cell differentiation and expansion. Their study provide evidence of butyrate that are produced by gut microbes could promote functional colonic regulatory T cells (T_{reg}) cells, specifically among $CD4^+$ T-cell subsets, via T-cell intrinsic epigenetic upregulation of the *Foxp3* gene (Furusawa et al. 2013). Some SCFAs such as propionate and butyrate were found to inhibit stimuli-induced expression of adhesion molecules and chemokine production, which could suppress monocyte/macrophage and neutrophil recruitment (Vinolo et al. 2011). On the contrary, there are reports that show pro-inflammatory roles. The differences could be due to the microorganisms that cause infection at anaerobic sites and lead to the accumulation of SCFAs. This would promote neutrophil accumulation and inflammatory processes (Vinolo et al. 2011). The immunomodulation by SCFAs occurs by activating the GPCRs, activating histone acetyltransferase, inhibiting histone deacetylase (HDAC), and stabilizing of hypoxia-inducible factors (Russo et al. 2019).

15.6 Long-Chain Polyunsaturated Fatty Acids

Obesity is a rising health concern worldwide as it increases the risk of metabolic syndrome. Visceral adipose tissue (AD) dysfunction has been associated with the onset of obesity and obesity-associated comorbidities. It is involved in the synthesis pro-inflammatory cytokines and chemokines, called adipokines (Philip C Calder et al. 2011). Apart from obesity, it is also associated with multiple other diseases (Lafontan 2014). AT has adipocytes and microvascular as well as immune cells in its stromal vascular fraction (SVF). It segregated into two compartments: the central for the subcutaneous upper abdominal and visceral fat mass, while the peripheral for the hip and gluteal-femoral fat. Excess central fat accumulation is associated with dyslipidemia, hypertension, and insulin resistance. However, excess femoral fat is associated with a reduced cardiovascular risk. Circulating hormones such as insulin and other signals originating in AT, including metabolites such as fatty acids (FAs) and adipokines such as leptin, adiponectin, retinol-binding protein 4 (RBP4), and apelin, circulate in proportion to body fat extent. They transmit signals to regulate fuel metabolism (Lafontan 2014). In obese individuals, visceral AT exhibited a dysfunctional feature in comparison to those derived from lean individuals (Landin et al. 1990). Immunomodulatory effects of *n*-3 PUFA occurred via the interaction between adipocytes and macrophages (De Boer et al. 2014), $(CD)8^+$ T cells (Monk

et al. 2015), and $(CD)4^+$ T cells (Liddle et al. 2017). Findings from experimental studies also support that EPA and DHA could lower the synthesis of adipokine by adipocytes (Ajuwon and Spurlock 2005). Therefore, therapeutic dietary modification of AT dysfunction could be an approach to lower the prevalence of obesity (Liddle et al. 2017). An interventional study using dietary *n*-3 PUFA in the form of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) exhibited improvement in function AT (Lancet 1999). It caused changes of AT immune cell function, synthesis of adipokine production, and metabolic pathways.

Diet with high ω-6 PUFA content enhanced the accumulation of myeloid-derived suppressor cells (Yan et al. 2013). Among the other types of disease related with high-fat diet is the non-alcoholic fatty liver disease (NAFLD). It occurs when there is an increase in the delivery of non-esterified fatty acids (NEFAs) to the liver from the adipose tissue. It is also contributed by imbalance due to increased hepatic fatty acid uptake and reduced excretion of lipids as lipoprotein from the liver (Byrne 2010). Furthermore, the disease is characterized by chronic inflammation that involves the Kupffer cells (Baffy 2009). Metabolism of ω-6 PUFA causes the synthesis of pro-inflammatory cytokines and eicosanoids, which causes Kupffer cell to secrete inflammatory cytokines. They will cause the activation of NF-κβ. The ω-6 PUFAs will be metabolized to form AA and followed by COX-/LOX to generate inflammatory lipid mediators (Patterson et al. 2012). However, supplementation with ω-3 PUFAs exhibited pro-resolving effects on both innate and adaptive immunity via multiple mechanisms (Schmitz and Ecker 2008) or anti-inflammatory like resolvins, protectins, and maresins (Serhan et al. 2009). Resolvins which are metabolites from LC ω-3 PUFAs exhibited anti-inflammatory activity, by which they protect from innate inflammatory responses (Isobe et al. 2012). Activation of dendritic cells (DCs) could promote adaptive immune response against tumor. It is also noted in activated macrophages (M1s), which are part of the tumor microenvironment. M1 macrophages contribute in the expression of iNOS and ROS and secretion of the NK and type 1 T-cell stimulating cytokine IL-12. Apart from these activities, they participate in the removal of pathogens and tumor cells (Heusinkveld and van der Burg 2011). They cause indirect cytotoxicity through the activation of adaptive immune responses (Biswas and Mantovani 2010).

Anti-inflammatory effects of PUFA have been extensively studied previously in trials for various diseases such as rheumatoid arthritis, inflammatory bowel diseases (IBD), and asthma (Philip C Calder 2006). There are two mechanisms of action of long-chain n-3 PUFAs. First is through the direct mechanism, where it replaces arachidonic acid as an eicosanoid substrate and prevents the metabolism of arachidonic acid. The second mechanism is indirect whereby it causes alteration of inflammatory gene expression (Calder 2006). Anti-inflammatory effects are most associated with the EPA (C20:5) and DHA (C22:6). Both interfere with the conversion of arachidonic acid (AA, C20:4) to prostaglandins (PGs) and leukotrienes (LTs). According to studies, LC ω-3 PUFAs will replace AA at the phospholipid bilayer. This alters the membrane composition, fluidity, signalling, and metabolism of pro-resolving mediators. A trial involving patients with colorectal cancer reported lowering of IL-6 level after receiving 0.2 g/kg fish oil after surgery, while

intervention during chemotherapy with 0.6 g per day for 9 weeks exhibited significant reduction of C-reactive protein and CRP:albumin ratio. These studies demonstrated anti-inflammatory effects; however, they recommended it was subject to various factors such as duration, dose, and mode of administration (Khadge et al. 2018).

The ratio of PUFA is an important factor in cellular responses and metabolic homeostasis (Wang et al. 2016). A reverse association of omega-3/omega-6 polyunsaturated fatty acid ratio with carotid atherosclerosis has been reported (Umemoto et al. 2016). This indicated that intake of an imbalanced diet could lead to cardiovascular disease. The pathogenesis of atherosclerosis involves a complex process which involves endothelial dysfunction, lipid accumulation, inflammation, vascular smooth muscle cell proliferation, matrix turnover, and also calcification (T. Wang and Butany 2017). Previous findings suggest that the oxidation of LDL could be initiated by the oxidation of omega-6 fatty acid contained within LDL particles (Simonetto et al. 2019). Lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) is a major receptor that binds to oxidized low-density lipoprotein (oxLDL) in endothelial cells. Its expression is practically undetectable in healthy arteries (Singh and Gautam 2019). Uptake of oxLDL by LOX-1 will activate intracellular signal pathways which leads to an increase in the expression of adhesion molecules and release of pro-inflammatory cytokines and matrix metalloproteinases (MMPs). It will promote neoangiogenesis, which is a key player in atherosclerotic plaque development and vulnerability (Markstad et al. 2019). Emerging clinical studies also show the relation between circulating oxLDL with cancer. This was supported by finding from an experimental study, where high expression of LOX-1 was present in metastatic colorectal cancer (Murdocca et al. 2019).

A clinical study tested the effects of low-fat fish oil on pro-inflammatory eicosanoids in men undergoing radical prostatectomy. Findings indicated there was a decrease in 15-S-hydroxyeicosatetraenoic acid (15-S-HETE) level relative to a Western diet. Further investigation is warranted to determine the pathways involved (Galet et al. 2014). Trials on leukotriene inhibitors have been done on patients with bronchiolitis (Liu et al. 2015) and chronic asthma (Chauhan and Ducharme 2012). A trial on leukotriene B4 (LTB4) receptor antagonist indicated there was no significant outcome when given in combination with gemcitabine and cisplatin in patients with advanced non-small cell lung carcinoma (Jänne et al. 2014). Evidence from experimental study showed that high omega-6/omega-3 ratio significantly increased lipid accumulation in macrophages and foam cell conversion. It also stimulated higher expression of pro-inflammatory cytokines (IL-6, IL-1β2, IL-4, IL-12β, IFN-γ, and IL-10) with corn and coconut oils, whereas oils with lower ratios of omega-6/omega-3 fatty acids (i.e., canola and fish oil) significantly reduced the levels of pro-inflammatory cytokines. This suggests that omega-3 fatty acids may improve immunoregulation and anti-inflammatory conditions (Alshatwi and Subash-Babu 2018).

15.7 Arachidonic Acid

Arachidonic acid (AA) is a major component in the membrane phospholipids, and their metabolic products are involved in multiple biological process. AA is formed after the activation of phospholipases which metabolize the phospholipid (Zeldin 2001). AA is necessary for the generation of lipid mediators which important biological function in inflammation and other diseases. They have been the focus as therapeutic targets (Zhang et al. 2014). AA will be metabolized into eicosanoids through three different pathways. In the COX pathway, AA is converted to the intermediate prostaglandin G_2 (PGG_2) and later to PGH_2, followed by the metabolism of PGH_2 to form prostaglandin and thromboxanes. In the LOX pathway, AA will be converted to leukotriene and hydroxyeicosatetraenoic acids (HETEs). The cytochrome P450 monooxygenase pathway involves the metabolism of AA to form epoxyeicosatrienoic acids (EETs), HETEs, and hydroperoxyeicosatetraenoic acids (HPETEs). Prostaglandins and leukotrienes bind to G protein-coupled receptor, nuclear peroxisome proliferator-activated receptor, and ABC transporter receptor (D. Wang and DuBois 2010).

Regarding the coronavirus pandemic, there was a report on the possible role of eicosanoids as a therapeutic approach. This is because of the critical role that eicosanoids play in both the initiation and resolution of inflammation. In the later stage of infection, prostaglandin E_2 and prostaglandin D_2 promote a lipid mediator class switching from leukotriene B_4 and 5-lipoxygenase pathway to lipoxins, resolvins, and protectins to promote resolution. This shows that subsequent activities of prostaglandins are essential for the resolution of inflammation (Das 2020).

EPA and DHA are n-3 fatty acids found in oily fish and fish oil supplements. They could suppress the inflammatory responses of leukocyte, adhesion molecules, cytokines, and T cells. Additionally, EPA could lead to the synthesis of eicosanoids with less biological potency compared to AA. The anti-inflammatory activity has been associated with fatty acid composition of the plasma membrane, disruption of lipid raft, and inhibition of nuclear factor kappa B (Philip C. Calder 2013). Therefore, among the strategies to increase EHA and DHA in their tissue concentrations is to incorporate such *n-3* FA into the diet (Combarros et al. 2020).

15.8 Eicosanoids

Eicosanoids have been shown to be involved in multiple diseases such as arthritis, atherosclerosis, pain, and cancer (Buczynski et al. 2009). They are biologically active lipids which can regulate cancer hallmark characteristics such as proliferation, apoptosis, progression, and invasion. They could stimulate epithelial cells to produce growth factors, pro-inflammatory mediators, and angiogenic factors. They also act upon stromal cells to stimulate tumor microenvironment through angiogenesis and evasion from the immune system (Wang and DuBois 2010). Prostaglandins (PGs) have structural relation but differ in their function. COX has been the target for non-steroidal anti-inflammatory drugs (Garavito and DeWitt 1999). The PGH_2

intermediate is then converted by prostaglandin E synthase (PGES), prostaglandin F synthase (PGFS), prostaglandin D synthase (PGDS), PGI synthase, and TX synthase to prostaglandin E_2 (PGE_2), prostaglandin D_2 (PGD_2), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), prostaglandin I, and thromboxane A_2, respectively (Garavito and DeWitt 1999; Yao and Narumiya 2019). For example, in colorectal cancer and breast cancer, PGE_2 causes the activation of prostaglandin E (EP) receptors which then stimulates cancer growth, metastasis, and angiogenesis (Mizuno et al. 2019; Qualtrough et al. 2007; Semmlinger et al. 2018).

In contrast a clinical study revealed that immunohistochemistry high positivity for PGD2 was associated with improved survival (Alves et al. 2019a, b). PGD2 is involved in the activation of DP (PGD2 receptor) and CRTH2/DP2 (chemoattractant receptor-homologous molecule expressed on Th2 cells). Previously, it was associated with inflammation, but recent studies reported it has anti-apoptotic effect in the human colorectal cancer cells, leukemic cells, and eosinophils (Wang and Mak 2011). Cell death was linked with its metabolic products that include PGJ_2, Δ^{12}-PGJ_2, and 15-deoxy-Δ12,14-PGJ_2 (15d-PGJ_2) (Uchida and Shibata 2008). Besides that, peroxisome proliferator-activated receptor gamma (PPAR-γ), endoplasmic reticulum (ER) stress, and oxidative stress were reported to be involved in the cell death (Elhassanny et al. 2019).

The immunomodulatory roles of prostaglandins and leukotrienes are shown by their ability to mediate the crosstalk between epithelial cells and the stromal cells (Wang and DuBois 2010). Chronic inflammation will lead to the recruitment of immune cells, synthesis of pro-inflammatory mediators, disruption of epithelial membrane integrity, and deficiency in the downregulation of mucosal response to antigen. Moreover, the newly recruited immune cells differ in their phenotype compared to those present in normal state. Such microenvironment would retain the inflammatory process and further promote angiogenesis (Wang and DuBois 2010).

There is a complex interaction between cancer and inflammation. The inflammation is involved in all the stages of cancer development that include susceptibility, initiation, progression, dissemination, morbidity, and mortality (Mantovani 2018; Trinchieri 2012). According to a previous study, PGE_2 had exacerbated inflammation and disease severity. It caused an increase in the recruitment of neutrophils and T helper 17 (TH17) cells to the colonic tissue of IBD. The study also showed that the increase of inflammatory T_H17 cells depends on T cells' and dendritic cells' activities. PGE_2 had shift the interleukin-12 (IL-12)/IL-23 ratio in dendritic cells through EP2 and EP4 receptors in favor of IL-23. This led to an increase of T_H17 cells. Each interleukin reacts in a different manner, where IL-12 stimulates T helper 1 (T_H1) responses and inhibits T_H17 development and function, whereas IL-23 is essential for T_H17 expansion and survival (Oshima et al. 1996). Similar findings were reported by other studies; PGE_2 stimulates IL-23-induced T_H17 volume from peripheral blood mononuclear cells and naive T cells (Boniface et al. 2009; Chizzolini et al. 2008), stimulates T_H1 cell differentiation, and increases inflammation through T_H1 and T_H17 cells (Yao and Narumiya 2019).

On the other hand, leukotrienes are involved in various inflammatory diseases. In asthma, the urinary LTE_4 is used as a biomarker (Hoffman and Rabinovitch 2018). Similarly, leukotriene B_4 (LTB_4) is another molecule synthesized through 5-lipoxygenation of arachidonic acid. It is produced by the leukocytes at inflammatory sites (Samuelsson et al. 1987). LTB_4 is a chemoattractant in human T-cell response. It could be a mediator between innate and adaptive immune response (Islam et al. 2006).

