Preetham Elumalai
Sreeja Lakshmi *Editors*

Lectins

Innate Immune Defense and Therapeutics

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Editors

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This book is lovingly dedicated to my late parents whose sacrifice, care and unconditional love brought me to this stage and to all my diligent students for being a huge factor in helping me to be a better teacher.

—Preetham Elumalai

Foreword by Dr. Devaraj & Dr. Niranjali Devaraj

It is our pleasure to write the foreword for the book titled *Lectins: Innate Immune Defense and Therapeutics*, which contains quality and informative chapters that enable the reader to understand the role of lectins in current science. In recent years, fruitful investigations have led to new insights into lectins, making them exciting topics of debate. The book provides a comprehensive overview of lectins, with special reference to their therapeutic applications and their role in immune defense. Each chapter is intended to provide specific aspects of lectins.

The immune system of vertebrates involves both innate and acquired immune responses. Innate immunity is more generalized with a robust response whereas the latter has a highly specific response to pathogens. The innate immunity components which identify sugars are called as lectins. The innate immune recognition process depends largely on the pattern-based recognition of microbial targets as “non-self” by host lectins and related proteins and their subsequent destruction by complement and phagocytic cells.

All lectins possess one carbohydrate recognition domain (CRD) which specifically and reversibly binds to a specific carbohydrate. Lectins are widely distributed in bacteria, fungi, viruses, plants and animals. Lectins are found in serum, plasma, mucosal surfaces and egg surface. The skin of several animal species, including fish, is assumed to be a rich source of novel and new unreported lectins. Different types of lectins such as ficolins, galectins, calnexin, pentraxin, F-type lectins, intelectins, and

mannose-binding protein (MBP) are known to play important roles in innate immunity and disease resistance.

The complement system plays an essential role in protecting against invading microorganisms and acts through three activation pathways, namely, the classic, alternative and lectin pathways. In the lectin pathway, binding of the lectin-Mannose Binding Serine Proteases (MASPs) complexes to carbohydrates on the surface of pathogens activates MASPs to acquire proteolytic activity against complement components C4 and C2, resulting in the elimination of pathogens after a chain reaction of proteolysis of complement components and protein assembly. However, this defense mechanism is poorly understood. Therefore, identification of immune-related genes and their expression studies are imperative.

The collective efforts of the authors and editors of this book are greatly appreciated. It is our hope that the book will be both engrossing and inspiring to the scientists so that they can gain more knowledge in this specific field.

Best wishes

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Foreword by Prof. Sadasivam J. Kaushik

It is my pleasure to write a foreword for this book entitled *Lectins: Innate Immune Defense and Therapeutics*, edited by Dr. Preetham Elumalai and Dr. Sreeja Lakshmi. The book covers a complete range of subjects dealing with lectins of diverse origin and on their potential roles in health and immune defense. I am convinced that this book will provide updated information on lectins of diverse origin to a wide audience of readers from academia, research and education.

As proteins which bind to specific carbohydrate structures, mono- or oligosaccharides, hundreds of "lectins" have been identified in almost all phyla, plants, animals or microbials. Knowledge on the identification, classification and their role as hemo-agglutinating agents has been increasing ever since the first discovery in the late nineteenth century.

The book covers indeed a full range of subjects dealing with these more or less ubiquitous proteins and their involvement in the immune system of higher animals and humans. In animal or human nutrition, lectins of plant origin are often considered as anti-nutritional, pathogenic factors. The book also covers the mechanisms involved in the actions of lectins within the target organisms. The implication of some lectins in eliciting innate-immune response is an aspect which is duly covered, adding new dimensions to the putative beneficial roles of specific lectins. The book also provides information on the possibility of producing lectins of interest using biotechnological tools. Given the phenomenal growth in knowledge on and

application of lectins, it is but timely to welcome such a complete book covering different aspects of lectins in an integrated and systematic manner.

I am sure this book will be well received with a wide readership, and I commend the sincere efforts by the authors and editors for bringing this compilation.

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Preface

Lectins: Innate Immune Defense and Therapeutics focuses on exploring the importance of lectins in immune defense and modern therapeutic approaches. Lectins are protein molecules, widely distributed in different species in almost all organisms including plants, animals, viruses, bacteria, cyanobacteria and yeasts. They serve as receptors for recognizing carbohydrate moieties present on the pathogenic surfaces and exert a specific interaction with them. Following the discovery of animal lectins, research in basic and applied biosciences experienced an accelerated thrust because of its role in mediating cell-cell interactions, host cell-pathogen interactions, antimicrobial activity, drug discovery, etc. Our book is anticipated to provide an overview on different types of lectins and future perspectives in scientific research as recognition and effector molecules in innate immunity or regulators of adaptive immune responses. We really appreciate the contributions of the authors for their expertise and skilful renditions. The facts about Lectins presented in this book will provide an overview to newcomers and a meticulous illustration to the specialists in the field.

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Preetham Elumalai
Sreeja Lakshmi

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About the Editors

Preetham Elumalai is an Associate Professor (Biochemistry) in the Department of Marine Biology, Microbiology and Biochemistry at Cochin University of Science and Technology (CUSAT), Cochin, and completed his master's degree from the University of Madras, Tamil Nadu. He has qualified the National Eligibility Test for Lecturership conducted by ASRB/ICAR/UGC. He received his PhD in Biochemistry and Molecular Immunology from the Institute for Immunology, University of Regensburg, Germany. He did his postdoctoral research in the same university and worked on lectin glycomics.

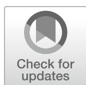
Dr. Preetham has worked at various universities and has quite a good experience in teaching and demonstrating concepts in Biochemistry and Immunology. His current research practice includes Proteomics and Functional genomic approach for the analysis of pathomechanisms of different aquatic diseases, application of nanotechnology for the regulation of nutrient uptake in fish using nutrigenomic approaches, genetic regulation of gene expression across tissues, time and environments.

He holds editorial positions in national and international journals and is a member of many prestigious societies, including Asian Fisheries Society, European Association of Fish Pathologists, International Veterinary Vaccinology Network, International Complement Society and Society of Neurochemistry. He has widely travelled to more than 20 countries on various teaching and research assignments. He was awarded with the prestigious INSA medal (2017) and MASTS (2019) UK visiting fellowship.

Sreeja Lakshmi holds a master's degree in Biochemistry from the University of Calicut, Kerala. She pursued her PhD in Biochemistry from Molecular Cell Biology, University of Regensburg, Germany. She did her Postdoctoral Research at the Institute of Tropical Medicine, University of Tuebingen, Germany. Dr. Sreeja is an awardee of HRD Fellowship for Women Scientist by the Department of Health Research and MASTS (Marine Alliance Science & Technology, Scotland) Award for Postdoctoral and Early Career Research Exchanges (PECRE). Currently she is pursuing her Postdoctoral Research in collaboration with Moredun Research Institute (MRI), UK, with International Veterinary Vaccinology Network (IVVN) Fellowship Grant, UK. Her Research interests extend through functional attributes of bioactive compounds for therapeutic applications, development of nano and glycovaccines against aquatic diseases, novel treatment strategies against neurodegenerative disorders and protein biochemistry.

Dr. Sreeja has published her work in many peer-reviewed journals and presented her works at national and international conferences. She holds memberships in International Veterinary Vaccinology Network (IVVN), Society of Biological Chemists, International Complement Society and Indian Academy of Neurosciences.

Chapter 1
Overview of Lectins



M. S. Prachi Vibhute, Mohamed Jaabir, S. Sangeetha Bharath, and Jeyachandran Sivakamavalli

Abstract Lectins are proteins that attach to carbohydrates and sugar-containing compounds in a particular and reversible manner, forming glycoconjugates in the process. Various evidences regarding their toxicity profile may be found throughout history, and it was once considered that lectins were only connected with poisonous components. However, recent research demonstrates that lectin science has advanced significantly, and their usage in studies of glycoprotein production, researching carbohydrates on cells/cell organelles, mapping neural pathways, anti-cancer medicines, and lymphocyte mitogenic activation is widely documented. In vivo and in vitro, lectins from viruses, bacteria, algae, mammals, and plants have been identified as modulators and tool markers; these molecules also have a role in mitotic induction and immunological responses, helping to the resolution of infections and inflammations. Despite the fact that lectins have piqued the interest of current researchers due to their therapeutic potential, there have been insufficient studies to date, and knowledge of lectins is limited to specific plants or animals. This chapter provides a detailed overview of lectins, beginning with their discovery and biological role, as well as an overview of their harmful consequences and therapeutic contributions.