The tumor epithelial cells release cytokines, chemokines, and pro-inflammatory eicosanoids. This will lead to the recruitment of leukocytes to establish an immunosuppressive tumor microenvironment. Among the eicosanoids, PGE_2 exhibited association with the modulation of tumor immunosuppression via T_H cells, $CD8^+$ cytotoxic T cells, regulatory T cells, dendritic cells, and myeloid-derived suppressor cells (MDSCs). They cause the shift from anti-tumor T_H1 response to immunosuppressive T helper 2 (T_H2) response. This occurs through the lowering of T_H1 cytokines (tumor necrosis factor-α (TNFα), interferon-γ (IFN-γ), and IL-2) and increasing of T_H2 cytokines (IL-4, IL-10, and IL-6) (Snijdewint et al. 1993). Additionally, PGE_2 could inhibit cytotoxic T cells via the stimulation of a CD94 and NKG2A complex and promote regulatory T-cell function (Zeddou et al. 2005). PGE_2 synthesized by tumor cells can indirectly halt the activity of T cells. This occurs through the downregulation of direct antigen presentation by tumor cells as well as cross-presentation by dendritic cells (Ahmadi et al. 2008). A previous study described that PGE_2 changes the function of dendritic cells from the induction of immunity to T-cell tolerance by upregulating CD25 and indoleamine-2,3-dioxygenase (von Bergwelt-Baildon et al. 2006). They could inhibit the differentiation of dendritic cells and proliferation of T cells (Yang et al. 2003). In the in vivo study of mammary tumor, PGE_2 promoted tumor progression through the induction of MDSCs (Sinha et al. 2007). These findings suggest that effects of PGE_2 may permit tumor cell survival (Wang and DuBois 2010).

Even though roles of PGE2 have been well established based on previous studies (Karpisheh et al. 2019; Miao et al. 2012; Moltu et al. 2017), further investigation is needed (Yoda et al. 2015). Inflammation is one of the factors which contribute toward the progression of breast cancer (Howe 2007). Cancer is a heterogeneous disease that is determined by the local tumor microenvironment (Basu et al. 2016; Gomes et al. 2016). Studies showed that prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is involved in hormone regulation and linked to the development of breast cancer (Basu et al. 2015). Its metabolite has been found to be increased in inflammatory response and *indicate* lipid peroxidation (Basu et al. 2016). Experimental studies demonstrated that COXs and free radicals were associated with breast cancer (Howe 2007). Clinical studies indicated that increased level of urinary PGE-M levels was also correlated with elevated risk of breast cancer (Cui et al. 2014). However, effects of F_2-isoprostane and $PGF_{2\alpha}$ in breast cancer need to be further investigated (Basu et al. 2015).

Cysteinyl leukotriene (cysLT) is another form of lipid mediator. It is synthesized from the arachidonic acid by the action of the enzyme 5-lipoxygenase (5-LOX). cysLT has inflammatory response, and inhibitors have been developed for the

treatment of asthma, allergic rhinitis, and other inflammatory disorders (Rahman et al. 2019).

15.9 Prostanoids

Prostanoids are synthesized when there is stimuli. They act by either a paracrine or an autocrine manner and have a significant role in normal physiology and disease (Smyth et al. 2009). For instance, PGE2 can induce fever, pain, and inflammation (Nakanishi and Rosenberg 2013), whereas PGD2 is involved in allergic reactions (Boyce 2007) While PGF2α regulates the cytokine response of mast cells, PGI2 regulates the function of T helper cells (Boswell et al. 2011; Kaneko et al. 2008). TXA2 is produced by platelets, activated macrophages, monocytes, and dendritic cells. The production of prostanoids in immune cells increases particularly during inflammation (Thomas et al. 2014; Tilley et al. 2001).

In most cells, COX plays an important role in the metabolism of arachidonic acid to form the prostanoids (Ricciotti and FitzGerald 2011). The two isoforms of COX are structurally identical but differ in substrate selectivity and their intracellular location. In regard to function, COX-1 maintains gastric and renal integrity (Vane et al. 1998; Laneuville et al. 1994), while COX-2 produces prostanoids that are involved in cancer, metabolic syndrome, and cardiovascular diseases (Aboonabi and Aboonabi 2020; Gartung et al. 2019). Due to these factors, COX2 has been a therapeutic target. Previous clinical studies showed promising results when given either non-steroidal anti-inflammatory drugs (NSAIDs) to non-selectively suppress COX-1 and COX-2 or selective suppression of COX-2 (Harris 2009).

The progression of cancer is associated with its microenvironment (Khadge et al. 2018). The microenvironment consists of various cells like fibroblasts and endothelial cells. The other types of cells are those involved in immune surveillance such as the T cells, macrophages, dendritic cells, mast cells, natural killer (NK) cells, myeloid-derived suppressor cells (MDSC), neutrophils, and others (Joyce and Pollard 2009). Lymphocytic infiltration is part of the attempt by the immune system to reject the presence of tumor. An example of alternative activation is the $CD4^+$ T helper lymphocyte type 1 and type 2 (T_H1 and T_H2, respectively) paradigm. Th1 leads to a complex cellular immune response involving T cells and macrophages, while Th2 or non-Th1 profile leads to a humoral immune response with typical involvement of B cells and antibodies. The uncontrolled and persistent Th2-type immune response may be associated with atopic diseases such as allergies or allergic asthma. Conversely, an uncontrolled and persistent Th1-type immune response is at the core of many chronic diseases in which inflammation leads to pathological changes and clinical symptoms (Padol and Hunt 2010). Stimulated T_H1 cells cause the release of cytokines that support cytotoxicity, while T_H2 cells contribute to adaptive immunity and humoral antibody production. T_H1-derived cytokines can suppress T_H2 cytokine responses, and vice versa (E. P. Chen and Smyth 2011). A study on early breast cancer patients found there was no association between the density of $CD3^+$ or $CD8^+$ tumor-infiltrating lymphocytes (TILs) and overall survival.

However, they found the median disease-free survival (DFS) of those with a high density of $CD8^+$ TILs to be significantly longer compared to those with lower density of $CD8^+$ TILs. According to their study, the density of $CD8^+$ TILs was an independent predictive factor for OS and/or DFS in these patients (Okabe et al. 2017).

According to WHO, ischemic heart disease (IHD) is one of the major causes of mortality worldwide (Nowbar et al. 2019). In relation to IHD, atherosclerosis plays a pivotal role in its development. Evidence show there is involvement of immune response in the development of atherosclerotic plaque. Obstructive atherosclerotic disease that is associated with the development of myocardial ischemia is characterized by the presence of stable plaque. On the other hand, unstable atherosclerotic plaque could rupture and has been related to thrombotic vessel occlusion, acute stroke, or myocardial infarction (Daghem et al. 2020). The unstable plaque has been associated with a Th1 immune response that involves T cells, neutrophils, and macrophages. In particular, macrophages are dense with cholesterol esters which are transported by low-density lipoprotein (LDL). Accumulation of lipids within the macrophages will lead to the formation of foam cells (Mazzolai et al. 2004). Structurally, cholesterol esters are formed through the esterification of free cholesterol with fatty acids (Ho et al. 2004; Nestler et al. 1990). Some of fatty acids present in cholesterol esters are polyunsaturated fatty acids, linolenic acid, arachidonic acid, docosahexaenoic acid, and docosapentaenoic acid (Tuckey and Stevenson 1979), while a stable plaque is correlated with a Th2 response with the involvement of smooth muscle cells and collagen-secreting cells along with collagen (Shah 2003). It is reported that the movement from Th1-driven to the Th2-driven inflammation will halt the progression of atherosclerosis and remodelling of plaque to form a stable smooth muscle- and collagen-composed plaque (Laurat et al. 2001). This would lower the risk of plaque rupture and the consequences of stroke or myocardial infarct MI (Adler et al. 2005).

COX-2-derived PGE_2 is able to suppress Th1-driven cellular responses and is necessary for Th2 responses (Pellicanò et al. 2007). Furthermore, PGE_2 could inhibit the activity of NK cells and stimulate B-lymphocyte Ig isotype switching to IgE (Roper et al. 1995). Consequently, inhibition of COX-2 by selective and tNSAIDs may cause an increase in Th1 response but instead impair the Th2 response. This would enhance pro-inflammatory cellular responses and suppress the humoral immune response (Haas et al. 2006). Therefore, modulation of the biosynthesis of prostanoids could be of therapeutic relevance in the treatment of atherosclerosis (Zhu et al. 2020).

15.10 Lipoxins

Lipoxins (LXs) are lipid mediators which are involved in the resolution of inflammation. They function by inhibiting neutrophil infiltration, promoting macrophage polarization, increasing macrophage efferocytosis, and restoring tissue homeostasis. LXs and their synthetic analogues are able to protect tissues from acute and chronic

inflammation by downregulating pro-inflammatory cytokines and chemokines (e.g., interleukin-1β and tumor necrosis factor-α), inhibiting pro-inflammatory pathway, and increasing the production of cytokines (Fu et al. 2020).

Diabetic patients with chronic kidney disease were treated with aspirin for 12 months. Finding from the trial showed there was an increase in the level of lipoxin epimer (15-epi-lipoxin A4). This had contributed to the reduction in inflammatory markers among the patients (Goicoechea et al. 2017). Similar finding was noted in coronary artery disease (CAD) patients in another study, where treatment with aspirin had increased the specialized pro-resolving lipid mediators (i.e., lipoxin, resolvin, and protectins). Their findings showed that patients with CAD had low level or absence of specific lipid mediators. Low level of lipid mediators may enable the progression of chronic vascular inflammation and may predispose to atherosclerosis and thrombosis (Elajami et al. 2016). In other diseases such as cancer, there was no association of lipoxin A4 with lowering risk of colorectal adenoma (Fedirko et al. 2017).

15.11 Triglycerides

Free fatty acid is the building blocks for various complex lipids like phospholipids, sphingolipids, and glycerolipids. An example of glycerolipid is the triglycerides (Balaban et al. 2015). Triglycerides are stored in the adipose tissue, which secretes metabolites that can either promote or resolve an inflammatory response (Kershaw and Flier 2004). They contain fatty acids as part of their chemical structure. Long-chain fatty acid is transported in the blood circulation either in the form of free fatty acids that are released from adipocytes bound to albumin or as triglycerides contained in VLDL and chylomicrons. These circulating triglycerides will be hydrolyzed by lipoprotein lipase to free fatty acids and then taken up into cells (Balaban et al. 2015).

Intake of excess dietary sources will cause further accumulation of triglycerides and form enlarged fat cells (hypertrophy) and increase in numbers of preadipocytes. Role of VLDL was also reported in cancer cells (Lu et al. 2017). As a consequence, this will be leading to hypoxic condition, activation of cellular stress pathways, and autonomous inflammation due to the formation of pro-inflammatory cytokine (de Luca and Olefsky 2008). This also results in myeloid infiltration of adipose tissue that will surround the adipocytes. Occurrence of a phenotypic shift of the adipose tissue macrophages will cause release of pro-inflammatory cytokines. It will promote the production of reactive oxygen species and activate inflammatory signalling pathways in neighboring adipocytes (Khadge et al. 2018).

15.12 Conclusion

In general, fatty acids play important roles in human physiology based on experiment and clinical studies. They display dual characteristics, where they are grouped as either pro-inflammatory or anti-inflammatory agents. In the clinical setting, anti-inflammatory fatty acids have been used to treat various ailments. Antagonist or inhibitors of pro-inflammatory fatty acids are commercially available for treatment purposes. Taken together, a better understanding of the immunomodulatory regulation by fatty acids particularly in chronic diseases such as cancer and diabetes is warranted to reduce the prevalence as well as the healthcare expenditure for treatment purposes.

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Immunomodulatory Effects of Endocrine-Disrupting Chemicals

16

Soma Mondal Ghorai and Hardeep Kaur

Abstract

Endocrine-disrupting chemicals or EDCs have become an integral part of human environment, be it in cosmetics, food, plastic packaging materials, toys, pesticides, and numerous other amenities. The last 10–15 years have seen a rekindled interest of the scientific community to determine their effect on human health especially their influence on the neuroendocrine immune system. EDCs like bisphenols, dioxins, phthalates, and phenols have been found to adversely influence the development and functioning of immune cells like monocytes, neutrophils, macrophages, etc. These compounds have been found to damage DNA, trigger chromosomal aberrations, disrupt cell cycle checkpoints, and cause cell death. The dosage as well as duration of exposure has also been found to play an important part in the effect of EDCs. This chapter provides a comprehensive overview on the EDCs, their mode of action and critical role as immunomodulators in allergy, inflammation and autoimmunity. The nexus between environmental EDCs and epigenetic regulation of gene has also been underlined. The article also stresses upon the need for enhanced research on existing and emerging EDCs along with advocating involvement of individual and scientific society and its stakeholders in communicating and implementing changes in public policy and awareness.

Keywords

Endocrine disruptors · Phthalate · Bisphenol · Epigenesis

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16.1 Introduction

16.1.1 Overview of Eco-immune-neuroendocrine System

Life throws continuous challenges in the form of intrinsic and extrinsic factors that tend to threaten survival. Every organism fights lifelong battle against extrinsic factors like bacteria, viruses, fungi, various other pathogens, and even certain food that are foreign to the body. Moreover, the intrinsic factors aptly termed as "molecular garbage" comprising cellular or metabolite debris, by-products of incomplete metabolisms, and non-enzymatic functions along with certain molecular aggregates also rage war within the body (Franceschi et al. 2017a). As the life evolved, every organism from invertebrates to vertebrates has recognized these dangers and adapted to neutralize such stressors that co-inhabit their *eco-space*. Thus, the cross-talk between both immune and neuroendocrine pathways, albeit using minimum energy, became the key to the survival of an organism in a particular ecological niche (Tauber 2017). Within a particular ecosystem, every living entity copes to maintain homeostasis by a cordial tri-directional communication between the central nervous system (CNS), the endocrine system, and the immune system (Elenkov et al. 2000; Weigent and Blalock 1987; Besedovsky and del Rey 1996; Ottaviani et al. 2008) (Fig. 16.1).