Keywords Lectins · Overview of lectins · Therapeutic applications of lectins

Abbreviations

ABL	Agaricus bisporus lectin
BSL	*Bryothamnion seaforthii*
CEA	Carcinoembryonic antigen
CFL	*Cratylia floribunda*

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ClcNAc	N-acetylglucosamine
Con A	Concanavalin A
CRD	Carbohydrate-recognition domain
CTLs	C-type lectins
ERGIC	53ER-Golgi intermediate compartment
Gal	Galactose
Glc	Glucose
IBS	Irritable bowel syndrome
IT'S	Immunotoxins
LacNac	N-Acetyllactosamine
LC-5	Levocetirizine-5 mg tablet
Man	Mannose
MBL	Mannose-binding lectin
MCF-7	*Michigan Cancer Foundation-7*
MMP-9	Matrix metallopeptidase
PHA	Phytohemagglutinin
PNA	Peanut agglutinin
SBA	Soybean agglutinin
SFL	*S. flavescens* lectin
Trp	Tryptophan
Tyr	Tyrosine
WGA	Wheat germ agglutinin

1.1 Introduction

Lectins are carbohydrate-binding proteins found in almost all plants, especially seeds and tubers like cereals, potatoes, and beans (legumes). Lectins could also be extracted from animal sources, according to recent studies. They have been employed as histologic and blood transfusion agents in the past. Lectins are found in many of our meals and are potentially poisonous, inflammatory, and resistant to cooking and digestive enzymes. Despite the fact that lectins are linked to toxicity, their value in aspects of cancer therapies, immunology, and antibacterial properties, among other things, cannot be overlooked. Lectins are proteins that can bind to certain carbohydrate groups in a non-covalent manner without altering them chemically. Binding is reversible, and every lectin has many carbohydrate-combining sites. There has yet to be any enzymatic activity linked with any pure lectin molecule. Individual lectin molecules can serve as cross-linking agents because they have more than one carbohydrate-combining site, and lectins were first discovered in extracts of plant seeds that included soluble components that agglutinate red blood cells. Phytohaemagglutinins (from the Greek word 'phyton', meaning 'plant') are the factors that cause hemagglutination. Boyd and Shapleigh (1954) coined the term 'lectin' to describe the fact that identical haemagglutinating substances can also

be derived from animal sources derived from the 'Latin' word 'legere', to pick, choose, or select (Boyd and Shapleigh 1954). Because most plant and animal species only have one type of lectin, lectins are called for the species from which they originated. Lectins bind specifically to carbohydrate-containing groups on the cell surface, and the consequences of this binding are studied using biochemical or microscopic methods. Surprising outcomes are frequently observed. Some lectins, for example, stimulate mitosis in quiescent lymphocytes, some lectins agglutinate neoplastic, malignant cells but not their regular, noncancerous counterparts, and several have been used to show major alterations in the cell surface organisation following viral infections and during growth.

1.2 History

Evidence indicating the occurrence of proteins capable of agglutinating erythrocytes in nature began to accrue towards the end of the nineteenth century. Because they were first discovered in plant extracts, these proteins were dubbed haemagglutinins or phytoagglutinins. Peter Hermann Stillmark, in his doctoral thesis delivered to the University of Dorpat in 1888, was the first to describe such a haemagglutinin (now Tartu, Estonia). Stillmark identified this highly deadly haemagglutinin from the seeds of the castor tree (*Ricinus communis*) and named it ricin (Sharon and Lis 2004). Paul Ehrlich frequently employed this haemagglutinin as a model antigen in immunological experiments (Franz 1988).

James B. Sumner discovered a protein from jack bean (*Canavalia ensiformis*) termed as concanavalin A, 31 years after Stillmark. This was the first time a pure haemagglutinin was discovered. Summer and Howell reported nearly two decades after ConA's isolation that it agglutinates cells like red blood cells and yeasts and also precipitates glycogen from solution. Furthermore, the observations of these published studies that sucrose suppressed ConA hemagglutination indicate for the first time the sugar specificity of lectins (Sumner and Howell 1936). Landsteiner and Raubitschek investigated the hemagglutination of red blood cells from diverse animals by different seed extracts in 1907 (Landsteiner and Raubitschek 1907). They discovered that the relative haemagglutinating activity of each extract tested was considerably variable. William Boyd and Karl Renkonen, working independently, discovered the key discovery of human blood group specificity for haemagglutinins only in the 1940s. They discovered that crude extracts of two leguminous plants, *Phaseolus limensis* and *Vicia cracca*, agglutinated blood type A erythrocytes but not blood type B or O cells, whereas *Lotus tetragonolobus* extract only agglutinated blood type O erythrocytes (Sharon and Lis 2004; Boyd and Shapleigh 1954).

In the research of the antigens associated with the ABO blood type system, the unique interaction between lectins and carbohydrates of erythrocytes was significant. Morgan and Watkins discovered that α-linked N-acetyl-D-galactosamine prevented the agglutination of type A erythrocytes by extracts of *Phaseolus limensis* the best,

whereas α-linked L-fucose decreased the agglutination of O cells by extracts of *L. tetragonolobus* (Morgan and Watkins 2000).

Plant agglutinins' ability to discriminate between erythrocytes of different blood types led Boyd and Shapleigh (1954) to coin the term lectins, which comes from the Latin word 'legere', which means 'to choose out or choose'. He coined this word to encompass all sugar-specific agglutinins of nonimmune origin, regardless of source or blood type specificity (Boyd and Shapleigh 1954).

In the early 1960s, two significant discoveries were pivotal in bringing lectins to prominence. The first was discovered by Nowell and Hungerford (1960) at the University of Pennsylvania in Philadelphia, who discovered that phytohaemagglutinin (PHA), a lectin found in red kidney beans (*Phaseolus vulgaris*), is mitogenic, meaning it can encourage lymphocytes to enter mitosis. This discovery revolutionised immunology since it disproved the previously held belief that lymphocytes are dead-end cells incapable of proliferating or differentiating (Nowell and Hungerford 1960). Several other lectins were found to be mitogenic in a short period of time. The discovery that concanavalin A serves as a mitogen was particularly significant since, unlike PHA, its action could be blocked by modest quantities of monosaccharides, such as mannose. This discovery proved that mitogenic activation is caused by lectins binding to sugars on the surface of lymphocytes, and it was one of the first proofs of a biological role for cell surface sugars. Mitogenic lectins quickly rose to prominence as instruments for investigating signal transmission into cells and the biochemical events that occur during lymphocyte stimulation in vitro. The discovery of T cell growth factor, now known as interleukin-2, in the conditioned media of normal human lymphocytes stimulated by PHA by Robert C. Gallo and his collaborators at the National Institutes of Health (Bethesda) in the 1970s was a particularly valuable conclusion of such studies (Morgan et al. 1976).

Joseph C. Aub of the Massachusetts General Hospital in Boston made the second finding (Aub et al. 1963, 1965). He discovered that wheat germ agglutinin (WGA) had the ability to agglutinate cancerous cells preferentially. Max M. Burger of Princeton University and Leo Sachs and Michael Inbar of the Weizmann Institute (Rehovot) each reported that concanavalin A has the same ability.

Haemagglutinins had been found in a variety of organisms, predominantly plants, until the early 1970s, but only a few had been purified, almost all using traditional methods. Plant lectins from soya beans, green peas, *Dolichos biflorus* seeds, wheat germ, and mushroom (*Agaricus campestris*) were among them (Sharon and Lis 1972), as were animal lectins from eel (Springer and Desai 1971), snail (Hammarstrom and Kabat 1969), and horseshoe crab (Marchalonis and Edelman 1968). As a result, a large number of lectins have been available, primarily from plants, which today number around 500. The demonstration that these lectins are invaluable tools for the detection, isolation, and characterisation of glycoconjugates, primarily of glycoproteins, for histochemistry of cells and tissues, and for the examination of changes that occur on cell surfaces during physiological and pathological processes, from cell differentiation to cancer, sparked a lot of interest in them. The eel's lectin was the first to be found to be specific for a sugar (L-fucose) (Watkins

and Morgan 1952). The first mammalian lectin, the galactose-specific hepatic asialoglycoprotein receptor, was discovered in 1974 as a result of Gilbert Ashwell's research at the National Institutes of Health and Anatol G. Morell's research at the Albert Einstein Medical School (New York) into the mechanisms that control the lifetime of glycoproteins in blood circulation (Hudgin et al. 1974; Stockert et al. 1974). Simultaneously, Vivian Teichberg reported the isolation from the electric eel of the first member of the galectin family of β-galactose-specific lectins, of which there are now over a dozen members (Teichberg et al. 1975). The number of pure animal lectins has been rapidly increasing since the early 1980s, owing largely to the development of recombinant techniques.

In the 1970s, researchers focused more on the molecular characteristics of individual lectins, which is necessary for a thorough knowledge of their molecular actions. These investigations spanned from determining the key physicochemical properties of lectins to peptide acid sequencing and 3D structural elucidation. The main structure of lectins was determined slowly until the development of recombinant technology, just half a dozen of lectins, all within plants, had been fully sequenced. Concanavalin A was the very first lectin whose main sequence had been determined in this case as well. Concanavalin A's 3D structure was solved by high resolution X-ray crystallography by Edelman's group and Karl Hardman with Clinton F. Ainsworth at Argonne National Laboratories (Argonne, Illinois), another first for this lectin (Edelman et al. 1972; Hardman and Ainsworth 1972). Christine Schubert Wright of Virginia Commonwealth University (Richmond) determined the structure of WGA and its complexes with its ligands (N-acetylneuraminic acid and β 4-linked N-acetylglucosamine oligomers) before the whole amino sequence of this lectin was known (Wright 1977).

Because the fundamental structure of many lectins is known, homologies between the sequences of lectins from taxonomically similar sources can be found, is proved for the legume lectins in collaboration with Donny Strosberg at the Free University of Brussels (Foriers et al. 1977). By the end of the decade, homologies for lectins from other families, such as galectins and C-type (Ca^{2+} needing) lectins, had been discovered (Drickamer 1988b). The number of both primary and 3D structures of lectins has exploded in recent years, with more than 200 of these having been discovered (Table 1.1).