The interdependence of neuroendocrine-immune system can be ascertained by the fact that these systems and their processes are mediated by a highly conserved common pool of molecules, suggesting that they have a common evolutionary origin (Besedovsky and del Rey 1996; Malagoli and Ottaviani 2014). A desirable ecological requirement often needs a common organizational architecture to understand complex biological processes. In this case, the immune system can be considered as the central system or element that integrates and mediates effective trade-offs with other systems of the body to maintain homeostasis. Metaphorically, it can be equated to the "knot of the bow" that forms a rigid core that is capable of integrating with

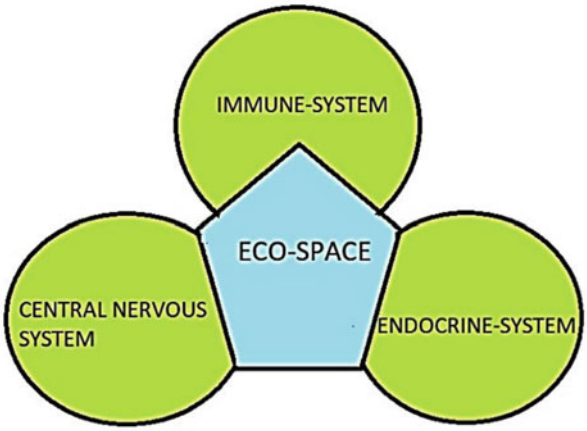


Fig. 16.1 Balance between neuroendocrine-immune systems: key for organismal survival

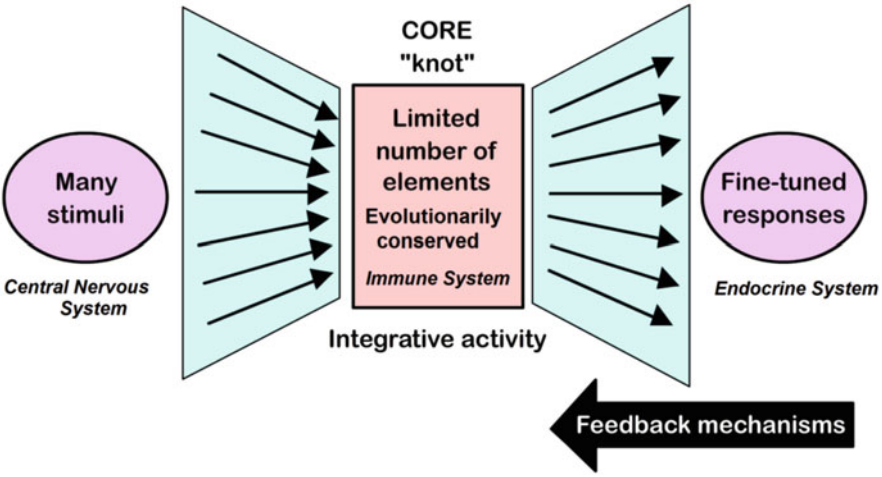


Fig. 16.2 Immune system as the "knot of the bow" with a conserved rigid core (Source: Adapted from Tieri et al. 2010)

other two systems. The molecules in the system are evolutionarily conserved and characterized by networking (ability to communicate with other systems), degeneracy (ability to interact with many different molecular mechanisms), and plasticity (ability to respond and adapt) (Csete and Doyle 2004; Tieri et al. 2010) (Fig. 16.2).

16.1.2 Neuroendocrine and Immune System Among Invertebrates and Vertebrates

The study of invertebrate and vertebrate neuroendocrine and immune system highlights high degree of conservation between the two, with the common objective of maintenance of homeostasis in the body of the organism (Malagoli and Ottaviani 2014). The story goes back to Elie Metchnikoff in 1882 and his discovery of specialized phagocytic cells in crustacean larvae (Tan and Dee 2009). Eventually later research in invertebrates led to an array of names given to such phagocytic cells. A consensus was reached to name such cells as *immunocyte*, a unique cell type that emerged first in invertebrates and then evolved in vertebrates. Immunocytes are phagocytic in nature; display pro-opiomelanocortin (POMC) peptides, corticotropin-releasing hormone (CRH), and other molecules; thus are involved in immune responses, infalmmation, as well as neuroendocrine or stress responses (Ottaviani 2011).

The POMC gene is translated into a protein called pro-opiomelanocortin (POMC) and cleaved into a distinct set of peptides that are expressed by different cell types in the body (Krude et al. 2003). The biological activity of POMC-derived peptides is further regulated by additional post-translational changes like glycosylation, phosphorylation, amidation, sulfation, and acetylation (O'Donohue and Dorsa 1982;

Farah et al. 1986; Vaudry et al. 1986). Adrenocorticotropic hormone (ACTH) is one of the main peptides formed from POMC which binds to melanocortin 2 receptor (MC2R) and stimulates the release of cortisol hormone. Cortisol is the major player that maintains normal blood sugar levels, protects the body from stress, and suppresses inflammatory reactions (Mains et al. 1977; Roberts and Herbert 1977). Another peptide is the beta-endorphin which binds to opioid receptors in the brain and further signals for pain relief (O'Donohue and Dorsa 1982).

It is now clear that the fundamental structure and function of most immune defense cells have remained the "same or similar" throughout the living system. Nature has just used the old molecules and evolved them into more complicated ones with defined functions (Malagoli et al. 2017). An increase in phagocytic activity under the influence of neuroendocrine molecules like ACTH, CRH, and other cytokines was seen in molluscan immunocytes (Ottaviani et al. 1995, 1997a, b). With the advent of new and sophisticated technical approaches like flow cytometry, in situ hybridization, radioimmunoassay, etc., the presence of POMC products, glucocorticoids, biologically active peptides, biogenic amines, and cytokines was established in invertebrates (molluscs and annelids) (Ottaviani et al. 1990, 1992a, b, 1995, 1998, 2004; Salzet et al. 1997; Grimaldi et al. 2012, 2014; Bidmon and Stumpf 1991; Tascedda and Ottaviani 2016). Changes in cell shape and mobility were observed in the immunocytes of *Mytilus galloprovincialis* when incubated with ACTH which is attributed to both cyclic AMP (cAMP) and protein kinase C pathway (Sassi et al. 1998). Immunocytes in molluscs (*Mytilus edulis*) and the insects (*Leucophaea maderae*) show increased adherence and chemotaxis with other opioid neuropeptides (Stefano et al. 1989a, b). Surprisingly, the fundamental phagocytic action of ACTH is maintained even in human as they transcend from urodele amphibians.

In higher vertebrates, neuroendocrine function is mainly carried out by cells of adaptive immune systems, the lymphocytes. The role of lymphocytes in the regulation of neuroendocrine functions in vertebrates is well-documented. The receptors for both adrenocorticotropic hormone (ACTH) and endorphins are expressed by lymphocytes in anuran amphibian (Ottaviani et al. 1992b), chickens (Siegel et al. 1985), reptiles (Harbour et al. 1991), and humans (Smith and Blalock 1981). Receptors for other different peptides like growth hormone (GH), somatostatin, thyrotropin hormone (TTH), corticotropin-releasing hormone (CRH), vasoactive intestinal peptide (VIP), vasopressin, and oxytocin are also displayed on lymphocytes and other immune cells like monocytes and lymphoid cell lines (Kiess and Butenandt 1985; Besedovsky and del Rey 1996).

The major mediators of immune system activity, the cytokines, also play a special role in interactions between the immune and neuroendocrine systems (Hughes Jr and Chin 1994). In invertebrates, cytokines such as interleukin-1 alpha (IL-1α), tumor necrosis factor alpha (TNF-α), IL-8, platelet-derived growth factor alpha beta (PDGF-αβ), and transforming growth factor beta-1 (TGF-β1) are involved in chemotaxis and cell adherence of immunocytes (Ottaviani et al. 2004). Employing different phosphoinositide signalling pathways, cytokines such as PDGF-αβ, TGF-β1, and IL-8 incite cell motility. In fact, IL-8 utilizes protein kinase A (PKA)

and protein kinase C (PKC) to induce cell motility (Ottaviani et al. 2000), while PDGF-αβ conducts its activity via Ca^{2+}-independent pathway (Ottaviani et al. 1997b) and TGF-β1 through a Ca^{2+}-dependent pathway (Kletsas et al. 1988).

In higher vertebrates, a complex network exists between many pro-inflammatory cytokines (e.g., IL-1, IL-6, and IL-18) and the central nervous system. Under specific stimuli, these cytokines induce multifarious molecular, systemic, and behavioral responses (Dantzer et al. 2008). These bind to specific receptors on neurons and microglial cells, incite distinct molecular signalling pathways, and control extremely complex functions (Alboni et al. 2013a, b, 2014; Elenkov 2008). IL-18 is demonstrated to directly contribute to loss of appetite during stress in rodents. It was shown that ablation of IL-18 gene produces obese phenotype, while the addition of the recombinant cytokine to the bed nucleus of the stria terminalis (BNST) causes anorexia (Francesconi et al. 2016).

Evolution has led to an intricate interdependence of the endocrine and immune system with each other, and there is clear-cut evidence that some immune-competent cells can secrete both functional substances like cytokines and some hormones (Smekens et al. 1983; Csaba et al. 2004; Csaba 2011; Lardone et al. 2011). It has also been seen that the immune cells get affected not only by endogenous hormones but also by certain compounds that structurally resemble these natural hormones. These compounds are termed as "endocrine-disrupting chemicals" or more commonly called EDCs. These compounds hence cause many health hazards, with the immune system constituting one of the main targets of EDCs' action. This chapter therefore focuses on EDCs as immunomodulators along with their role in (1) innate and adaptive immunity, (2) allergic diseases and inflammation, (3) autoimmune diseases, and (4) epigenetic regulation.

16.1.3 What Are Endocrine-Disrupting Chemicals (EDCs)?

Endocrine disruptors or endocrine-disrupting chemicals (EDCs) are exogenous compounds that mimic the normal endocrine system or its hormones and cause untoward effects on homeostasis, reproduction, and developmental processes in human. These chemicals are also found to play an adverse role in the environment affecting both wildlife and marine life (Fig. 16.3). EDCs are normally derived from industrial and agricultural sources and are ubiquitously found in the environment. They are also present in most of the consumer products like personal care products and cosmetics as well as in pesticides, fungicides, insecticides, herbicides, and chemicals used in the plastic industry. To date, there are thousands of compounds that can be classified into EDCs, but according to the European Union, there are 150 listed EDC compounds. There is still much to know about the mechanism of their action, and surprisingly, EDCs are yet to be defined correctly as different regulatory bodies keep changing their definition (Table 16.1) (EC 1996; Kavlock et al. 1996; Damstra et al. 2002).

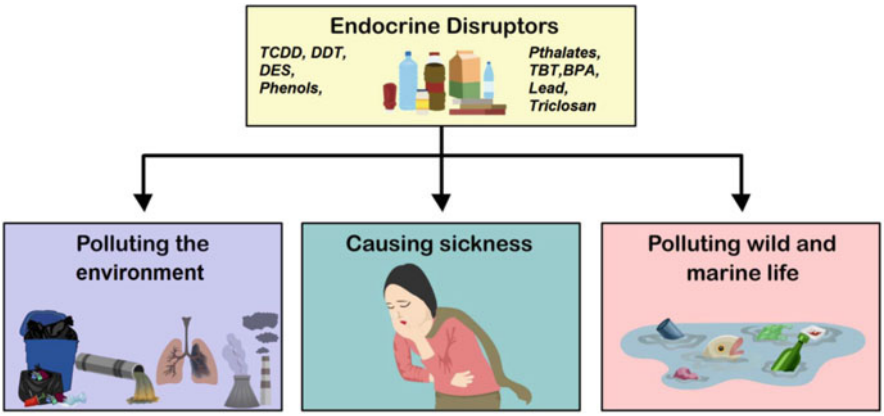


Fig. 16.3 Examples of endocrine disruptors

Table 16.1 Different definition of EDCs given by different agencies

S. no.	Definition of EDCs	Agency	Year
1	"An exogenous agent that interferes with synthesis, secretion, transport, metabolism, binding action, or elimination of natural blood-borne hormones that are present in the body and are responsible for homeostasis, reproduction, and developmental process" (Kavlock et al. 1996)	US Environmental Protection Agency	1996
2	"An exogenous substance that causes adverse health effects in an intact organism, or its progeny, secondary to changes in endocrine function. A potent endocrine disrupter is a substance that possesses properties that might be expected to lead to endocrine disruption in an intact organism" (European Commission 1996)	European Union	1996
3	"Exogenous substance or mixture that alters function (s) of the endocrine system and consequently causes adverse effects in an intact organism, or its progeny, or (sub) populations" (Damstra et al. 2002)	World Health Organization	2002

EDCs are known to cause many endocrine and metabolic disorders like neurobehavioral disorders, impaired thyroid and sex hormone functioning, obesity, diabetes, metabolic syndrome, polycystic ovarian syndrome, irregular menstruation and ovarian failure, cancer of breasts, cancer of ovaries and cervix, disrupted spermatogenesis, autoimmune disorders, allergy, asthma, and inflammation. All living species encounter health hazards due to EDCs. These compounds are further classified based on their sources as given below (Tavares et al. 2016; Zawatski and Lee 2013):

(a) **Natural EDCs** which enter the ecosystem through food: coumestrol, genistein, and phytoestrogens.

(b) **Synthetic EDCs** which are released into the environment as food preservatives, plasticizers, lubricants, industrial solvents, herbicides and pesticides, pharmaceuticals, personal care products, etc.: vinclozolin and diethylstilbestrol (DES), dichlorodiphenyltrichloroethane (DDT), phthalates, bisphenol A (BPA), polychlorinated biphenyls (PCBs), polybrominated biphenyls (PBBs), dioxins, etc.

EDCs can also be classified based on the system or organ they affect (Whaley et al. 2001; Lee et al. 2013).

1. EDCs that affect the central nervous system.
2. EDCs that affect the thyroid.
3. EDCs that affect the pancreas.
4. EDCs that affect the reproductive system.
5. EDCs that affect the immune system.

16.1.4 Mode of Action of EDCs

Endocrine disruptors can exert their action via mimicking a natural hormone or by blocking the receptors of the natural hormones. They can also affect the enzymatic pathways that synthesize the hormones or by disturbing their signalling mechanism. Broadly EDCs' mode of action can be outlined as given below and also shown in Fig. 16.4.

(a) **Binding to hormone receptors:** The general understanding is that most of the endogenous hormones exert their action via their respective receptors. Traditionally, EDCs are known to exert their action by binding to the steroid hormone nuclear receptors (pancreas receptors (paRs), thyroid receptors (TRs), retinoid receptors, estrogen receptors (ERs), androgen receptors (ARs), and progesterone receptors (PRs)). But recent research has proven that the extent of EDCs' toxicity does not limit to just binding to steroid hormone receptors but has expanded to the horizons of both non-nuclear steroid hormone receptors (membrane ERs), non-steroid receptors (neurotransmitter receptors such as serotonin receptor, dopamine receptor, and norepinephrine receptor), and orphan receptors (aryl hydrocarbon receptor (AhR)) (Lee et al. 2013; Hampl et al. 2016; Kolšek et al. 2014).

(b) **Effect on enzymatic pathways:** The physiological behavior underlying both sex-specific endocrine and reproductive systems occurs due to various enzymatic pathways. EDCs can affect the enzymatic pathways that have substantial roles in steroid biosysthesis and /or biotransformation and several other mechanisms taht influence sex-specific physiology/behavior and the endocrine and reproductive systems. EDCs can specifically inhibit or disrupt the enzymatic pathways involved in steroidogenesis, particularly in the metabolism of estrogens (Chen et al. 2013; Piché et al. 2012; Andric et al. 2006). For example, an increase in circulating estradiol is often noted when the enzyme sulfotransferase is inhibited by polychlorinated biphenyl (PCB) metabolites.