1.3 Definition of Lectins

The operational definition of lectins has undergone multiple revisions. As more information about these compounds became available, transitions occurred. The basic definition of lectins as plant agglutinins has been broadened to include other lectins by 1972, agglutinins derived from sources other than plants, with a focus on the carbohydrate-binding abilities of these molecules. In the years since efforts have been made to put other carbohydrate-binding proteins in this category (Sharon and

Table 1.1 A brief history of lectin

Scientist(s)	Year	Discovery	References
Stillmark	1888	Haemagglutinating activity in *Ricinus communis* seed extracts	Sharon and Lis (2004)
Ehrlich	1890	Use of abrin and ricin in immunological research	Franz (1988)
Landsteiner and Raubitschek	1907	Haemagglutinating activity in non-toxic plants	Landsteiner and Raubitschek (1907)
Sumner	1917	Isolation and crystallisation of Concanavalin A (Con A)	Sharon and Lis (2004)
Sumner and Howell	1936	Sugar specificity of Concanavalin A	Sumner and Howell (1936)
Boyd and Renkonen	1940s	Blood group specificity of plant haemagglutinins	Boyd and Shapleigh (1954)
Watkins and Morgan	1952	Inhibition of lectins by simple sugars	Watkins and Morgan (1952)
Boyd and Sharpleigh	1954	Introduction of the term lectin	Boyd and Shapleigh (1954)
Nowell	1960	Mitogenic stimulation of lymphocytes by *Phaseolus vulgaris* lectin	Nowell and Hungerford (1960)
Aub	1963	Agglutination of malignant cells by lectins	Aub et al. (1965)
Ashwell and Morel	1974	Role of animal lectins in endocytosis of glycoproteins	Hudgin et al. (1974)
Gallo	1976	Interleukin 2 dissolved in medium of lectin stimulated lymphocytes	Morgan et al. (1976)

Lis 1972). A lectin was defined as a ‘sugar-binding protein or glycoprotein of nonimmune origin that agglutinates cells and/or precipitates glycoconjugates’ in an attempt to clarify the term in 1980 (Goldstein 1980). This definition, which was embraced by the IUB Nomenclature Committee and the IUB-IUPAC Joint Commission on Biochemical Nomenclature in 1981, excludes carbohydrate-binding proteins with only one carbohydrate-binding site, such as carbohydrate-specific enzymes, transport proteins, hormones, and toxins. Concerns were raised almost immediately about this classification since it excluded carbohydrate-binding proteins that have not been shown to agglutinate cells but are structurally related to lectins from the same plant. Despite these difficulties, the Nomenclature Committee stuck to its 1981 decision because it wanted to keep other carbohydrate-binding proteins out (Dixon 1981).

After that, Kocourek and Horejsi published a new definition. They defined lectins as ‘proteins of non-immunoglobulin nature capable of specific recognition and reversible binding to carbohydrate moieties of complex carbohydrates without altering covalent structure of any of the recognised glycosyl ligands’. This definition eliminates chemotactic proteins and glycosyl ligands due to its emphasis on complex carbohydrates. Some poisons are only able to bind to simple carbohydrates (Kocourek and Horejsi 1983).

1.4 Carbohydrate-Binding Specificity of Lectins

The fact that most plant (and animal) lectins can be categorised into a small number of carbohydrate-binding groups is both fascinating and significant. Mannose/glucose-binding lectins, N-acetylgalactosamine/galactose-binding lectins, N-acetylglucosamine-binding lectins, L-fucose-binding lectins, sialic acid-binding lectins, and lectins bearing 'complex' binding sites are among these (Liener 2012) (Fig. 1.1).

Kurt Drickamer of Columbia University (New York) claimed in 1988 that the carbohydrate-binding activity of most animal lectins is contained in a restricted polypeptide segment known as the carbohydrate-recognition domain (CRD), based on an examination of the then-known amino acid sequences (Drickamer 1988a). S-CRD is really the CRD found in galectins, while C-type CRD is present in C-type lectins. In addition to the ones just described, several other forms of CRD have also been identified, each of which has a pattern of unique and evolutionarily conserved amino acid residues spaced at a specific spacing. On this basis, the majority of animal lectins could be classified into structurally related families and superfamilies, the most common of which is the C-type lectins (CTLs). The P-type lectins and the siglecs are two more groups of relevance. The most of CTLs are large, asymmetric transmembrane lycoproteins with a varying number of structurally and functionally distinct polypeptide domains linked to the CRD. Galectins, however, are tiny, soluble, nonglycosylated proteins that, unlike CTLs, do not require Ca^{2+} to function.

1. Mannose

2. N-acetylgalactosamine

3.N-acetylglucosamine

4.Fusose

5. N-acetylneuraminic acid/Sialic acid

Fig. 1.1 Carbohydrate binding sites for lectins (Created with BioRender.com)

Selectins, collectins, and endocytic lectins are the three families that make up the CTL superfamily (Drickamer 1988a; Sharon and Lis 2004).

Binding with simple or complex carbohydrate conjugates is reversible and non-covalent. The 'Hapten inhibition test,' in which various sugars or saccharides are examined for their ability to block the property of hemagglutination of erythrocytes, can be used to determine the specificity of lectins towards carbohydrates. Because each lectin molecule contains two or more carbohydrate-binding sites that are crucial for their capacity to agglutinate cells or react with complex carbohydrates, more than one carbohydrate moiety can impact the binding characteristic of many lectins (Wu et al. 1988; Singh and Sai 2012). In today's world, the hapten inhibition test is routinely employed to determine lectin specificity.

1.5 Role of Lectins

The biological activity of lectins is based on speculation rather than fact. Plant-derived lectins, which were essentially the only ones most likely known for a long time, have piqued researchers' interest in the potential physiological significance of lectins (Liener 1986). According to one study, feeding bruchid beetles a food containing the lectin 'the black bean' led to the death of the bruchid larvae (Janzen et al. 1976). Scientists concluded that the principal function of lectins in legumes could be connected to protection from insect seed predators. Furthermore, WGA, PNA, and SBA were observed to limit the sporulation and growth of fungi such as *Trichoderma viride*, *Penicillium notatum*, and *Aspergillus niger*, implying that lectins may be involved in plant protection against harmful microbes (Barkai-Golan et al. 1978). The primary carbohydrate specificity groups of roughly 11 lectins were all observed to cause growth disruption during germination of *Neurospora crassa*, *Aspergillus amstelodami*, and *Botryodiplodia theobromae* spores, according to the researchers (Brambl and Gade 1985). Furthermore, because of their role in adhesion and agglutination, lectins may be implicated in sugar transport or carbohydrate storage. Lectins are also thought to have a role in both symbiotic and pathogenic interactions between microbes and their hosts. They also play a crucial function in microbial adherence to diverse surfaces; they can bind to mucosal membranes and withstand acid and proteolytic enzyme denaturation (Liener 1986; Lis and Sharon 1986a). Nearly three decades ago, it was discovered that lectins are responsible for the unique interaction between nitrogen-fixing rhizobia and leguminous plants, which provides the plant with the necessary nitrogen (Bohlool and Schmidt 1974). It was predicated on the idea that lectin from one legume was attached to the appropriate rhizobial species' surface polysaccharide or lipopolysaccharide, but not to bacteria that were symbionts of other legumes. As a result, it has been proposed that rhizobial attachment onto plant roots is caused by an interaction between bacterial surface sugars and lectins found in leguminous plant roots. This is known as the lectin recognition theory, and it is still a source of debate due to a lack of conclusive proof. Lectins are proteins that connect different types of cells and

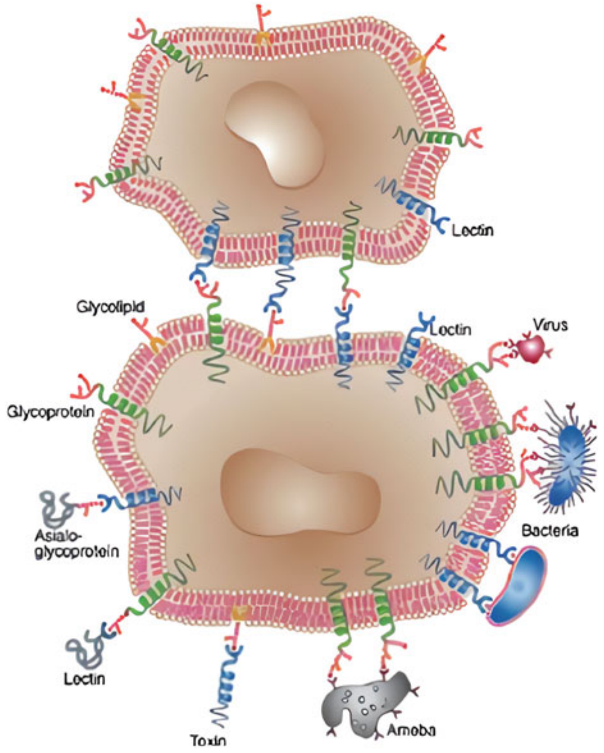

Fig. 1.2 Specificity of cell surface carbohydrates towards various biomolecules (Sharon and Lis 2004)

viruses to other cells by binding to the surface carbohydrates of the cells to be attached (Liener 2012; Lis and Sharon 1986a). Cell surface lectins bind to specific glycoproteins in some cases, while in others, the carbohydrates of cell surface glycoproteins or glycolipids serve as attachment sites for biologically active molecules with carbohydrate specificity, such as microorganisms, various plant toxins, galactic, and so on (Fig. 1.2).

After the 1950s, the importance of lectins and their role in identification and cell surface interaction became clear, with the discovery that influenza haemagglutinin is responsible for the virus's attachment to the host cell prior to infection (Sharon and Lis 2004). A few years later, studies revealed that lectins can provide innate immunity in animals. For example, methyl a-D-mannoside can prevent urinary tract infection in mice caused by mannose-specific Escherichia coli; various other lectins have also been shown to confer innate immunity. The mannose-specific receptor, which is found on the surface of macrophages, binds to infectious organisms that expose mannose-containing glycans on their surface, causing the foreign organism to be consumed and killed (Sharon and Lis 2004). Dectin-1, which is specific for b1, 3, and/or b1, 6-glucans found on fungi, is a newly identified lectin of this type. Calnexin, calreticulin, ERGIC-53, collectins, dectin-1, galectins, macrophage mannose receptor, selectins, to mention a few, have all been demonstrated to

Table 1.2 Role of lectins (Sharon and Lis 2004)

Lectin	Role	References
Animals		
Collectins, dectin-1	Innate immunity	Epstein et al. (1996), Saijo and Iwakura (2011)
Calnexin, calreticulin, ERGIC-53	Control of glycoprotein biosynthesis	Molinari et al. (2004), Kamiya et al. (2008)
L-selectin	Lymphocyte homing	Lasky et al. (1989)
E- and P-selectins	Leukocyte trafficking to sites of inflammation	Ley (2003)
Macrophage mannose receptor	Innate immunity; clearance of sulphated glycoprotein hormones	Gage et al. (2011)
Plants		
Various legumes	Defence, symbiosis with nitrogen-fixing bacteria	Bohlool and Schmidt (1974)
Microbes		
Amoeba, bacteria, influenza virus	Infection	Sharon and Lis (1989)

be important animal lectins in identifying critical biological features. They have been linked to defence mechanisms, lymphocyte homing, and immunological cell interactions. They are also important in cell biology, including as cell–cell interactions, cell proliferation, apoptosis, cell division, and the cell cycle (Lis and Sharon 1986b) (Table 1.2).

1.6 Toxic or Therapeutic?

The toxic potential of lectins has long been recognised. Some lectins are resistant to gut enzymes and are difficult to break down; they may adhere to the gut wall and cause harm to the lining. Diseases like colitis, Crohn's disease, Celiac Sprue, and IBS may be linked to this. When rats and humans were provided dietary sources rich in these proteins, Lectins were active in their faeces, according to a study conducted by P.G Brady. Brady and his coworkers used affinity chromatography to identify wheat germ agglutinin. They also extracted, purified, and characterised wheat germ agglutinin from faecal samples (Brady et al. 1978). According to research, lectin-derived amino acids are unavailable to animals, and partially digested lectins can adhere to the intestine's epithelial cell lining (King et al. 1980). Jaffe was the first to link impaired performance in rats to *Phaseolus vulgaris* consumption (kidney beans lectins) (Jaffé 1960). Years of research have demonstrated that after lectin interacts with the intestine, it is endocyted, causing several systemic problems. De oliveria and his colleagues conducted a few similar experiments and found that feeding rats lectin produced from pure *Phaseolus vulgaris* resulted in intestinal, liver, and

pancreatic enlargement (De Oliveira et al. 1988). This enlargement of the pancreas appears to be the cause of the rats' observed decrease in insulin levels. It was also discovered that kidney bean lectin-fed rats seemed to have thymus atrophy; this atrophy is thought to have developed as a result of the abnormal proliferation of gut bacteria, as the rats' immune systems were depressed by the lectins' toxic effects. The consumption of kidney bean lectin disrupts the intermediate metabolism, resulting in weight loss, poor development, and eventually death of the rats in the study (De Oliveira et al. 1988). Insulin-dependent diabetes, rheumatoid arthritis, IgA nephropathy, and peptic ulcers are some of the other disorders linked to lectins. The failure of certain types of barrier methods in the body, notably SIgA barrier protection, could cause lectin sensitivity. It might be claimed that lectins are unique to certain carbs, and that when they bind to their carbohydrate substrate, they disrupt the cellular membranes and cause cell death. Despite the fact that lectins have a number of harmful components, their benefits have been reported in the literature, making them a topic of therapeutic interest for a number of researchers (Singh and Sai 2012).

In the famous politically driven 'umbrella murder' of Georgi Markov, a Bulgarian opposing party writer and exiled broadcaster, ricin was used as a weapon. Attempts to use ricin as a possible weapon of war were made by the USA during World War I, and the British military created and tested a ricin bomb during World War II, but it was never used as a weapon of mass destruction. Ricin has recently entered the arsenals of extreme people, groups, and governments (Sharon and Lis 2004).

In recent years, remarkable progress has been made in understanding the critical functions of lectins in a variety of biological processes (Nowell and Hungerford 1960). The significance of lectins as biotechnological tools was acknowledged early in the biological application investigations. In 1960, a significant step forward in immunology was made by determining the significance of these proteins in lymphocyte cell division. Phytohaemagglutinin (PHA), a lectin found in red kidney bean, has been discovered to induce lymphocytes to enter mitosis (Nowell and Hungerford 1960). In addition to immunological investigations, current work has looked into the impact of lectins in the field of microbiology, because lectins can be used to verify the role of pathogen-host cell interaction and its importance in disease development. For example, it has been suggested that the bacterium *Helicobacter pylori* attacks human cells via a lectin-lectin interaction (Bennett and Roberts 2005). Certain lectins are also utilised to deliver chemotherapeutic drugs and to study cell surface receptors in bacteria, protozoa, and fungus. Bacterial cell wall components and bacteriophage receptors can also be determined using lectins (Sharon and Lis 1989). Because of the unusual ability of lectins to bind non-covalently to simple sugars and polysaccharides, lectin research has sparked the interest of microbial taxonomists. Many microbes rely on lectins for identification and taxonomic classification in clinical laboratories (Sharon and Lis 1989; Lis and Sharon 1986b).

Lectins are usually monoclonal proteins with a broad range of specificities and molecular mass; as a result, they are considered important diagnostic tools in microbiology. One of the benefits of using lectins in clinical microbiology is that

hapten inhibition experiments can be used to investigate cellular or membrane receptor regions (Nicolson 1974; Lis and Sharon 1986a).

The ability of lectins to bind to cells causes a broad range of biological phenomena. For example, lectins have been used to fractionate animal cells, including B and T lymphocytes and to show alterations in cell surface structure following virus or parasite infection. They are vital and adaptable instruments which can be used as fluorescence and electron microscopy probes, as well as in gel diffusion tests (Nicolson 1974). During the separation of glycoproteins, immobilised lectins are employed for affinity chromatography because they have an advantage over other purification procedures in that elution may be done with a reasonably affordable monosaccharide and the glycoprotein to be purified is not denaturated (Lis and Sharon 1986b).

Lectins have been discovered to have anticancer effects in recent years (Mody et al. 1995). Several studies have demonstrated that lectins can limit tumour growth by inducing cytotoxicity, apoptosis, telomerase activity downregulation, and angiogenesis inhibition (Mody et al. 1995). Furthermore, lectins have been discovered to sequester the pool of accessible polyamines in the body, assisting in the prevention of cancer cell proliferation. Some lectins are powerful poisons, but because of this, they could be employed as therapeutic agents. For example, lectins like Recin and Abrin have been linked to specific monoclonal antibodies and are used in cancer treatment (Lord 1987). By connecting ricin to cell type-specific monoclonal antibodies, ricin could be used to generate selectively cytotoxic chemotherapeutic drugs. Cancer is a fatal disease in which the abnormal behaviour of a single cell type makes chemotherapy difficult to cure (Lord 1987). It is critical in cancer treatment that the medicine targets only the cancerous cells while leaving the healthy cells alone, which can be difficult, especially with chemotherapy. Incorporating a hybrid reagent with selectivity for target cells with possible cytotoxicity is a viable technique. The most common expression of such hybrids is immunotoxins (ITs), which are conjugates in which cell reactive monoclonal antibodies are chemically attached to strong toxins (Pastan et al. 1986). A monoclonal antibody that has been generated against the tumour cell-specific surface antigen confers target cell selectivity (Lord 1987). Ricin is the toxin of choice since it is well characterised and has simple purification methods, and also the fact that human immunity to it is unusual, making one of the most effective poisons known (Olsnes and Pihl 1982). *Abrus precatorius* seeds, *Adenia digitata* roots, *Viscum album* leaves, and *Adenia volkensii* roots are other plant lectins that are structurally and functionally similar to ricin (Stirpe and Barbieri 1986).

1.7 Applications of Lectins

Lectins have piqued the curiosity of biologists, who are particularly interested in their studies and uses in agriculture and medicine (Movafagh et al. 2013). These proteins with distinct properties have found use in a variety of domains of biology,

and as more lectins are isolated and their natural roles understood, they will continue to play an essential part in agricultural and medicinal research.