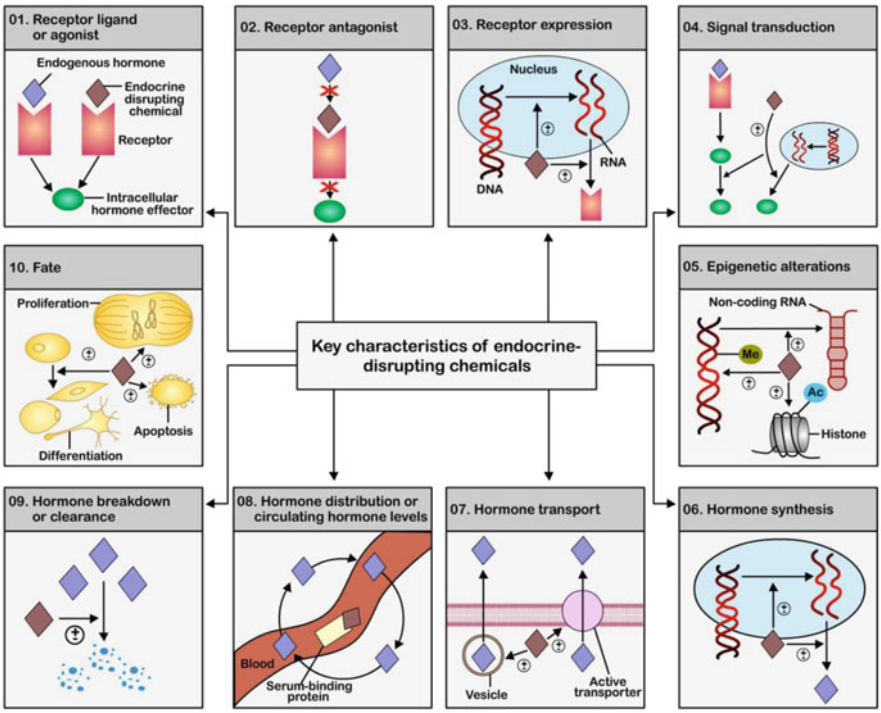


Fig. 16.4 Mechanism of action of endocrine disruptors (Source: Adapted from La Merrill et al. 2020)

(c) **Effect on signalling pathways:** Most of the hormone action takes place via their nuclear and non-nuclear receptors which in turn follow specific signalling pathways to perform specific functions. EDCs can control many cellular signalling pathways by either downregulating or upregulating the genes involved in the regulation of signal transduction (Zhang et al. 2015; Hsieh et al. 2012a). The details can be checked later in the chapter (Sect. 16.7).

(d) **Effect on other mechanisms:** Many synthetic and natural estrogens and antiandrogens cause toxicity via other mechanisms which may be both genotoxic and reprotoxic. These can be either epigenetic mechanisms that usually involve imprinting, RNA interfering, histone methylation, DNA modification, release of oxidative reactive radicals, cytotoxicity, apoptosis, peroxisome dysfunction, and hormonal imbalances. Various other mechanisms are also reported which disturb the cellular metabolisms or functions of several cell organelles like the peroxisomes, mitochondria, and cytoskeletons. The highly intricate signalling web and cellular communication of endocrine-immune system enable EDCs to employ the mechanisms/pathways that cause harm to the main immune organs like the spleen, liver, and kidney. These compounds damage DNA; cause chromosomal aberrations; disrupt cell cycle checkpoints; cause cell death by autophagy, apoptosis, or necrosis; and release pro-inflammatory cytokines (Kiyama and Zhu 2014; Kiyama and Wada-Kiyama 2015).

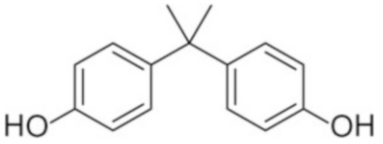
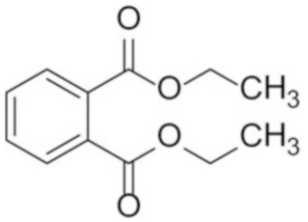
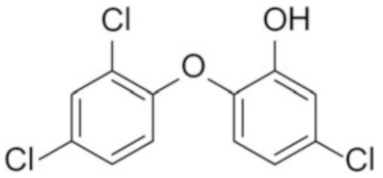
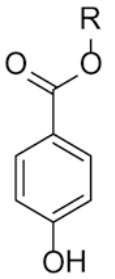
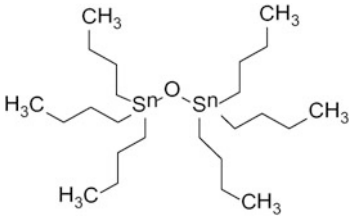
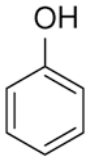
[EDC can up- or downregulate the processes which are represented as (±) symbol. EDC can antagonize hormone receptors as well as alter hormone receptor expression. EDC can alter signal transduction (including changes in protein or RNA expression, post-translational modifications, and/or ion flux) in hormone-responsive cells. EDC can induce epigenetic modifications in hormone-producing or hormone-responsive cells. ECD can alter hormone synthesis, hormone distribution, or circulating hormone levels and hormone transport across cell membranes. EDCs can epigenetically damage DNA, cause chromosomal aberrations, disrupt cell cycle checkpoints, and cause cell death by autophagy, apoptosis, or necrosis].

16.2 Effect of EDCs on the Immune System

Homeostasis and physiologic development of the body depend on a complex and intricate mechanism that requires a successful organization of the hypothalamus and pituitary gland in the brain (Gore 2008). To maintain this balance, neuroendocrine systems control the immune responses, and in turn, many immune cells become sensitive to endogenous hormones; thus, they get easily affected by endocrine disruptors. Various researches have shown that EDCs may modulate the immune system at different levels of the immune regulatory network, including maturation and differentiation of various immune cells, production of cellular and humoral response, and cytokine synthesis by the immune cells (Chalubinski and Kowalski 2006). Unfortunately, limited work has been done to understand the effect of EDCs on the immune system despite the fact that these compounds can have a profound effect either alone or in combinations under a wide concentration range. Dysfunction within the immune system on exposure to EDCs is now noted in certain diseases like lupus, diabetes, allergy, asthma, etc. It has been proven that the development and functions of various immune cells (natural killer cells, dendritic cells, eosinophils, monocytes, neutrophils, mast cells, etc.) can get affected by many EDCs (Nowak et al. 2019). Nonetheless, it must be noted EDCs do not follow the traditional toxicology dose-dependent linear graph. Some EDCs seem to be effective at very low doses, and their adverse effect decreases at high dose levels; thus, they follow non-linear, inverted U-shaped dose-response curves (Myers et al. 2009). Though EDCs cause many health hazards affecting different systems, the immune system constitutes one of the main targets of EDCs' action. List of EDCs which are known to directly affect the immune system is given in Table 16.2.

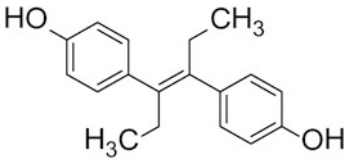
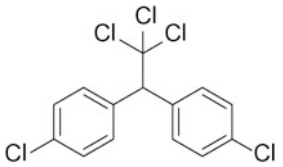
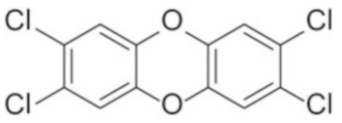
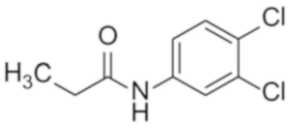
The immune system comprises innate and adaptive immunity and is capable of remembering all the immunological experiences it was exposed to. This memory is given a new term called *immunobiography*. The varied heterogeneous responses can be attributed to the lifelong learning of the immune system and its response to a variety of foreign components it had encountered, thus building a memory within adaptive immune responses. This is also defined as providing a "trained immunity" to the innate branch of the immune system (Franceschi et al. 2017b). Thus, the development of our immune system right from birth decides the proper functioning of our body at later stages of life. It had been noted that the development and

Table 16.2 List of compounds which directly affect the immune system

Name of compound	Source	Chemical structure
Bisphenol A (BPA)	Polycarbonate plastics used in food and drink packages, epoxy resins in metal cans, sports and toys, medical equipment (dental monomers; eye lenses, plastic tubes, and pipes used in hospitals), and consumer electronics	
Phthalates: – Diethyl phthalate (DEP) – Di-(2-ethylhexyl) phthalate (DEHP) – Diisononyl phthalate (DINP)	Cosmetics and personal care products, PVC (polyvinyl chloride), plastics	
Triclosan	Soaps and detergents, deodorants and facial tissues, wound disinfectants and antimicrobial preservatives, kitchenware and utensils, toys	
Parabens: – Methylparaben – Ethylparaben – Propylparaben – Butylparaben	Cosmetics and personal care products, food preservatives, pharmaceutical preservatives	
Tributyltin (TBT)	Paints with anti-fouling biocides	
Phenols: – Nonylphenol (NP) – Octylphenol (OP) – Diisononylphenol (DiP)	Cosmetics and personal care products, soaps, plastics, and pesticides	

(continued)

Table 16.2 (continued)

Name of compound	Source	Chemical structure
Diethylstilbestrol (DES)	Pharmaceutics	
Dichlorodiphenyl-o-trichloroethane (DDT)	Herbicide	
Tetrachlorodibenzo-p-dioxin (TCDD)	Herbicide	
Propanil	Herbicide	

maturation of the system are affected by exogenous agents like EDCs, thus hampering the effector functions of both innate and adaptive branches of immunity (Csaba 2018; Malaisé et al. 2018).

16.2.1 Effect of EDCs on the Immune System of Neonates and Infants

It is a well-known fact that neonates and infants possess immature immune system and hence are more prone to certain diseases like common cold and asthma. This can be attributed to increased immune system susceptibility to xenobiotics which can produce an adverse effect even at a low-dose exposure. A study by Luebke et al. in 2006, who demonstrated that exposure to five EDCs, namely, diethylstilbestrol (DES), diazepam (DZP), lead (Pb), 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), and tributyltin oxide (TBTO), rendered greater risk to children than adults and the immunotoxicity remained persistent throughout life. Another study in Inuit population (Inuit are a group of culturally similar indigenous peoples inhabiting the Arctic regions of Greenland, Canada, and Alaska) showed that infants developed acute otitis media on exposure to p,p′-DDE (dichlorodiphenyldichloroethylene, a metabolite of DDT) (Dewailly et al. 2000). Other studies showed that bisphenol A (BPA) could immunomodulate innate immunity against influenza virus type A (Roy et al.

2012) and parasitic *Nippostrongylus brasiliensis* (Ménard et al. 2014). Contrary to this, few studies showed that BPA exposure to perinatal animals does not cause significant changes in immunity and does not impact the hematopoiesis in the fetal liver (Holladay et al. 1993; Heilmann et al. 2006).

EDCs are also found to affect the adaptive immune system, and the fetal lymphoid organs and tissues are shown to be quite vulnerable (Fine et al. 1989; Gehrs and Smialowicz 1997; VanLoveren et al. 2003; Dietert 2009). Exposure to dioxins and propanil can cause thymus atrophy and splenomegaly (Cuff et al. 1996; Xie et al. 1997). Additionally, perinatal introduction to DES and BPA resulted in reduced lymphocytes in the thymic cortex and decreased levels of T-helper cells, T-regulatory cells, and dendritic cells in the spleen and mesenteric lymph nodes. A dose-dependent decrease in the secretion of cytokines like IFN-γ by T_H1 cells and IL-13, IL-4, and IL-10 by T_H2 cells was also observed (Yoshino et al. 2004; Ménard et al. 2014). Nonyphenols and Bisphenols are shown to shift the immune responses from T_H1 type towards T_H2 type, predisposing an individual to allergic conditions in later life (Rees Clayton et al. 2011; Pisapia et al. 2012). Environment levels of TBT (1 nM–10 μM) did induce apoptosis in developing B-cell lines by directly affecting the B cells or changing the hematopoietic microenvironment in the bone marrow (Baker et al. 2017). DEHP metabolite and mono(2-ethylhexyl) phthalate metabolite both work as an agonist to the PPAR-γ signalling pathway, thereby leading to the inhibition of developing B lymphocytes and eventual apoptosis (Schlezinger et al. 2004; Agas et al. 2018). A sex-related observation on the perinatal exposure of EDCs like dichlorophenol, BPA, parabens, and triclosan was made which showed boys to be more sensitive toward respiratory allergy rather than girls, thus shedding light on the potential of EDCs to act via estrogen receptors for the biological mechanism (Buckley et al. 2018; Lee-Sarwar et al. 2018).

16.2.2 Effect of EDCs on Cells of Innate Immune System

16.2.2.1 Macrophages and Monocytes

Large phagocytic cells of innate immunity that are essentially derived from the bone marrow precursor cells differentiate into either monocytes in the blood or macrophages in the tissue. Macrophages and monocytes possess characteristics to migrate, phagocytose, and kill microorganisms and are also known to release reactive oxygen and nitrogen species during the inflammation process. Dormant macrophages are activated through their high- and low-affinity receptors for IgG (FcR/RI/II) and receptors for complement proteins (CR1). Thus, they provide important links between innate and adaptive immunity by expressing Toll-like receptor-mediated pathogen recognition molecules by releasing a variety of pleiotropic pro-inflammatory cytokines such as IL-1, TNF-α, and IL-8, as well as lipid mediators (e.g., leukotrienes and prostaglandins) which are accomplished in programming adaptive immune responses (DeBrosse and Rothenberg 2010).

Prominent EDCs have shown a differential pattern of dose, time, and route of exposure to affect macrophages and monocytes, which constitute the first line of

defense in the immune system. Surprisingly, initial work on the effect of EDCs (NP, DiP, and BPA) did not demonstrate any harmful impact of such compounds on cell viability of monocytes or macrophages (Segura et al. 1999; Bennasroune et al. 2012). However, with increasing research on environmental pollutants, EDCs like BPA were found to exhibit undesirable effects on innate immune cells. It was observed that BPA at the concentration of 10^{-5} to 10^{-8} M suppresses macrophage ability to adhere and modulate inflammatory responses (Segura et al. 1999). Other EDCs like propanil, nonylphenol (NP), and diisononylphenol (DiP) also showed decreased phagocytic activity in human macrophages and monocytic cell line (THP-1), which in turn directly affected the phagocytosis of many bacterial pathogens as seen in *Listeria monocytogenes* infection (Ustyugova et al. 2007; Bennasroune et al. 2012). Apart from phagocytosis, macrophages are also involved in the autophagy process as a defense mechanism against pathogens by delivering them for lysosomal degradation. Wang and coworkers in 2018 investigated the property of autophagy in RAW 264.7 macrophages on exposure to triclosan, and it was noted that triclosan led to excessive autophagy and subsequently to carcinogenic tendency.

Macrophages regulate reactive oxygen and nitrogen species (the key molecules for defense against pathogenic agents) by expressing genes and proteins involved in the production of nitric oxide (NO) synthase and inducible nitric oxide synthase (iNOS). Several EDCs like BPA, NP, propanil, and octylphenol were noted to suppress NO production in both in vivo animal models and in vitro human cell cultures (Ustyugova et al. 2007; You et al. 2002; Lee et al. 2017). Inhibition of NO in macrophages occurs via estrogenic nuclear receptors (ER) and is similar to the manner as it happens in the endocrine system (Byun et al. 2005; Yoshitake et al. 2008; Lee et al. 2017). Additionally, macrophages reduce the expression of the nuclear transcription factor NF-κβ, an important component of intracellular signalling involved in the release of nitric oxide as well as other inflammatory mediators (You et al. 2002; Ustyugova et al. 2007; Frost et al. 2011; Lee et al. 2017). Studies also indicated that few EDCs like OP, NP, and DiP inhibit the phosphorylation of ERK1/2 kinase (Yeh et al. 2010; Bennasroune et al. 2012). Thus, there is an intricate relationship between the ER and NF-κβ signalling, and EDCs are shown to act by multiple signalling pathways in a dose-dependent manner (Yang et al. 2015; Rastgar et al. 2018).