1.7.1 Antimicrobial and Antifungal Activity of Lectins

To induce adhesion and infection, several human pathogens use cell surface glycans as either receptor or ligand (Sharon and Lis 1989; Zem et al. 2006; Mukhopadhyay et al. 2009). *E. coli* binds to mannosides in the host, whereas influenza virus attaches to sialic acids in the host (Mukhopadhyay et al. 2009). Various *E. coli* strains have been identified that have specificities for other carbohydrate moieties on the host cell surface, such as galabiose (Gal-α-4-Gal) and NeuAc-α-2,3-Gal-β-3-GalNAc (Khan et al. 2000; Buts et al. 2003). The genital pathogen *Neisseria gonorrhoea* binds N-acetyllactosamine (Gal-β-4-GlcNAc, LacNAc), while *Streptococcus pneumonia* binds the pentasaccharide NeuAc-α-3-Gal-β-4-GlcNAc-β-3-Gal-β-4-Glc as well as the internal tetra- and trisaccharides Galβ-4-GlcNAc-β-3-Gal-β-4-Glc and GlcNAc-β-3-Gal-β-4-Glc. Fucose is particularly bound by *Pseudomonas aeruginosa* (L-Fuc) (Barthelson et al. 1998). Bacteria can distinguish between two identical glycans with only one hydroxyl group difference (Sharon 2006). Because such host–pathogen interactions are multivalent, the binding events have a high affinity and are well suited for host invasion (Nimrichter et al. 2004; Mukhopadhyay et al. 2009).

The antitumoural and antiviral actions of lectins, as well as their negative effect on microbes, can disclose their cytotoxic effects; lectins with varied carbohydrate specificities can cause growth inhibition or death of fungi and bacteria. Table 1.3 explains how lectins could be used to detect, type, and control bacteria and fungus that cause harm to plants and humans. Antibacterial activity on Gram-positive and Gram-negative bacteria is mediated by lectin interactions with bacterial cell wall components such as teichoic and teichuronic acids, peptidoglycans, and lipopolysaccharides; research revealed that isolectin I from Lathyrus ochrus seeds binds to muramic acid and muramyl dipeptide via hydrogen bonds formed between the ring hydroxyl oxygen atoms of sugar and carbohydrate-binding site of lectin and hydrophobic interactions with the side chains of residues Tyr100 as well as Trp128 of isolectin I (Bourne et al. 1994). Despite the high number of purified lectins and haemagglutinins, only a few of them showed antifungal activity. Fungi growth can be inhibited by lectin binding to hyphas, leading to inadequate nutrition absorption, or by interfering with the spore germination process (Lis and Sharon 1981). Chitin, a polysaccharide, is a component of fungus cell walls, and chitin binding lectins have antifungal activity; disruption of chitin synthesis and/or deposition in cell wall may be the cause of antifungal action (Selitrennikoff 2001). The carbohydrate-binding property of lectins is most likely implicated in antifungal processes, and lectins with different specificities can induce varied effects. Plant agglutinins are thought to have a function in plant defence mechanisms against phytopathogenic microorganisms (Sá et al. 2009b).

Table 1.3 Antimicrobial and antifungal activity of lectins (Hamid et al. 2013)

Plant	Lectin specificity	Antimicrobial activity	References
Araucaria angustifolia (seed)	GlcNAc	*Clavibacter michiganensis*, *Xanthomonas axonopodis* pv. *Passiflorae*	Santi-Gadelha et al. (2006)
Artocarpus incisa (seed)	GlcNAc	*Fusarium moniliforme, Saccharomyces cerevisiae*	Trindade et al. (2006)
Myracrodruon urundeuva (heartwood)	GlcNAc	*B. subtilis, Corynebacterium callunae, E. coli, Klebsiella pneumoniae, P. aeruginosa, S. aureus, Streptococcus faecalis, fusarium solani, F. oxysporum, F. moniliforme, fusarium decemcellulare, fusarium lateritium, fusarium fusarioides, fusarium verticillioides*	Sá et al. (2009b)
Phaseolus coccineus (seeds)	Sialic acid	*Helminthosporium maydis, Gibberalla sanbinetti, R. solani, Sclerotinia sclerotiorum*	Chen et al. (2009a)
Pisum sativum (seed)	Man	*Aspergillus flavus, F. oxysporum, Trichoderma viride*	Sitohy et al. (2007)
Urtica dioica (rhizome)	GlcNAc	*B. cinerea, C. lindemuthianum, Phoma betae, Phycomyces blakesleeanus, Septoria nodorum, Trichoderma hamatum, T. viride*	Broekaert et al. (1989)

Recent research has shown that lectins of various origins and carbohydrate specificities can be used as antifungal and antiparasitic medicines. Plant lectins studied for antifungal potential, primarily versus phytopathogenic species, have by far the most reported antifungal activity binding to hyphae, leading to growth inhibition and spore germination prevention. A lectin extracted from the heartwood of *Myracrodruon urundeuva*, for example, was able to reduce the growth of *Fusarium oxysporum*, *F. decemcellulare*, and *F. fusarioides* by more than 50% (Sá et al. 2009b). A galactose-specific lectin isolated from *Bauhinia monandra* secondary root likewise inhibited *Fusarium* growth, with the greatest effect (30 percent suppression) on *F. solani* (Souza et al. 2011). Lectins have also been shown to prevent the growth of phytopathogens from other genus. Lectins isolated from the seeds of *Phaseolus vulgaris* suppressed the development of *Coprinus comatus*, *Rhizoctonia solani* (Ye et al. 2001), and *Valsa mali* (Ang et al. 2014). At 40 g/mL, a mannaose-specific lectin of *Ophioglossum pedunculosum* roots severely inhibited the development of *Sclerotium rolfsii* (He et al. 2011).

Antifungal lectins also affect fungi that are human and animal diseases. *Candida tropicalis*, *Candida parapsilosis*, *Candida albicans*, and *Pichia membranifaciens* were all inhibited in growth and had their membrane permeability altered by *Helianthus annuus* seed lectin. This protein could also cause pseudohyphae to develop and reactive oxygen species to be produced in *C. tropicalis* (Regente et al. 2014). A lectin isolated from *Cladonia verticillaris* lichen inhibited the growth of the dermatophyte *Trichophyton mentagrophytes* by 35% (Ramos et al. 2014). The impacts of lectins on human and animal pathogens have been studied using many methodologies, including the detection of parasiticidal action, infection prevention,

and the research of carbohydrate–receptor interactions' role in the infective activity. The lectins both Con A and ricin caused tegumental damage (basal vacuolation and inflammation of basal membrane invaginations) in mature *Schistosoma mansoni* worms, which was avoided when assays were conducted in the presence of carbohydrates that inhibited these lectins (Simpson and McLaren 1982). *Strongyloides ratti* (rat threadworm) migratory pattern was disrupted by Con A, *Triticum vulgaris*, and *Glycine max* lectins along a sodium chloride gradient, indicating the involvement of carbohydrate moieties in the chemosensory activity of labial sensilla in this nematode (Tobata-Kudo et al. 2005). Lectins from *Coprinopsis cinerea*, *Aleuria aurantia*, and *Laccaria bicolor* had a larvistatic impact on *Haemonchus contortus* (Barber's pole worm), causing the larva to be arrested at the L1 stage; the lectin from *Marasmius oreades* increased larval death (Heim et al. 2015). Cramoll 1,4 was tested in infected mice with *S. mansoni* for in vivo antihelminthic action; treatment with such a lectin reduced the amount of expelled eggs, restored adult worms, as well as liver granulomas (de Melo et al. 2011).

Lectins were also tested for their ability to protect hosts and vectors from parasites and viruses. At 100 g/mL, the *Microgramma vacciniifolia* rhizome lectin was found able to cause the death of *Biomphalaria glabrata* embryos and grownups; also, the snails treated with the lectin deposited a small number of eggs, some of which had abnormalities (de Albuquerque et al. 2014). Adult *B. glabrata* snails were also killed by lectins of *Cratylia floribunda* (CFL) as well as *Dioclea guianensis* (Dgui) (dos Santos et al. 2010). The mosquito *Aedes aegypti* (carrier of the causative agent of dengue, chikungunya, and zika fever) was found to have larvicidal action against lectins of *Myracrodruon urundeuva* bark, heartwood, as well as leaves (Sá et al. 2009a; Napoleão et al. 2012). Furthermore, lectins obtained from whole seeds plus seed cake of *Moringa oleifera* have not only larvicidal, ovicidal, but also oviposition-stimulant activities on *Aedes aegypti*, making them promising candidates for mosquito population control, even in egg traps (Coelho et al. 2009; de Lima Santos et al. 2014).

1.7.2 Anti-insect Activity of Lectins

Lectins play an interesting role in the host's defence against diseases and predators (Fitches et al. 2008; Hakim et al. 2010). Several alternative techniques, including the use of plant lectins, have been tried to replace traditional insect control measures that produce pollution and disrupt the food chain. Plant lectins' anti-insect activity against a wide range of insect species has been thoroughly established, suggesting that they could be used as naturally occurring insecticidal agents against pests that limit agricultural output (Fitches et al. 2008; Hogervorst et al. 2006). In many regions of the world, *Bactrocera cucurbitae* is a serious pest of cucurbitaceous vegetables and fruits. The insect has so far eluded practically all traditional control efforts, with damage to the standing crop reported to be as high as 100% in certain cases (Singh et al. 2009). Lectins have been successfully engineered into a range of

crops, including wheat, rice, tobacco, and potatoes, and have been identified being one of the potential agents against insect pests. This method could be employed as part of an overall pest management strategy or to combat caveat pests. In general, it appears that widespread use of transgenic insecticidal and herbicide-tolerant plants has few detrimental consequences for the environment. Furthermore, at least some transgenic plants can benefit the corresponding habitats and human health because their production significantly reduces the load of chemical insecticides and herbicides. Lectins have anti-insect properties (Velkov et al. 2005). They either increase insect mortality or cause insect development to be delayed. *Arisaema jacquemontii* lectin prevented the formation of *Bactrocera cucurbitae* larvae when added to an artificial diet (Kaur et al. 2006). The lectin from *Arisaema helleborifolium* had anti-insect action against *B. cucurbitae* second instar larvae (Singh et al. 2006). The insecticidal function of lectins could be owing to the larvae's enzymatic activity being orchestrated. The action of esterases in larva increased after treatment with various lectins, whereas the action of acid phosphatase as well as alkaline phosphatase declined. Galectin-1 treatments of *Plutella xylostella* larvae resulted in microvilli disruption and defects in these epithelial cells (Chen et al. 2009b). By avidly adhering to the larval brush boundary and peritrophic membrane, *Dioscorea batatas* lectin hindered the development of *Helicoverpa armigera* larvae into adults (Ohizumi et al. 2009).

Bactrocera cucurbitae was found to be resistant to a lectin from *Colocasia esculenta* (L.) Schott corms (Thakur et al. 2013). The lectin was discovered to be specific for N-acetyl-D-lactosamine (LacNac), and asialofetuin, a desialylated serum glycoprotein, had anti-insect potential against *Bactrocera cucurbitae*. In comparison to the control, the lectin drastically reduced the % pupation and emergence. The effect on various enzymes was investigated using LC5 (51.6 g mL^{-1}) CEA in a second instar larvae artificial diet bioassay. Esterases, phosphatases (acid and alkaline), superoxide dismutases, catalase, and glutathione-S-transferase were all shown to have significantly increased enzyme and specific activity ($p < 0.01$, $p < 0.05$). CEA disrupted normal growth and development, causing stress in the larvae and activating their detoxification and antioxidant systems, according to these findings. As a result, the lectin appears to be a promising candidate for *Bactrocera cucurbitae* control. In terms of integrated pest management, the lectin gene is a promising choice. The usefulness of this candidate gene is determined by the fact that it produces an edible protein and hence is unlikely to offer any severe health risks to humans if produced in a transgenic plant.

1.7.3 Healing Applications of Lectins

Many studies have shown that lectins have a healing effect. Healing is the process of tissue repair following trauma, in which a controlled group of cells and molecules initiates a series of events that lead to the anatomical and functional restoration of wounded tissues (Cordeiro and Jacinto 2013). Haemostasis, inflammatory phase,

tissue development (proliferation), and extracellular matrix remodelling are all consecutive phases of injury repair (tissue maturation). Coagulation factors and platelets, for starters, enhance blood coagulation in injured tissue. Inflammatory cells such as neutrophils and macrophages phagocyte injured cells and extracellular matrix, resulting in the regeneration of new tissue and the creation of scars (Velnar et al. 2009). The role of lectins as healing agents is unclear; nonetheless, throughout the healing process, lectins may influence the immune response, cytokine generation, inflammatory response, and cell antiproliferative action (Nascimento da Silva et al. 2014). Lectins have been shown to have a positive influence in the healing of cutaneous wounds and the modification of the scarring process, with promising results and therapeutic potential.

Induced wounds in the dorsal thoracic region of rat had been treated with a lectin extracted from the marine rhodophyte *Bryothamnion seaforthii* (BSL). During the treatment, BSL displayed a proinflammatory impact and accelerated wound reduction. Treatment with BSL enhanced fibroblast collagen synthesis and the exceptionally fast of young skin annexes following 7 and 12 days, promoting luminal epithelium restructuration and wound closure. BSL has an immunomodulatory influence on immune cells during the inflammatory and proliferative stages, with stimulatory effects on polymorphonuclear cell migration to wounded sites and fibroblast activation, resulting in a prohealing effect (Gonzaga do Nascimento-Neto et al. 2012).

In order to assess the regulation of scar formation, the antiproliferative action of an edible mushroom Agaricus bisporus lectin (ABL) was studied in vitro using human ocular fibroblasts (Batterbury et al. 2002). At 100 g/mL of ABL, ocular fibroblast proliferation had been reduced by 40% and collagen lattice contraction became totally eliminated. These findings suggest that ABL has the ability to influence the healing process as well as scar formation in human ocular tissue. Growth factors such as epidermal growth factor as well as insulin may alter ABL's ability to suppress proliferation (Batterbury et al. 2002; Yu et al. 1993).

The use of mannose-binding lectin (MBL) replacement therapy as a therapeutic method for a radiation-induced chronic ulcer has been reported. In this investigation, a patient with an inadequate level of MBL and a persistent radiation-induced ulcer after breast cancer treatment was observed, with no good healing after 15 months of conventional therapy and plastic surgery. Thus, for 6 weeks, an experimental intravenous therapy using human plasma-derived MBL was administered; after the treatment, the patient was completely healed. MBL is a part of innate immunity, and also its role in microbial clearance and immune response modulation could aid wound healing (Maaløe et al. 2011).

Previous research has shown that plant lectins can increase the creation of matrix metallopeptidase-9 (MMP-9) which is involved in several stages of wound healing, as well as act as chemoattractive factors for inflammatory cells, inducers of cytokine release, and collagen synthesis (Nagase et al. 2006). Healing impairment is a significant therapeutic concern in tissue repair, particularly for patients with diabetes and other disorders who have chronic wounds and need additional cicatrisation

therapy. Some lectins are prohealing natural source compounds that are highly effective at inducing quicker reepithelialisation and cicatrisation in this situation.

1.7.4 Lectins in Cancer Research

Lectins have quite a wide range of uses in biological sciences, including cancer research, due to their precise specificity. Lectins are have anticancer properties. Plant lectins are a well-defined and innovative non-traditional anticancer chemical source. A number of plant lectins (mostly galactoside and galNAc specific) have also been tested as potential cancer treatments in preclinical and clinical trials (Ernst 2006). Lectins have now become a well-established method for understanding several areas of cancer and metastasis in recent years. Lectins are now known to play a role in tumour cell identification (surface markers), cell adhesion as well as localisation, signal transduction across membranes, mitogenic activation, host immune system enhancement, cytotoxicity, and apoptosis. To learn more about these lectin-dependent activities, researchers are looking for new lectins with one or more of these roles, as well as developing lectin- (or glycoconjugate-) based tools to target tumour cells. For several decades, legume lectins have been one of the most intensively investigated plant lectin families regarding their molecular foundation of protein–carbohydrate interactions. The potential applicability of this lectin family as anti-tumour medicines that could bind specific cancer cell surface glycoconjugates has piqued researchers' curiosity in recent years (Damodaran et al. 2008). Concanavalin A (ConA), a standard legume lectin with the mannose/glucose-binding affinity, has been shown to trigger apoptosis into murine macrophage PU5–1.8 cells via mitochondrial clustering and cytochrome c release. ConA causes apoptosis in human melanoma A375 cells via a caspase-dependent mechanism, according to a recent study (Liu et al. 2009). ConA triggered mitochondrial transmembrane potential (MMP) breakdown, cytochrome c release, caspase activation, and eventually mitochondria-mediated death as a result. Furthermore, other recent studies have shown that legume lectin called *S. flavescens* lectin (SFL) can promote cancer cell death via a caspase-dependent apoptotic pathway, with the death-receptor pathway being one of the apoptotic pathways (Liu et al. 2008). Another classic legume lectin, isolated from *Phaseolus coccineus* L. (*Phaseolus multiflorus* wild) seed and specific for sialic acid, exhibits significant antiproliferative action (Chen et al. 2009b). In murine fibrosarcoma L929 cells, this lectin caused caspase-dependent death. Furthermore, when the sialic acid-specific function was totally suppressed, its antineoplastic effect was reduced rapidly, implying that such a sugar-binding specificity is the primary cause of antineoplastic activity as well as apoptosis (Chen et al. 2009b). Hemagglutinin from *Flammulina velutipes* reduced the multiplication of leukaemia L1210 cells (Ng et al. 2006). On HeLa and FemX cells, *Haliclona crater* lectin revealed was found to be cytotoxic (Pajic et al. 2002). Hemagglutinin from dark red kidney beans inhibited cell growth of leukaemia L1210 cells (Xia and Ng 2006). Breast cancer

MCF7 cells as well as hepatoma HepG2 cells were both inhibited by a glycine max lectin (Lin et al. 2008). The proliferation of (L1210) cells and hepatoma (HepG2) cells was slowed by Del Monte banana lectin (Cheung et al. 2009). Autumn purple bean lectin induced the creation of apoptotic bodies, which hindered the proliferation of hepatoma HepG2 cells (Fang et al. 2010). Cancer patients can benefit from mistletoe lectin to improve their quality of life (Semiglazov et al. 2006). The mechanism of action of antitumour lectins was unravelled in order to broaden their application. Apoptosis is induced by lectins in a variety of cancer cell types. Korean mistletoe lectin-treated B16-BL6 melanoma cells are one example (Park et al. 2001). Another examples are human A253 cancer cells treated with Korean mistletoe lectin (Choi et al. 2004), Agrocybe aegerita lectin-treated HeLa cells (Zhao et al. 2009).