To maintain homeostasis within the immune system, macrophages and monocytes are involved in the controlled regulation of cytokine production such as TNF-α. Any disturbances leading to either the overproduction or low production of pro-inflammatory cytokines can lead to the diseased condition, though their production by macrophages is mainly to destroy pathogenic invasions in the body. EDCs are demonstrated to disrupt this delicate balance as some are shown to either overintensify or suppress TNF-α production. Exposure of propanil, NP, DiP, and diethyl phthalate to THP-1 cell lines and macrophages causes high production of TNF-α (Xie et al. 1997; Luebke et al. 2006; Ustyugova et al. 2007; Ochiai et al. 2014), while some EDCs showed a decrease in lipopolysaccharide (LPS)-induced TNF-α production (Byun et al. 2005; Lee et al. 2017). The inhibition of TNF-α by

certain EDCs can be catastrophic to the body with increased chances of cancer due to the inhibition of the tumor-preventing abilities of the immune cells. EDCs can also interrupt cellular communication by modulating cytokines like IL-1β, IL-4, IL-6, and IL-8 (Xie et al. 1997; Nishioka et al. 2012; Ochiai et al. 2014). Exposure to DEHP showed increased production of chemokines (e.g., CXCL1, CXCL2, CXCL3, CXCL6, CCL3), whereas BPA reduced the production of monocyte chemotactic protein (MCP-1) (Nishioka et al. 2012).

It has been observed that EDCs cause oxidative stress or DNA damage leading to cell cytotoxicity in a dose-dependent manner. For instance, BPA causes cell cytotoxicity in the cell lines of RAW 264.7 macrophages and U937 monocytes in a dose- and time-dependent manner (Hwang et al. 2013; Huang et al. 2018). Polybrominated diphenyl ethers (PBDEs) and PBA cause damage to DNA due to apoptosis by the activation of oxidative stress-associated mitochondrial apoptotic pathway (Lv et al. 2015; Huang et al. 2018). The other possible mechanism of action by BPA is by activating the nuclear enzyme poly(ADP-ribose) polymerase-1 (PARP-1) and releasing powerful mitochondrial cytotoxins, thus causing lysis of chromatin and eventual cell death (Mokra et al. 2015; Huang et al. 2018; Chiarugi and Moskowitz 2002). Additionally, another reason for concern is that some other analogues of BPA like BPS, bisphenol F (BPF), and bisphenol AF (BPAF) cause oxidative stress and show genotoxicity even at very low concentrations of 1 ng/mL (Mokra et al. 2015).

16.2.2.2 Neutrophils

Neutrophils are polymorphonuclear cells (PMNs) that remain in good quantity in the circulation, patrol tissues, and initiate aggressive responses upon encountering danger signals. They are the ones to migrate first to the damaged tissue where they undergo phagocytosis along with the release of reactive chemicals and proteases. Thus, neutrophils endow tremendous defensive capacities, but that also renders them as circulating "grenades" which can cause extreme tissue damage if they overreact (Phillipson and Kubes 2011). EDCs are not directly known to affect neutrophils, but they suppress PMN activities like chemotaxis, adhesion, phagocytosis, and killing by oxidative stress in people exposed to DDT (Hermanowicz et al. 1982). Also, exposure to BPA reduced the capability of neutrophils to effectively kill and phagocytose *Staphylococcus aureus* (Balistrieri et al. 2018). Similarly, TBT also induce apoptosis in neutrophils and reduce their life span in circulating blood (Lavastre and Girard 2002).

16.2.2.3 Mast Cells and Eosinophils

Mast cells, basophils, and eosinophils are essential components of allergic inflammation. The initiation and propagation of hypersensitive reactions are due to the binding of IgE to FcεRI receptors on mast cells and basophils/eosinophils (Stone et al. 2010). It has been shown that environmental doses of BPA are capable of activating mast cells without involving IgE, leading to the enhanced release of histamine and leukotrienes (O'Brien et al. 2014a). Similarly, TBT at doses commonly present in the environment can cause inflammation of the respiratory tract (Kato et al. 2006). DEHP treatment caused a surge of eosinophils in the

bronchoalveolar lavage fluid with increased concentrations of IL-4, suggesting that this EDC has the potency to develop asthma-like conditions (You et al. 2014). In certain EDCs like parabens, the impact on mast cells and eosinophils depends on the chemical structure and the dose of its exposure. Short-linear or branched parabens do not cause degranulation of mast cells even at a high concentration of 100 μM, whereas long alkyl chains (e.g., heptyl, octyl, nonyl, and decyl paraben) induce the release of histamine at very low concentrations. Changes in functional groups in parabens also show differential effects, as methylparaben does not elicit any allergic reactions, butylparaben induces a weak skin reaction, but heptyl paraben induces strong histamine release (Uramaru et al. 2014).

16.2.2.4 Natural Killer (NK) or Null Cells

NK cells are large granular lymphocytes (LGL) with an abundance of 10–15% in peripheral blood, and these cells kill the virally infected and malignant cells, without previous sensitization. NK cells destroy transformed cells by necrotic process, and this cytotoxic effect is due to the release of proteolytic enzymes: perforin, phospholipases, chondroitin sulfate, serine esterase (granzymes A and B), and other lytic molecules (Geering and Fussenegger 2015). NK cells express surface antigens like CD3, TCR, surface Ig, CD56, CD16, CD94/NKG2D, CD158a, CD158b, CD161, and FasL for effective killing. Moreover, NKs are a vital source of inflammatory cytokines, such as TNF-α, IL-1β, and interferon-γ (IFN-γ) (Hurt et al. 2013; Brown and Whalen 2015). EDCs like TBT is reported to dampen the NK cells' action against the tumor cells by downregulating CD16, CD18, and CD56 and thus decreasing the expression of perforins and granzyme B (Whalen et al. 2002; Thomas et al. 2003; Dudimah et al. 2007). Others like DDT, triclosan, atrazine, and NP decreased CD-16 and inhibited NK cell cytotoxic activity against tumor cells in a dose-dependent manner (Hurd-Brown et al. 2013). Also most of the EDCs work as immunosuppressors at low doses, for instance, TBT increases TNF-α production at low doses (5–100 nM) and decreases TNF-α production at high dose (200 nM TBT) (Hurt et al. 2013). Similarly, Brown et al. (2018) demonstrated that dibutyltin suppressed IL-6 release at high concentration (2.5–5 μM) and increased IL-6 release at low concentration (0.05 and 0.1 μM). Thus, it is quite evident that EDCs exhibit strong immunosuppressor responses toward NK cell's activity at non-monotonic low doses, but not at high doses (Vandenberg 2013).

16.2.3 Effect of EDCs on Cells of Acquired Immune System

16.2.3.1 Dendritic Cells

Dendritic cells (DCs) are one of the most important components of the cellular immune system and are the prominent antigen-presenting cells, thus forming a bridge between the innate and adaptive immune branch. DCs can acquire a varied functional capability as they are modulated by several cytokines and growth factors like GM-CSF, M-CSF, FMS-like tyrosine kinase 3 (Flt3), and TGF-β. Further different DCs secrete specific subsets of cytokines depending upon the

immunological responses. DCs mainly occur in two forms, immature and mature, wherein immature DCs are involved in immune tolerance and mature DCs are the major antigen-presenting cells, capable of inducing T_H2 or T_H1 immune responses. Misbalance in DCs may lead to many pathophysiological conditions like hypersensitivity, autoimmune diseases, or cancer (Castell-Rodríguez et al. 2017). The phenotypes and functional capabilities of DCs are affected by exposure to EDCs like atrazine and herbicides, as these are found to directly interfere with the expression of important markers (MHC-I, CD86, CD11b, CD14, and CD11) on DCs' cell surface (Pinchuk et al. 2007). It was noted that DCs' phenotypic characters also change considerably under the influence of many EDCs like BPA, BPF, and BPAF in a dose-dependent manner and reduce DCs' capability to endocytosis (Švajger et al. 2016). Very low doses (0.1 μM) of BPA were found to have no effect on cell maturation, but at a dose of 1 nM, BPA decreases the expression of HLA-DR and CD86 on the surface of DCs, while at a high dose (100 nM), BPA significantly increases the expression of CD1a and HLA-DR on monocytic DCs (Guo et al. 2010). It was also observed that BPA at a concentration of 0.001–1 μM modulated the mature DCs to release TNF-α-induced cytokines like IL-5, IL-10, and IL-13 and also increase the expression of mRNA and protein synthesis of the chemokine CCL1 (Guo et al. 2010). Another group of EDCs like DEHP and butyl-benzyl-phthalate decreases the expression of IFN-α/IFN-β in plasmacytoid DCs (pDCs) by suppressing the ERK1/2 and NF-κβ pathways, essential for DCs' maturation (Švajger et al. 2016).

16.2.3.2 Lymphocytes

Adaptive immune responses are elicited due to unique cells called lymphocytes which specifically recognize, respond, and carry out effector functions against foreign antigens. The B lymphocytes and the T lymphocytes express clonally distributed antigen receptors with a single specificity, each specific for a different antigenic determinant. Genes encoding the antigen receptors unique for either B or T lymphocytes are formed by somatic hypermutation and recombination of specific DNA segments during the maturation and differentiation stages of these cells. They are the mediators of humoral and cellular immunity and represent 20–40% of circulating white blood cells and 99% of cells in the lymph.

Literature has ample examples of various EDCs affecting the lymphocytes in both its count and functional activities. Some researchers have established that EDCs enhance apoptosis of T and B cells in vitro (Krug 2012; Gostner et al. 2015; Lee et al. 2017). A high concentration of DDE in the human blood causes an increase in total lymphocyte count (Vine et al. 2001). Atrazine reduces the expression of CD25 and CD69 markers for lymphocyte activation, thus inhibiting the $CD4^+$ T-cell proliferation (Thueson et al. 2015). Parabens, NP, and DES inhibited mitogen-induced lysosomal enzyme secretion from peripheral blood lymphocytes (Bairati et al. 1994). Parabens also showed genotoxic and cytotoxic effects on human lymphocytes in vitro in a dose-dependent manner (Güzel Bayülken and Ayaz Tüylü 2019). Other EDCs like benzophenone, OP, and TBT were shown to stimulate the differentiation of T cells to T_H2 via the increased expression of specific cytokines IL-4, IL-10, and

IL-13 (Tada-Oikawa et al. 2008; Kuo et al. 2013; Feng et al. 2016). The increased IL-4 can be related to increased predisposition to allergic conditions and IgE levels in the blood (You et al. 2014). Phthalates at environmental concentrations, present in dust from the floor of flats or carpets, as well as DEHP concentration at permissible limits did result in developing asthma, allergy, atopic dermatitis, conjunctivitis, and allergic rhinitis in children (Larsson et al. 2010; Ait Bamai et al. 2014).

Most prominent effects were seen with exposure to BPA on lymphocyte functions, though it more effectively modulates B cells rather than the T cells (Sakazaki et al. 2002). BPA inhibited many biochemical pathways of phytohemagglutinin-stimulated peripheral blood mononuclear cells' T_H1 immune responses in a dose-dependent manner (Gostner et al. 2015). BPA disrupts immune stem cells and cytokine levels, thus causing many autoimmune disorders. It enhances IgM production by B1 subpopulations of cells in the autoimmune disorder, systemic lupus erythematous (SLE) (Yurino et al. 2004). Children with type 1 diabetes mellitus show increased BPA in their urine (İnce et al. 2018). BPA induces diabetes by stimulating the spleen and lymph nodes to modulate the release of IL-4, IL-6, IL-10, TNF-α, and IFN-γ cytokines (Bodin et al. 2015). BPA and OP disrupt calcium homeostasis and stimulate insulin resistance. Ca^{2+} imbalance results in oxidative stress that leads to apoptosis of pancreatic cells (Ahn et al. 2018).

16.3 EDCs as Immunomodulators in Allergy

The higher incidence of allergy all around the globe can be ascertained with many epidemiologic studies that link exposure of EDCs to the development of allergic diseases (Lang et al. 2008). The presence of occupational settings with the exposure to polyvinyl chloride (PVC) fumes has increased the onset of respiratory disorders within many populations (Jaakkola and Knight 2008). Exposure to EDCs from gestational age to adult contributes to the development of many chronic inflammatory diseases, particularly allergic diseases. The prevalence of asthma and airway diseases among pediatric population also established the relation between EDCs' exposures during gestational period. Increased consumption of phthalates in the plastic industry has caused asthma and allergic rhinitis among children (Larsson et al. 2010). Recent study has also suggested that increased occurrence of wheezing among preschool kids is due to the presence of diethylhexyl phthalate in indoor dust (Kolarik et al. 2008).

16.3.1 Mechanism of EDCs' Action in Allergic Diseases

Environmental concentrations of EDCs like benzophenone, p-octylphenol, and tributyltin chloride promote T_H2 polarization by suppressing T_H1 immune responses (Lee et al. 2004a). This polarization directs the naive $CD4^+$ T cells primed with anti-CD3 to enhance T_H2 development by inhibiting IL-12 and augmenting IL-10 production by splenic APCs (Kato et al. 2004). Thus, under normal antigenic

challenge, the body elicits T_H1, T_H2, Treg, or T_H17 immune responses (Rescigno et al. 2001), which when exposed to EDCs induce TNF-α production by human peripheral blood myeloid DCs via the epigenetic regulation of the TNFA gene promoter and promote T_H2 polarization of T cells (Hung et al. 2010). A study by Kuo et al. (2012) showed that exposure of butylbenzyl phthalate (BBP), bis (2-ethylhexyl) phthalate (BEHP), dibutyl phthalate (DBP), and diethyl phthalate (DEP) to human bronchial epithelial cells led to IL-8 production and enhanced secretion of chemokines (RANTES; CCL5) from activated T cells. Thus, EDCs are known to negatively affect APCs, mainly the dendritic cells, by depleting the glutathione levels and causing exaggerated inflammation of the airways through increased bronchial smooth muscle cell proliferation and migration (Kato et al. 2006).

It has been noted that EDCs usually have an adjuvant effect on basic mechanisms of allergic reaction. The T_H2 polarization and response cause the release of cytokines and increase the production of IgG1 and IgE immunoglobulins (Bornehag and Nanberg 2010). Treatment with BPA, OP, and NP reported high production of IL-4 (Lee et al. 2003, 2004a), and also diethylhexyl phthalate (DEHP) and diisononyl phthalate (DINP) enhance IL-4 release from the activated $CD4^+$ T cells (Lee et al. 2004b). Taken together, these findings suggest that EDCs have the ability to augment allergic processes by expanding or even shifting the immune processes toward an IgE-related response through enhanced IL-4 production in T cells (Fig. 16.5).