Mistletoe lectin was stabilised using alginate/chitosan microcapsules with a biodegradable polymer wall that can protect the lectin from stomach acidity (Lyu et al. 2004). The use of lectins in immunofluorescence and/or immunohistochemistry can identify the early premalignant stage of prostate cancer. Glycoconjugate expression is frequently changed in tumour cells. In dysplastic epithelial tumour cells, abundant N-acetylglucosamine (α1,3) N-acetylglucosamine/galactose and galactose (β1,4) N-acetylglucosamine (α,2) mannose (α1,6) residues were found, as demonstrated by labelling by N-acetylgalactosamine-specific and complex type oligosaccharide-specific lectins. These lectins bind to androgen-independent rat prostatic cancer, demonstrating that sugar residues are present in some dysplastic and neoplastic prostatic cells (Chan et al. 2001).

In general, the above-mentioned lectin discoveries show that they may share some biological activities and anti-tumour processes that are closely linked to their matching molecular structures. As a result, these findings could lead to new insights into the anti-tumour mechanisms of lectins.

1.8 Conclusion

Lectins are a subject with a lot of potential and a lot of research. As more lectins are isolated and more research is done on their biological activities and methods of action, lectin production can be enhanced, and new uses for lectins can be discovered and investigated for important contributions in numerous sectors of biology. Despite the fact that lectins have been marketed as potential toxins, there is now a large body of literature reporting their activity in a variety of tissues and processes, a diversity of reports that demonstrates the widespread importance of lectins in cell biology and as a potential therapeutic agent, particularly for cancer, molecular/cell biology, and immunology. However, our understanding of lectins is confined to particular plants or animals, and more studies are needed to find lectins and their significance in as many different sources as feasible. There should be a plethora of experimental research avenues that can be pursued. The end may not be in sight in the quest for their roles, but it is conceivably around the corner. If effective research is contributed to their comprehension, lectins could represent the next generation of medications.

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Conflict of Interest. The authors have no conflicts of interest to declare.

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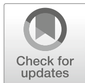
Chapter 2
Structure, Biosynthesis, and Biological Properties of Lectins



N. S. Kaviyarasi

Abstract Lectins are often complex, multidomain proteins with single protein module for sugar binding, termed as carbohydrate recognition domain. Numerous cells in the body synthesis lectins include hepatocytes, activated macrophages, dendritic cells, bone marrow, and epithelial cells in the intestines and lungs. The signal peptide-containing lectins can be synthesized and processed through the traditional endoplasmic reticulum/Golgi network, while those without the signal peptide follow non-traditional pathway or transport route. Some native lectin monomers are oligomerized during post-translational modification, a characteristic of most carbohydrate-binding proteins. The oligomeric lectin has multiple binding sites, which enhances the affinity of binding for multivalent or clustered ligands on pathogenic organisms. During infection, interferon-γ, interlukin-4, and interlukin-6 induce the expression of lectin genes. Several types of cancer cells express elevated levels of lectin mRNA. The expression of functionally active lectin depends on factors like promoter activation, mRNA stability, and post-translational modification. Human lectin genes and its polymorphisms play a crucial role in disease resistance. Moreover, lectin plays a major role in regulating immunity and the process of apoptosis.

Keywords Lectin · Lectin biosynthesis · Lectin structure · Galectin · Mannose-binding lectin · DC-SIGN · Gene regulation · Lectin mRNA · Lectin biological properties

Abbreviations

CRD	Carbohydrate recognition domain
CTL	C-type lectin
CTLD	C-type lectin domain

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DAMP	Damage-associated molecular patterns
DCs	Dendritic cells
DC-SIGN	Dendritic cell-specific ICAM-3-grabbing non-integrin
DC-SIGNR	Dendritic cell-specific ICAM-3-grabbing non-integrin related
ER	Endoplasmic reticulum
ICAM	Intercellular adhesion molecule
MASPs	Mannose-binding lectin-associated serine proteases
MBL	Mannose-binding lectin
MGL	Macrophage galactose-type C-type lectin
NK cell	Natural killer cells
PAMP	Pathogen-associated molecular patterns
PRR	Pattern recognition receptors
SP-A	Surfactant protein A
SP-D	Surfactant protein D
SRP	Signal recognition particle
TM	Transmembrane

2.1 Introduction

In the innate immune system, interactions between lectins and carbohydrates play a crucial role. In addition to classical complement cascade, lectin also activates the complements in invading potential pathogen via phagocytosis. Lectins are carbohydrate-binding protein, expressed by plant, animal, and microbial cells, hence ubiquitous in nature. In plants, highly abundant constitutively expressed lectins are found in seed and vegetative tissues, which function as storage lectins. In contrast, inducible lectins are expressed throughout the plant at a low basal level in nucleocytoplasmic compartment of the cell, which will be upregulated under stress (Van Damme 2014). Numerous animal lectins also exhibit inducible expression patterns, which will be detected at high levels under infectious conditions, just like plants (Manigandan and Ramar 2012). Increased levels of animal lectins have been found during infection and inflammatory response. During inflammatory response the host defense cells such as mast cell, polymorphonuclear neutrophils, macrophages, and dendritic cells sense the pathogen epitope and increase the expression of lectin gene. In addition to immune modulation, lectin plays important role in cell development, cell signaling, cell cycle, as molecular chaperones for glycoprotein quality control.

Most of the animal lectins are polypeptide ranging from ~100 to 400 amino acid residue, encoded by specific gene and translated inside chosen site. Protein synthesis occurs in endoplasmic reticulum or in cytosol, with or without signal peptide. However, lectins with signal peptide can use the conventional ER/Golgi network for synthesis and processing, whereas lectin that lacks signal peptide follows unconventional transport or non-classical pathways. Some lectins are oligomeric protein

and possess multiple binding sites, which recognize multivalent glycans expressed by contact cell.

This chapter briefs about the biochemical properties and synthesis process of selected lectins, at least one example for each mode of synthesis. C-type lectin includes mannose-binding lectin (soluble lectin) and DC-SIGN (membrane bound lectin) and it has been synthesized by classical ER based protein synthesis, whereas galectin (soluble S-type lectin) has been synthesized by non-classical export pathway.

2.2 Role of Lectin in Innate Immunity

Innate immunity is a first-line defense mechanism for the host and it is the process by which soluble and membrane bound host compounds, such as lectins, are bound to the surfaces of pathogenic organisms such as bacteria, viruses, parasites, and fungi. In these interactions, immune responses can be triggered and hence infectious organisms can be neutralized (Van Kooyk and Rabinovich 2008; Vasta 2009). A host's innate immunity is mediated by molecules such as lectins, toll-like receptors, nuclear oligomerization domains, and NK cell receptors. They recognize epitopes specific to pathogenic organisms (Kawai and Akira 2010). As a result, they are referred to as pathogen-associated molecular patterns (PAMPs), and the host molecules that recognize them are called pattern recognition receptors (PRRs). PRR is also capable of identifying endogenously occurring glycans called damage-associated molecular patterns (DAMP). The release of DAMP by host cells occurs upon tissue damage due to infection, inflammation, or tumor growth. PAMPs include glycolipids, glycoproteins, and nucleic acids resulting from bacteria, viruses, and parasites, while DAMPs are high mobility group protein-1, heat shock proteins, interleukin-1 (IL-1), and defensins (Matzinger 2002). In addition, lectins play a major role as PRRs against PAMPs, since many glycoprotein structures (glycans) are recognized by them. Most of the PRR have been expressed by myeloid cells, including macrophages, mast cells, monocytes, polymorphonuclear neutrophils, and dendritic cells. Lectin can act as a secreted PRR, an endocytic PRR or a signaling PRR. The principle functions of these PRR interactions with PAMP of pathogens include opsonization, phagocytosis, activation of complement pathway and proinflammatory signaling pathways, and induction of apoptosis.

2.3 Occurrence of Lectin

Lectins are found in both plant and animal kingdoms. Their biological role differs depending on organism of origin. The information about manually curated lectin 3D structure is available in Unilectin 3D curated database. As of March 2021, the database contains 2278 three-dimensional X-ray structures of lectins. Based on

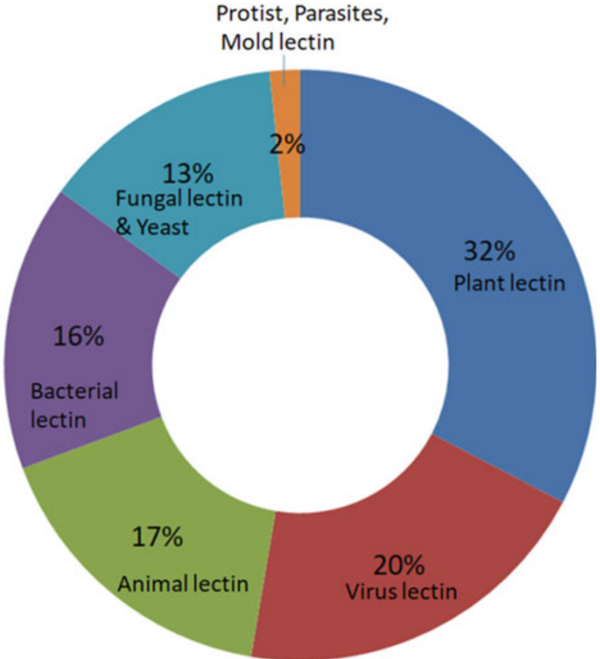


Fig. 2.1 An overview of all 2278 structures accessible in the 3D-lectin database (March 2021)

origin, lectin has been divided into 6 groups (Fig. 2.1). This covers almost 300 different proteins.