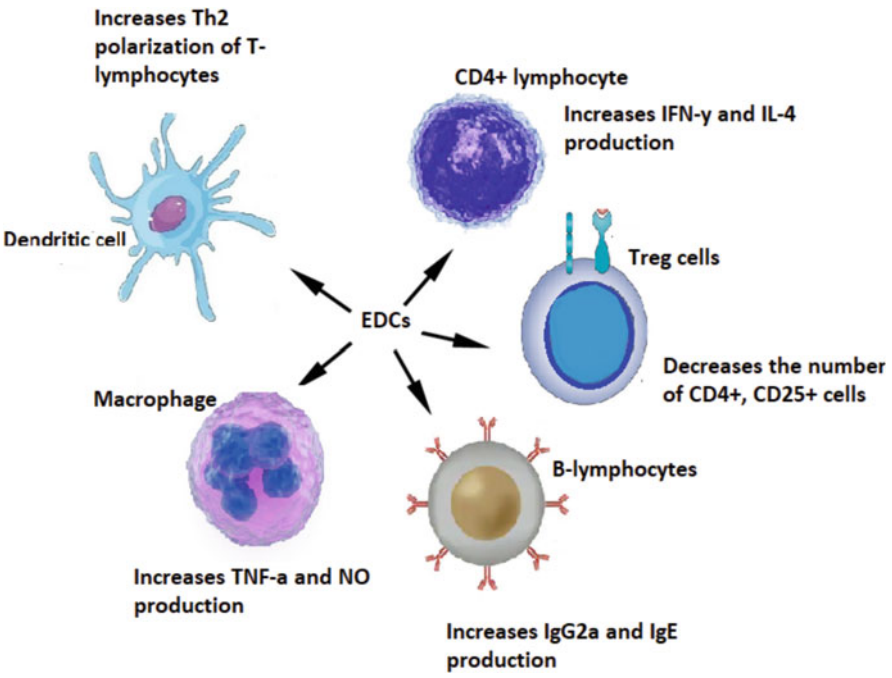


Fig. 16.5 Presumptive action of EDCs on different cell types causing shift in effector functions

16.4 EDCs as Immunomodulators in Inflammation

Approximately 1000 EDCs examined to date produce the suppression of some immune responses or result in inappropriate enhancement of others by disrupting the homeostasis of the immune system. These EDCs cause tissue inflammation by acting on innate immune cells which depends on (1) the specific EDC, (2) the age of exposure, (3) the sex of the exposed, (4) the age at assessment, and (5) the types of inflammatory pathways examined (e.g., neutrophil-, macrophage-, or eosinophil-driven inflammation). The inflammatory responses in the body occur due to mainly three key interconnected, often overlapping, intracellular pro-inflammatory signalling pathways. These are nuclear factor: NF-Kβ-inhibitor of Kβ kinase; c-Jun N-terminal kinase and activator protein-1 (JNK-AP1); and inflammasomes which are activated by various surface receptors like the Toll-like receptors (TLRs), TNF-α receptor, or cytoplasmic NOD-like receptors (NLRs) (Lackey and Olefsky 2016; Osborn and Olefsky 2012). These inflammatory signalling can also be triggered by the release of reactive oxygen species from dysfunctional mitochondria and also by endoplasmic reticulum stress due to misfolded protein. This leads to the increased serine kinase phosphorylation of IRS1 or IRS2 and subsequent transcription of inflammatory genes (i.e., cytokines, chemokines, and components of the inflammasome). Moreover, the inflammasome which is a multiprotein complex activates caspase-1, thereby cleaving IL-1 family of cytokines to form bioactive components like IL-1b and IL-18 leading to inflammatory cell death (Li et al. 2014; Henao-Mejia et al. 2012; Guo et al. 2015). This is followed by the activation of anti-inflammatory signals brought by the G protein-coupled receptor 120 (GPR 120), estrogen receptor a (ERa), and interleukin receptor 10 (Lackey and Olefsky 2016; Osborn and Olefsky 2012; Miller et al. 2012) to maintain the fine balance between pro- and anti-inflammatory cytokine levels and is essential in maintaining metabolic homeostasis.

16.4.1 Major EDCs Associated with Inflammation

Literature provides some minimal immune-related exposure information for a significant number of the EDCs, but extensive immune-related research and evaluation involves a handful of EDCs that are of greatest environmental concerns. Exposure during perinatal stage can have a long-lasting effect on health of both children and adults. These purported drugs are chemicals that harm the endocrine and target the immune system including the cells and non-lymphoid tissues at exposure to very low doses. These exclusive EDCs are atrazine, BPA, dioxin, phthalates, perchlorate, polybrominated diphenyl ethers, lead, arsenic, mercury, perfluorinated chemicals, organophosphate pesticides (OPs), and glycol ethers. Apart from the "dirty dozens," a few additional EDCs like PCBs, heavy metals (e.g., cadmium), antibacterial soap products (triclosan), estrogenic food components (e.g., genistein), and drugs (valproate) also are known potent inflammatory agents.

16.4.1.1 Arsenic

Arsenic is a widespread pollutant present in many chemical compounds like the rodenticides, insecticides, weedicides, paints, wallpaper, ceramics, and certain chemotherapeutic agents. Exposure to arsenic may happen due to accidental ingestion or inhalation of insecticides containing arsenious oxide, copper acetoarsenite, or calcium or lead arsenate. The ubiquitous presence of arsenic in the environment causes subtle and long-term diseases such as cardiovascular disease, diabetes, various cancers, neurotoxicity, and immune alterations. Arsenic has been reported to cause immune diruption mainly in hosts with reduced protection against viral infection and cause exaggerated and inappropriate inflammatory responses (Farzan et al. 2013; Ramsey et al. 2013). Reports have shown that prenatal exposure to arsenic is associated with increased production of pro-inflammatory cytokines such as interleukin-6 (IL-6) (Qi et al. 2014) due to altered pro-inflammatory signalling (Fry et al. 2007; Bailey et al. 2014). The overall disproportionate inflammation facilitates the activation of redox pathways which contributes to tissue pathology and development of cancer in later life (Bourdonnay et al. 2011).

16.4.1.2 Atrazine

Atrazine is a widely used herbicide that kills weeds in crop fields and in warm-season turf lawns. Research on the rampant use of atrazine has revealed many of its harmful effects both on aquatic lives and on humans. Workers at the chemical manufacturing plants exposed to atrazine showed increased incidence of cancer that led to its ban by the US Environmental Protection Agency in 2013. Prenatal and lactational exposure to atrazine leads to heightened immune response causing autoimmune disorders and allergic reactions (Rowe et al. 2006). Exposure to atrazine also alters the regulation of inflammation in tissues, for instance, in specific prostate inflammation in the adult (Filipov et al. 2005; Stanko et al. 2010). However, atrazine is also reported to cause immunosuppression, including reduced NK cell activity (Zhao et al. 2013).

16.4.1.3 Bisphenol A

Bisphenol A (BPA) is a prevalent EDC found in food and beverage cans and plastic containers. The leaching out of BPA has been the cause of major health defects, especially in infants and children where it has been associated with increased airway inflammation (Spanier et al. 2012) and allergic inflammation (Bauer et al. 2012). Studies in rodents have also shown that BPA exposure results in long-term mast cell dysregulation that enhances the production of pro-inflammatory mediators in allergic asthma (O'Brien et al. 2014b).

16.4.1.4 Dioxin

One of the most studied groups of chemicals, dioxins, such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) mediate biological activities via interaction with the aryl hydrocarbon (Ah) receptor (Hogaboam et al. 2008). It has been observed that TCDD exert a profound effect on immune function and regulate inflammation with viral infection (Jin et al. 2010) via the cross-talk between the

pathways for AhR induction (Vogel et al. 2014). There is evidence suggesting that dendritic cells are direct targets of ligands for the AhR and the skewing of dendritic cell function by exposure to dioxin may play a significant role in altered host responses (Jin et al. 2014). TCDD also induce alterations in gene expression in adipose tissue contributing to an elevated risk of inflammation-driven obesity and diabetes (Kim et al. 2012; Warner et al. 2013). Other alterations caused by TCDD are as follows: (1) it negatively affects the hematopoiesis process by interfering with the differentiation process of B and T lymphocytes (Ahrenhoerster et al. 2014); (2) it results in the loss of immune tolerance causing disruption in mucosal immunity and allergy (Ishikawa 2009); and (3) it causes imbalance in cytokine production and increased inflammation leading to high risk of liver fibrosis (Pierre et al. 2014).

16.4.1.5 Glycol Ether

Glycol ethers are alkyl ethers of ethylene glycol or propylene glycol used as popular solvents in paints and cleaners. Though only few studies are done on glycol ethers, they were reported to cause immune dysbiosis, with some parameters elevated and others suppressed. Studies in mice have shown that 2-methoxyethanol (ME) has caused extensive immune alterations than 2-butoxyethanol (2-BE) (Exon et al. 1991). Singh et al. (2001) reported differential immune response to glycol ether exposure in effector cells and in a sex-dependent manner. It was noted that glycol ether suppressed T-cell activity but did not affect the functional capacity of B lymphocytes. Also, exposure of 2-BE increased hemangiosarcomas in male but not in female mice (Klaunig and Kamendulis 2005). Development of hemangiosarcoma by 2-BE exposure happens due to increased macrophage activation and inflammation in the liver involving Kupffer cells (liver macrophages) as well as elevation of tissue hypoxia combined with hematopoietic signalling in the bone marrow (Kamendulis et al. 2010; Laifenfeld et al. 2010).

16.4.1.6 Lead

Lead is a potent neurotoxic agent found in paints and many chemicals that hampers both the neurological system and immune system. Gestational lead exposure causes key immune alterations and inflammation. For example, lead shifts the immune response toward T_H2 type from T_H1-driven responses (Gao et al. 2007), increases immunoglobulin E (IgE) production (Heo et al. 2004), increases risk of allergic reactions such as asthma (Pugh Smith and Nriagu 2011), and enhances autoimmunity in an animal model of lupus (Hudson et al. 2003). In terms of inflammation, the shift from T_H1- to T_H2-type responses causes shunted response to virus infection and cancers and produces acute inflammation. The inflammation due to lead can also be attributed to elevated arachidonate on cell surfaces, contributing to high prostaglandin production (Knowles and Donaldson 1990) and enhanced release of reactive oxygen species by innate immune cells (Pineda-Zavaleta et al. 2004). This exaggerated inflammation finally contributes to increased risk of both hypertension and cardiovascular disease in the exposed person (Vaziri 2008).

16.4.1.7 Mercury

Along with other heavy metals, mercury also shows many health risks like immunotoxicity and autoimmune diseases (Motts et al. 2014). Mercury causes inflammation within body by inducing the expression of both cyclooxygenase-2 and inducible nitric oxide synthase (iNOS) genes (Park and Youn 2013). It affects the innate immune cells and increases pro-inflammatory cytokine production leading to cardiac inflammation and enhanced autoimmunity (Nyland et al. 2012; Fujimura et al. 2012). Organs such as the brain and neurological system which are vulnerable to oxidative-induced damage are most affected by mercury exposure (Fujimura et al. 2012). Methylmercury also disrupts mitochondrial function, thus leading to a cascade that reduces adenosine triphosphate (ATP) synthesis and increases lipid, protein, and DNA peroxidation. It concurrently reduces glutathione levels and increases the risk for inflammatory-driven oxidative damage in tissues (Carocci et al. 2014).

16.4.1.8 Organophosphate Pesticides

Most of the research on harmful effects of the organophosphate pesticides (OPs) revolved around its extreme sensitivity of the neurological system. So, less priority was given to OPs as immunomodulators, and more focus was conferred on their neurotoxicological evaluation. Recently, studies have linked altered immune responses to neurotoxicity as an endpoint of OP exposure. Sunkaria et al. (2012) have suggested that OP activation of resident microglia (brain macrophages) and their successive apoptosis cause the release of pro-inflammatory cytokines that contribute significantly to OP-induced neurotoxicity. Early exposure to OPs also disrupts the immune function of innate immune cells, particularly of dendritic cells (Schäfer et al. 2013) and macrophages (Proskocil et al. 2013).

16.4.1.9 Perchlorate

Mostly found in drinking water, perchlorates are used as oxidants in rocket fuels and in some fertilizers. Perchlorates have been prominent in targeting thyroid causing hypothyroidism (Yu et al. 2002; United States Environmental Protection Agency, DRAFT 2012). Though very limited research is reported in terms of immunotoxicity, perchlorates show reduced in vitro phagocytic activity of macrophages, enhanced plaque-forming cell (PFC) assay response to sheep erythrocytes as an antigen, and enhanced local lymph node assay response to 2,4-dinitrochlorobenzene (United States Environmental Protection Agency, DRAFT 2012).

16.4.1.10 Perfluorinated Chemicals

Perfluorinated compounds (PFCs) are widely used in a number of industrial applications like stain repellents for textiles, additive to paper products, and aqueous film-forming foams used to fight electrical fires. Recently, they have been declared as persistent, bioaccumulative, and toxic compounds by the Stockholm list of hazardous chemicals. Immunotoxicity by perfluorooctanoic acid (PFOA) is quite prominent, and it disrupts not only the endocrine system but also the immune

homeostasis (Vested et al. 2013; DeWitt et al. 2012). Immunotoxicity of PFOA is mediated by alterations in inflammatory gene transcription and activates alpha isotype of peroxisome proliferator-activated receptors (PPARs) (DeWitt et al. 2009). This leads to differential lymphoid population and cytokine levels and reduced adaptive immune responses (DeWitt et al. 2009; Rosen et al. 2008; Yang et al. 2002). In utero exposure to PFOA causes reduced antibody responses to routine childhood vaccines contributing to childhood immunosuppression and elevated risk of infections (Grandjean et al. 2012; Granum et al. 2013). PFOA is also seen as a potent agent to cause inflammation, pathologies, and disease in neurological and other systems (Hu et al. 2012). PFOA epigenetically alters glutathione transferase expression and depletes glutathione reserves (Tian et al. 2012) increasing the risk of hepatic inflammatory damage (Tan et al. 2013). Liver damage is exacerbated by pro-inflammatory cytokines like IL-6, cyclooxygenase-2, and C-reactive protein (Yang et al. 2014). PFOA also affects mast cells and increases the release of TNF-α, IL-1β, IL-6, IL-8, and histamine, thereby increasing the inflammatory components of allergic responses (Singh et al. 2012).

16.4.1.11 Phthalates

Polyvinyl chloride (PVC) is a solid plastic made to soften by the addition of phthalates and BPA. Both phthalates and BPA are potent endocrine disruptors and are excessively used in the manufacturing of pipes, polyvinyl flooring and siding, hoses, cable coatings, medical devices, and plumbing and automotive parts. These are also used in upholstery, housewares, shower curtains, raincoats, toys, school supplies, food packaging, and shoes. Among numerous studies related to phthalates, di-(2-ethylhexyl) phthalate (DEHP) was found to be the most potent immunomodulator and causes tissue inflammation and allergy (Hoppin et al. 2013). Increased IgE production, altered activity of macrophages, and their altered antigen presentation capacity were reported due to phthalate exposure (Li et al. 2013). Phthalates alter gene expression in both immune response and inflammatory-associated genes, thus leading to increased inflammatory oxidation, suppressed T-dependent antibody response and NK cell activity, and increased macrophage production of TNF-α (Campioli et al. 2014).

16.4.1.12 Polybrominated Compounds

Initially thought to be less toxic than polychlorinated compounds, polybrominated diphenyl ethers (PBDEs) were replaced in a variety of industrial and home-use products as burn inhibiting flame retardants. But later, PBDEs were also reported to be toxic to both endocrine and immune system and became a significant concern to human health (Hood 2006). PBDEs suppressed T_H1-driven acquired immune function and reduced IFN-γ production, as well as antigen-specific $CD8^+$ T-cell numbers, proliferation, and function (Lundgren et al. 2013). Exposure to PBDEs supremely compromises against viral infection and reduces pro-inflammatory cytokine release (e.g., TNF-α and IL-6) (Watanabe et al. 2010). PBDEs also reduce the number of peripheral blood monocytes and certain T-cell subpopulations as well as diminish NK cell activity (Fair et al. 2012). Exposure to PBDE in early life adversely

affects the structure and function of both primary and secondary lymphoid organs in the children (Hong et al. 2010).

16.5 Endocrine Disruptors and Autoimmune Diseases

After World War II, the Industrial Revolution generated lots of plasticizers, agricultural pesticides, and herbicides in the environment. The alarming increase in the level of these EDCs in water, food, and personal care products led to high incidences of autoimmune disorders like type 2 diabetes, thyroiditis, SLE, rheumatoid arthritis (RA), multiple sclerosis (MS), etc. (Asher et al. 2006). Extensive work by Harpsoe et al. (2014) in Denmark has shown interesting observation, where adiposity was noted to precede the diagnosis of a range of autoimmune diseases among women. It was also outlined the common etiology linking adiposity with a more generic underlying explanation to the mechanism operating via estrogen receptors and autoimmune disease, providing a guide to potential intervention targets like immune subsets, leptin, or perhaps other mechanisms. Thus, a focus to maintain healthy body weight is substantiated by the removal of estrogenic endocrine disruptors, such as dioxins, phthalates, and polychlorinated biphenyls, from the environment to prevent autoimmune diseases in women (Schooling and Zhao 2015). During pregnancy, women, if exposed to EDCs through diet, experience weight gain accompanied with insulin resistance and immune cell dysfunction, thus leading to autoimmune disorders like type 2 diabetes (Filardi et al. 2020) (Fig. 16.6). Role of sex hormones (especially estrogen, prolactin, progesterone, and testosterone), total microbiome, susceptible genes, and epigenetic modifications are the main cause for gender disparity in autoimmune diseases.

The higher incidence of autoimmune disorders can be attributed to three major endocrinological transitions that women undergo during puberty, pregnancy, and menopause. At all these transitions, changes in hormone milieu influence both innate and adaptive immunity and also release of pro- and anti-inflammatory cytokines. A delicate balance between defense against pathogens, immunological tolerance, and autoimmunity is maintained by a balanced interplay between various hormones and T_H1 and T_H2 immune responses. Mechanistic and animal studies as well as human epidemiological data have established a strong connection between endocrine transition states in women and progress of certain autoimmune diseases such as multiple sclerosis, SLE, type 1 diabetes mellitus, RA, and psoriasis. Greater understanding of endocrine transitions and their role in autoimmune diseases could aid in the prediction, prevention, and cures of these debilitating diseases in women (Desai and Brinton 2019). Moreover, people with precedence for autoimmune disorders are more prone to infectious diseases (Abedi-Valugerdi et al. 2005). For instance, people with type 2 diabetes get infected by coxsackievirus disease and also succumb to autoimmune thyroiditis as a most common comorbid disease (Jugan et al. 2010).

Females have greater incidence of SLE, MS, and RA, probably due to the predominance of estrogen in their system. It can be clearly noted that EDCs are sex hormone linked and are influenced by endogenous pituitary-gonadal axis

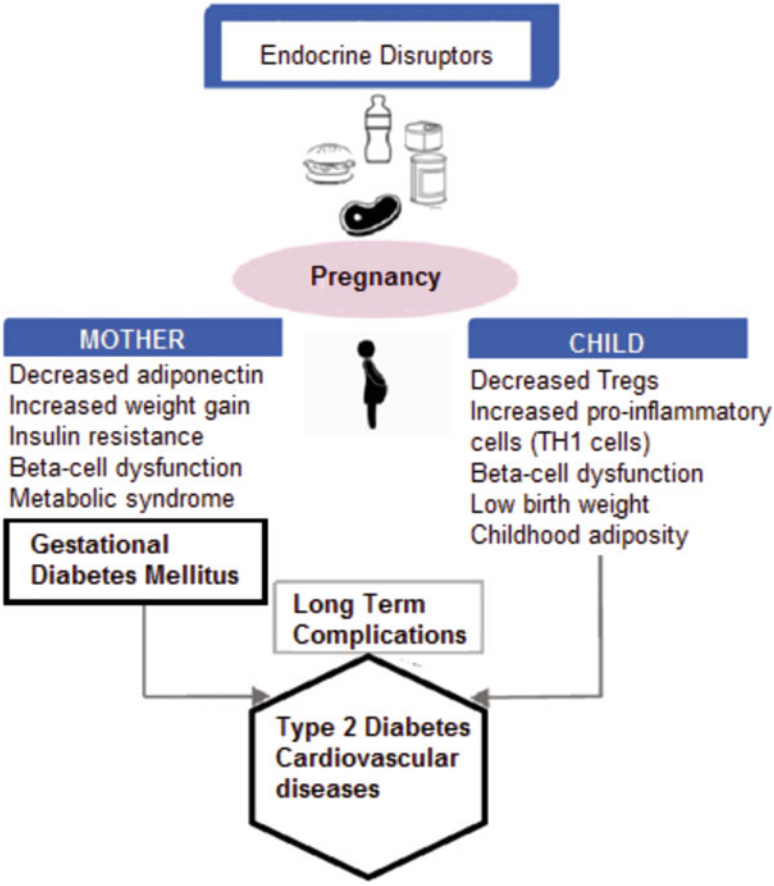


Fig. 16.6 Effect of EDCs present in diet during pregnancy

suggesting their intervention in the incidence of autoimmune diseases (McMurray 2001). Experimental evidences showed that exposure to DDT caused low uterine weight and albuminuria in SLE females and organochlorine accentuates most of the autoimmune disorders (Li and McMurray 2009). Notably, all EDCs do not affect in a dose-response manner but show differential activity. BPA acts as an immunosuppressor by downregulating IFN-γ products, thereby inhibiting albuminuria. Similarly, TCDD is seen to reduce mortality in SLE mice by decreasing anti-DNA antibodies and serum IgG levels. Low numbers of $CD4^+/CD8^+$ T cells are seen in the thymus and $CD4^+$ T cells in the spleen but higher percentage of $B220^+/sIgM^+$ B cells along with high concentration of IFN-γ in the serum, suggesting the immunosuppressor role of TCDD (Li and McMurray 2009). This dual nature of EDCs regarding autoimmune disorders is due to their affinity for various receptors (E2 or aryl hydrocarbon receptors) and subsequent signalling after activation, to these receptors (Nilsson et al. 2001).

16.6 Endocrine Disruptors and Epigenetic Regulation

The term "epigenesis" has a long history of usage and was first coined by German physician and naturalist C. F. Wolff (1734–1794) and defines the way a gene changes in the face of environmental influences (Wessel 2009). Environmental components thus can impact the way genetic material expresses itself either positively or negatively, without changing the DNA sequence. The major mechanisms of the epigenome are DNA methylation, post-translational modifications of histone proteins (histone modifications), and non-coding RNA (ncRNA) (Jirtle 2013). All these mechanisms are known to interact with each other and influence development, differentiation, and function at the cellular level (Li 2002; Costa 2008; Namihira et al. 2008). Additionally, DNA methylation, histone modifications, and ncRNAs complement together and lead to X-chromosome inactivation (Avner and Heard 2001) and genomic imprinting (Bartolomei 2009; Brosnan and Voinnet 2009).

DNA methylation: Cytosine residues in CpG dinucleotides usually undergo methylation of DNA and assist in genomic imprinting and also reduction in gene expression by interfering with binding of transcription factors. Also, it leads to X-chromosome inactivation as well as suppression of retro transposons (Ehrlich 2003). Segments of DNA which are rich in CG content usually possess CpG islands (CGIs) that are mainly associated with the regulatory sequences of the genes but are quite understated in the genome (Turner 2009). In the absence of DNA methylation, transcription factors can bind to the regulatory sequences leading to upregulation of gene expression (Yagi and Koshland 1981; Ehrlich 2003). Otherwise, it causes alteration within the genome and subsequent gene regulation (Suzuki and Bird 2008).

Histone modifications: The most important phenomenon in genomic DNA packaging is the modification of histone proteins. The structural and functional fate of the chromatin is based on the post-translational modifications of histones at lysine, arginine, serine, and threonine amino acid residues (Hirose et al. 1985; Turner 2009). Acetylation of histone at the lysine residue is associated with relaxation of chromatin, allowing access to transcription factors and active transcription, whereas deacetylation leads to gene silencing. Methylation of lysine and arginine or phosphorylation of serine and threonine is associated with either gene activation or silencing, depending on the position of the amino acid altered (Sharma et al. 2005; Yoo and Jones 2006; Turner 2009).

Non-coding RNA: Non-coding RNAs or ncRNAs do not code for any proteins as they lack clear open reading frame but are relatively being hailed to possess important biological processes (Chang et al. 2006). The ncRNAs differ in size from microRNAs, or miRNAs (15–21 nucleotides, nt), and small RNAs (100–200 nt) to large RNAs (>200 nt). Recently, they have been shown to regulate gene expression in "cis" and "trans" manner; thus, they are involved in genomic imprinting, developmental patterning and differentiation, X-chromosome inactivation, and transposon virus silencing (Costa 2008; Brosnan and Voinnet 2009).

16.6.1 Role of EDCs in Epigenesis

Genetics alone could not explain the reckless upsurge in chronic diseases over the past several decades wherein some epidemiologic research clearly suggests significant role of environmental effects on the onset of diseases. Environmental EDCs are known to epigenetically regulate the expression of certain diseases by modifying histone proteins or DNA (Kuo et al. 2011), thereby setting in a disease without actually altering the DNA sequence (Edwards and Myers 2007). Autoimmune diseases or chronic inflammation are usually attributed with the overexpression of multiple inflammatory genes, which are controlled many times through epigenesis. Some early studies showed that exposure of neonatal mice to EDCs caused demethylation in the lactoferrin promoter area, indicating epigenetic changes (Li et al. 1997). EDCs like BPA and vinclozolin also alter DNA methylation patterns and interfere with related gene transcription (Edwards and Myers 2007; Jirtle and Skinner 2007). Neonatal mice exposed to environmental doses of BPA become susceptible to prostate carcinogenesis due to modulation in DNA methylation (Ho et al. 2006), whereas pregnant mice exposed to BPA cause alteration of methylation in the forebrain of fetal mice and changes the behavior of the offspring (Yaoi et al. 2008; Palanza et al. 2008). Exposure to EDCs affects the reproduction via DNA hypomethylation, histone modification, and ncRNAs in the ovary and various female reproductive organs, leading to disruption/altered gene expression (Zama and Uzumcu 2009; Bredfeldt et al. 2010; Luense et al. 2011; Bromer et al. 2010).

EDCs not only impact the endocrine, neural, and reproductive systems via epigenesis but also affect the immune system (Yeh et al. 2010; Hung et al. 2010). NP and 4-OP (4-octylphenol) act on human monocytes and alter histone H4 acetylation, modulating the expression of chemokines (Yeh et al. 2010), and render histone trimethylation and acetylation, leading to differential TNF-α expression (Hung et al. 2010). Surprisingly, it is now documented that EDCs cause changes in the epigenome and cause epimutations in the germline stem cells, thus leading to trans-generational inheritance of epigenetic markers, stimulating the onset of autoimmune diseases in offspring (Skinner et al. 2010).

16.7 Signalling Pathways as Targets of Immunomodulation by EDCs

The potential of EDCs to interfere with hormonal systems has raised considerable concern in recent years. These chemicals have global presence and can expose children and adults including pregnant women. EDCs contribute to the dysfunction of the immune system and also affect the functioning of other metabolic pathways. Most of the endocrine system pathways function due to the binding of specific hormones to their receptors (nuclear or non-nuclear). EDCs interfere, temporarily or permanently, in the normal functioning of such pathways, by binding to hormone receptors and modifying gene expression (Ghassabian and Trasande 2018), thus

leading to several endocrine and reproductive disorders in both animal and human studies. The hormone receptors can be broadly categorize into steroid receptor like estrogen receptor (ER), or thyroid receptor, or aryl hydrocarbon receptor (AhR). Some of the important signalling pathways that are prone to action by EDCs are given below.

16.7.1 Estrogenic Action of EDCs

Many environmental contaminants exert estrogenic actions in wildlife as well as laboratory animals by binding to nuclear estrogen receptors (ERs). This further affects the transcription of estrogen-responsive target genes in the nucleus by binding to the estrogen response element (ERE) of target genes. This comprises the genomic pathway of EDC action. The "non-genomic pathway" of action of EDC may take place through a novel seven-transmembrane estrogen receptor called G protein-coupled receptor (GPR30) that acts as ER and is located in the cytoplasmic membrane. Binding of EDCs to GPR30 leads to its activation that initiates rapid downstream cellular signalling. This further leads to the subsequent stimulation of protein kinase activation and phosphorylation that has an effect on the transcription of target genes. Therefore, interaction of EDCs through either ERs or GPR30 along with their mutual interaction can adversely affect gene expression and intracellular signalling leading to severe adverse effects on organs eventually affecting human and animal health (Lee et al. 2013) (Fig. 16.7).

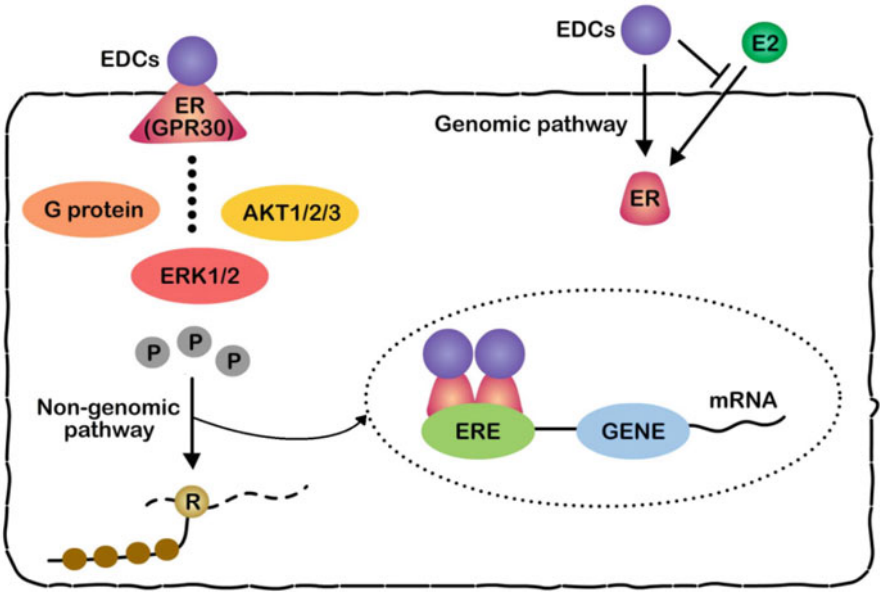


Fig. 16.7 Action of EDCs on estrogen pathways

In an ER-negative cell line (HEK293) stably transfected with the receptor GPR30, it was found that several environmental estrogens bind effectively with this receptor protein. As compared to estradiol-17beta (E2), Genistein was found to bind very effectively at 13% relative binding affinity (RBA). BPA, zearalonone, and nonylphenol also had high binding affinity to the receptor protein. Kepone, p,p′-DDT, 2,2′,5′,-PCB-4-OH, and o,p′-DDE had lower binding affinity to GPR30, while o,p′-DDT, p,p′-DDE, methoxychlor, and atrazine were able to displace [(3)H]-E2. It was also seen that the binding affinity of these compounds to GPR30 was almost comparable to their binding affinity to ERs. Genistein, bisphenol A, nonylphenol, and kepone were also able to act as estrogen agonist in in vitro assay of membrane-bound adenylyl cyclase activity, a GPR30-dependent signalling pathway that is activated by estrogens. These experiments therefore confirmed that nontraditional estrogen actions mediated through GPR30 can also be effectively disrupted by the addition of a variety of environmental estrogens (Thomas and Dong 2006).

16.7.2 Action of EDCs on Thyroid Signalling Pathway

Thyroid hormones, triiodothyronine (T3) and thyroxine (T4), are very important for normal human behavior and intellectual and neurological development. Hypothyroidism can have extreme health-related problems. Congenital hypothyroidism however can cause irreversible brain damage if left untreated. Deficiency of thyroid hormones during pregnancy could also be responsible for retarded neurological development of the child (Haddow et al. 1999). It has been seen that exposure to certain EDCs such as dioxins and PCBs, especially during the perinatal period, can severely affect the normal thyroid functioning. By using animal model studies, it was found that PCBs tend to reduce circulating and tissue concentration of thyroid hormone (Goldey et al. 1995; Morse et al. 1993). A given EDC therefore can interfere with thyroid hormone functions and homeostasis by any of the following mechanisms: inhibiting hormone synthesis, production of thyroid hormone by the thyroid gland, altering serum transport proteins, or increasing breakdown of thyroid hormones (Fig. 16.8) (Table 16.3).

16.7.3 Effect of EDCs on AhR/HDAC6/c-Myc Signalling Pathway

Aryl hydrocarbon receptor (AhR) is a transcription factor that belongs to the basic helix-loop-helix family and gets activated by binding of ligands (Matsumura 2009). In the cytoplasm of resting or non-activated cells, AhR is bound by chaperone proteins. However, on binding with its stimulating ligands (e.g., phthalates and dioxins), AhR undergoes conformational change that leads to the release of AhR from its cytoplasmic chaperones. The activated AhR can now regulate gene expression through both non-genomic and genomic mechanisms (Puga et al. 2005). The non-genomic mechanism leads to the translocation of AhR to the nucleus. In the nucleus, AhR gets associated with aryl hydrocarbon receptor nuclear translocator to

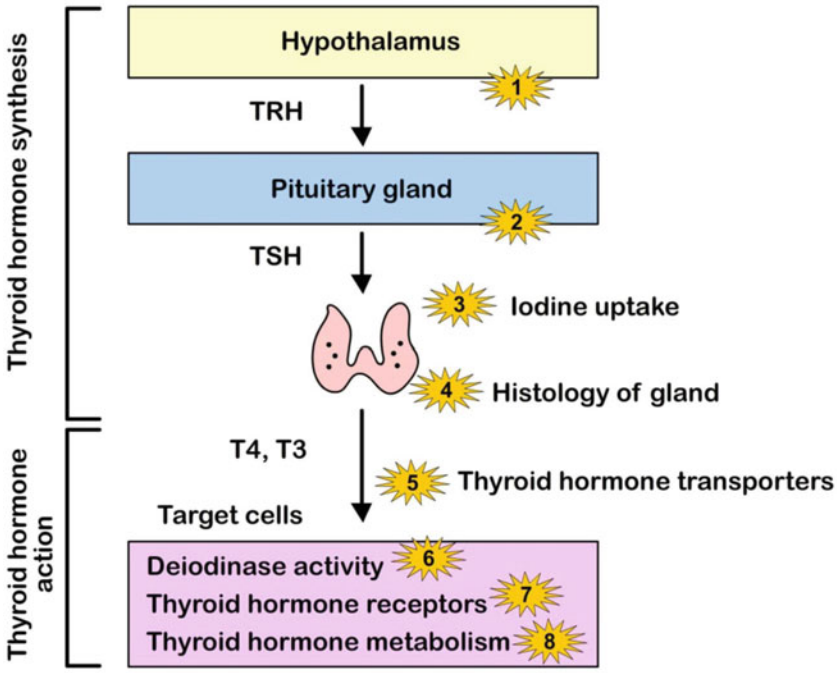


Fig. 16.8 Effect of EDCs on thyroid signalling pathway. Groups of chemicals act at PCB and PCDD, 5 and 7; PBDEs, 5, 6, 7, and 8; pesticides, 4, 5, and 7; PFASs, 5 and 6; NIS, 3; BPA, 2 and 7; and phthalates, 1, 2, 5, and 8. *BPA* bisphenol A, *NIS* sodium iodide symporters, *PBDE* polybrominated diphenyl ethers, *PCB* polychlorinated biphenyl, *PCDD* polychlorinated dibenzodioxins, *PFAS* perfluoroalkyl substances, *TRH* thyroid-releasing hormone, *TSH* thyroid-stimulating hormone, *T4* thyroxine, *T3* triiodothyronine. (Source: Adapted from Ghassabian and Trasande 2018))

form a heterodimer that binds to AhR response elements present on the promoter region of target genes (Reyes et al. 1992). In the non-genomic mechanism, AhR gets translocated to the cytoplasmic membrane. Here, it induces intracellular calcium and cyclic AMP and triggers a rapid signalling response that ultimately leads to transcriptional activation in the nucleus (Monteiro et al. 2008). It has been found that in either mechanism, the environmental ligands are able to cause AhR activation, which regulates gene expression in the nucleus. This eventually can confer a significant effect on the pathophysiology of human cancer (Ishida et al. 2010). In one such study, it was seen that phthalates that are an important class of EDCs stimulate the cell surface AhR which further trigger the downstream cAMP-PKA-CREB1 (CREB-cAMP-response element binding protein-transcription factor) signalling cascade. The pathway will lead to the enhanced expression of HDAC6 (histone deacetylase 6) that further facilitate the assembly of the β-catenin-LEF1/TCF4 transcriptional complex and transactivation of the *c-Myc* oncogene in the nucleus. This non-genomic pathway emanated from the phthalate-induced AhR

Table 16.3 Target of action of some important EDCs in thyroid signalling pathway

Endocrine disruptors	Target of action
PCDD—polychlorinated biphenyls and polychlorinated dibenzodioxins	Thyroid hormone transportationThyroid hormone receptors
Polybrominated diphenyl ethers	Thyroid hormone transportersDeiodinase activity in the thyroid glandThyroid hormone receptorsThyroid hormone metabolism
Pesticides	Histology of the thyroid glandThyroid hormone transportationThyroid hormone receptors
PFASs—perfluoroalkyl substances	Thyroid hormone transportationDeiodinase activity in the thyroid gland
NIS—sodium iodide symporters	Iodine uptake into the thyroid gland
Bisphenol A and other phenols	Expression of thyroid receptor genes in the pituitaryThyroid hormone receptors
Phthalates	Thyroid-releasing hormone receptor in the hypothalamus and pituitaryThyroid-stimulating hormone receptor in the thyroid glandExpression of genes related to thyroid hormone metabolism, synthesis, and transportation

Source: Ghassabian and Trasande (2018)

promoted tumorigenesis of ER-negative breast cancer cells (Hsieh et al. 2012b) (Fig. 16.9).

16.8 Future Need for Clinical Research in Indian Scenario

Exposure to EDCs has a detrimental effect on the metabolism of an organism. It can also adversely affect the endocrine and reproductive systems that can persist for generations to come. EDCs have also been found to promote carcinogenesis and potentially affect neuronal and immune system functioning. Therefore, more sensitive and accurate in vitro and in vivo strategies and techniques are essential to detect the adverse actions and effects of EDCs. This knowledge would ensure better human health. Further, the combined impact of multiple EDCs must also be unraveled as these EDCS are generally released into the environment as mixtures rather than individual reagents.

In the USA, the large numbers of possible EDCs are delimited by certain laws like "the Toxic Substances Control Act"; "the Food Quality Protection Act"; "the Food, Drug and Cosmetic Act"; "the Clean Water Act"; "the Safe Drinking Water Act"; and "the Clean Air Act." The US Congress has ensured improved practices of the evaluation and regulation process of EDCs and other drugs and chemicals. By the year 2016, the EPA (Environmental Protection Agency, USA) had accomplished estrogen screening results for nearly 1800 chemicals.

In Europe, the European Commission has been actively involved in setting the criteria for identifying endocrine-disrupting chemicals (EDCs) in thousands of products including disinfectants, pesticides, and toiletries that are thought to act as

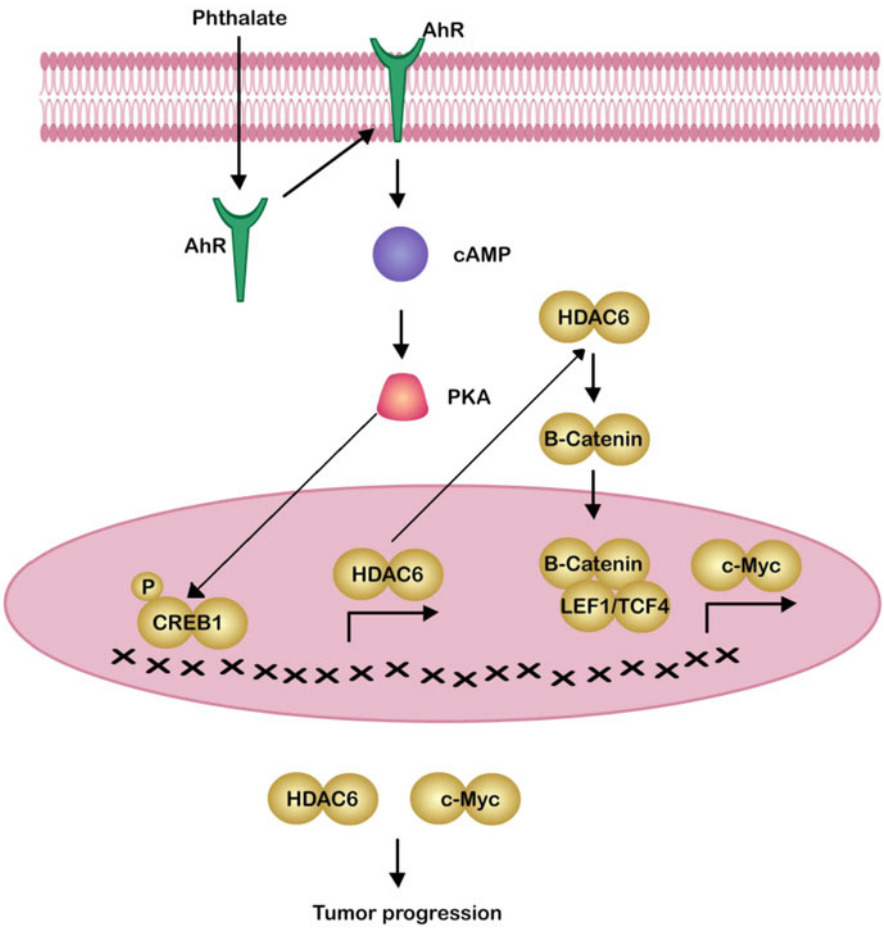


Fig. 16.9 Effect of EDC (phthalates) in the progression of tumors (Source: Hsieh et al. 2012b)

carcinogens or are linked to birth defects and development disorders in children. The subject of EDCs is a global issue, and the countries across the globe are trying to phase out these chemicals from consumption as far as possible. However in India, the public information on EDC is very limited, and people are ignorant about their adverse effects. This is despite the fact that India is one of the biggest markets for the use of these chemicals and EDCs. For example, the issue of BPA in baby feeding bottles has been raised and discussed in the Parliament of India many times. The Bureau of Indian Standards (BIS) has also reviewed and revised the standards for baby feeding bottles in 2015 as per IS 14625:2015 and banned the use of BPA in baby feeding bottles. The Ministry of Child Welfare also proposed phasing out BPA from cups, spouts, and straws with the possible amendment into the definition of feeding bottles in the infant milk substitutes, feeding bottles, and infant foods (Regulation of Production, Supply and Distribution Act, 1992). Researchers in

India have also identified 686 potential EDCs by searching and analyzing the available literature. Most of the potential EDCs are used in consumer products, with evidence for adverse reproductive or metabolic effects. To create the Database of Endocrine Disrupting Chemicals and their Toxicity Profiles (DEDuCT), a team from India's Institute of Mathematical Sciences (IMSc) in Chennai computationally mined and evaluated more than 16,000 published research articles to identify those with information on EDCs. The compounds included in DEDuCT are those that had in vivo rodent, in vitro human, or in vivo human experimental evidence for endocrine disruption (Karthikeyan et al. 2019). They also created a public online tool to aid further EDC research. DEDuCT classifies the chemicals based on the type of experimental evidence, exposure sources, chemical structures, and physiological systems they affect. The database also includes the compounds' known and predicted pharmacokinetic properties, including absorption, distribution, metabolism, excretion, and toxicity. In addition, it lists observed adverse effects with corresponding dosage information.

The evaluation of EDCs has led to a deduction that the vast diversity of EDCs and their association or link with numerous disorders calls for more scientific information and extensive research in the days to come. Besides policies at local, state, national, and international level should be formulated to ensure best public health. Early identification and timely intervention is the key to protect the exposure of mankind especially the at-risk groups.

16.9 Conclusion

With the advent of industrialization and modernization, more than 100,000 new chemicals as consumer products are pumped into our environment. Many of these chemicals are now known as endocrine-disrupting chemicals (EDCs) and have become a cause of anxiety owing to their toxicity in animal studies and their impacts on human health. EDCs are omnipresent in the environment, including the hydrosphere, lithosphere, and biosphere. These EDCs mimic many molecular receptors (particularly of steroid hormones) and not only disrupt several endocrine and reproductive systems but also adversely affect the immune system. This chapter unravels the immunomodulatory effects of EDCs and summarizes EDC's interference with the synthesis of cytokines, immunoglobulins, and inflammatory mediators and also the activation and survival of immune cells. The alteration within the immune system caused by EDCs may lead to the immunodeficiency against infection or immune-enhanced responses like allergy and autoimmune diseases.

Although research to date has focused on the in vitro and animal studies suggesting potential mechanisms by which EDCs overwhelm immunity, augment allergic reaction, and modulate autoimmune response, these results cannot be directly applied to humans. In the case of humans, the frequency and duration of exposure to chemicals, dose and chemical properties, routine exposure, and interactions with other chemicals as well as lifestyle factors may also play a role in their biological effects. There are critical windows during early life in which the

immune system is vulnerable to disruption, and exposure during these periods may increase the risk of immune system dysfunction later in life. Environmental EDCs can alter the immune system by affecting the ability of individuals to mount well-regulated immune responses to microbial and vaccine antigens, allergens, self-antigens, and tumor antigens. The challenges that still persist need an explanation to understand the environment and human interaction. Firstly, the molecular targets should be identified which have clinical relevance of real-life exposure to EDCs; secondly, the exposure period and time of the EDCs that is required to induce immunodeficiency, allergy, and autoimmune diseases need to be established; and lastly, the in vivo studies in human clinical trials are of paramount importance to establish the clear picture of immunomodulatory effects of environmental endocrine-disrupting chemicals.

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