The plant lectin makes up a crucial portion of the root nodulation process and in active defense against pathogens and insects (Martínez-Alarcón et al. 2018). In animal system, lectins are involved in cell–cell communications, protein trafficking, and primitive defense reactions (Lepenies and Lang 2019). Furthermore, the microbial lectin acts as adhesion molecules, which facilitates the microbes to adapt the host environment and leads to colonization processes (Esko and Sharon 2009). Some bacterial toxins belonging to lectin family can recognize host glycans and these toxic substances exert their toxic action by anchoring themselves to their target (Martínez-Alarcón et al. 2018).

2.4 Cellular Location of Lectins

Lectin can be categorized into two types based on cellular location, viz. intracellular and extracellular lectins (Santos et al. 2014). The extracellular lectins may either free soluble form or membrane bound. However, the major functions of lectin include cell adhesion, cell signaling, glycoprotein clearance, and pathogen recognition (Fig. 2.2). Extracellular lectins of the host bind to foreign pathogen glycans, including C-type lectins, siglecs, and galectins (Davicino et al. 2011). Galectin is found

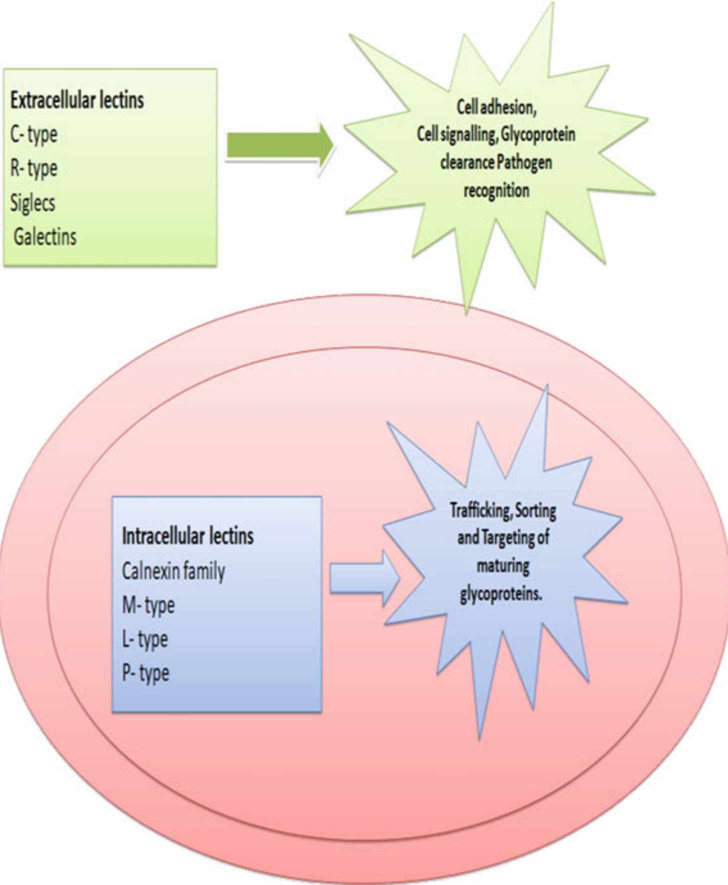


Fig. 2.2 Cellular location of lectins

inside the cytosol and nucleus and controls many intracellular functions, including the intracellular cell signaling, RNA splicing, signaling programmed cell death, endocytic process, and trafficking (Yang et al. 2008).

2.5 Structure and Its Biological Properties

The structural differences among lectins start from its primary structure to their degree of molecular organization. A divergence could occur in amino acid sequence, in the number of subunits or in the nature of the polypeptides. Lectins are often multidomain proteins with a single protein module for binding sugar, referred to as a carbohydrate recognition domain. In general, five distinct lectin groups can be distinguished based on structure (and often on function): C-type lectins, P-type

lectins, S-type lectins, pentraxins, and I-type lectins (Gabius 1997). Of these, C-type and S-type have conserved CRD with a wide range or very narrow range of carbohydrate specificities (Anderson et al. 2008).

Fujimoto et al. (2014) have classified lectins into 48 families based on their three-dimensional structure as shown by X-ray crystallography or NMR analysis. In addition to providing insight on similarities and differences among lectin families, 3D structural analyses can reveal how carbohydrates are bound and the carbohydrate-binding pose of lectins. A number of studies have shown that the different topology of β-structure found in lectins. In lectin families, the most frequent fold is the β-sandwich. Several types of folds exist in the β-sandwich, including the Greek key, jelly roll, or immunoglobulin-like fold, and it consists of two antiparallel β-sheets arranged as a globular structure (Fujimoto et al. 2014). These super secondary structures of CRD play a variety of functional roles. It features a partial dileucine zipper, like that found on the human macrophage galactose-type C-type lectin (MGL), containing YENF internalization motifs. These motifs has role in the recognition of a large number of pathogens by dendritic cells, the maintenance of homeostasis and its interaction with tumor associated antigens derived from abnormal glycosylation processes (Zizzari et al. 2015).

It is evident that how lectins bind to carbohydrates via hydrophobic and hydrogen-bonding interactions, but amino acids involved may vary apart from lectin specificity (Sharon 1993). Depending on the molecule and oligomerization state, these lectin proteins can present 2–12 sites of interaction (Balzarini 2006). Essentially most of the side chains of lectin are capable of forming hydrogen bonds with sugars within one kind of lectin–carbohydrate complex.

2.5.1 C-type Lectin (CTL)

In contrast to other lectins, CTL is specifically regulated by Ca^{2+} for ligand binding, and it has a unique domain structure (C-type lectin domain, CTLD), which allows it to bind ligands otherwise inaccessible to it (Zelensky and Gready 2005). Carbohydrate ligands are typically bound to the C-type CRD by Ca^{2+} ions. In addition to stabilizing protein conformation, these ions form coordination bonds with the ring surface of sugar and the hydroxyl groups along its surface. In addition, amino acid residues coordinating Ca^{2+} can also form hydrogen bonds with sugar hydroxyl groups. CRDs that contain a recognizable consensus sequence are an approximately 115–130 amino acid segment found in C-type lectins. There are two loops in CTLD. β strands at N- and C-terminal (β1, β5) are oriented to form an overall loop structure with antiparallel β-sheet and within the domain lies the second long loop region, which is accessed and exited from the core domain at the same point as the first loop region. Cysteines (C1, C2, C3, C4) are the most conserved residues on the CTLD, form disulfide bridges at the bases of the loops: the whole domain loop connects β5 and α 1 by linking C1 and C4, similarly the long loop region connects β3 and β5 by

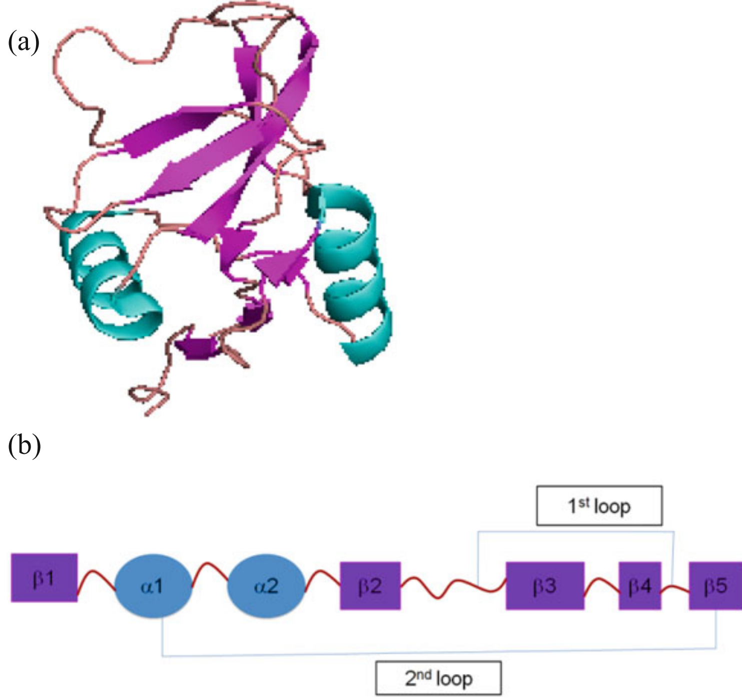


Fig. 2.3 (**a**) Structure of CLDT fold (pdb: 1KX1) (**b**) Linear structure of CTLD showing double loop

linking C2 and C3. These cysteines have a highly conserved "WIGL" motif and play a structural role in maintaining CTLD folds (Zelensky and Gready 2005) (Fig. 2.3).

In total, 17 distinct protein structures with CTLDs have been identified. Collectins, endocytic receptors, selectins, and proteoglycans are all members of the large C-type lectin family, in which some are embedded on membrane and other secreted out by cell (Varki et al. 2015). Collectin family belongs to secretory lectin and has vital role in innate immunity. Among the most studied collectins are mannose-binding lectin, surfactant protein-A, and surfactant protein-D. Each polypeptide chain of a collectin consists of four modular domains, namely a cysteine-rich N-terminal domain, a collagen-like region, α-helical neck region, and a C-terminal CRD (Fig. 2.4a).

Collagen-like regions consist of a Gly-Xaa-Yaa amino acid sequence that forms triple helices of different lengths. In addition to the collagen-like domain, the neck domain region is made-up of α-helical coiled-coils that are required for the formation of the collagen-like triple helices. In the N-terminal region, cysteines form disulfide bonds that allow triple helices to oligomerize in different orders depending on the collectin type (Fig. 2.4b). MBL and SP-A composed of a six trimeric subunit that forms octadecamer, to form "bouquet" organization, whereas SP-D lectin composed

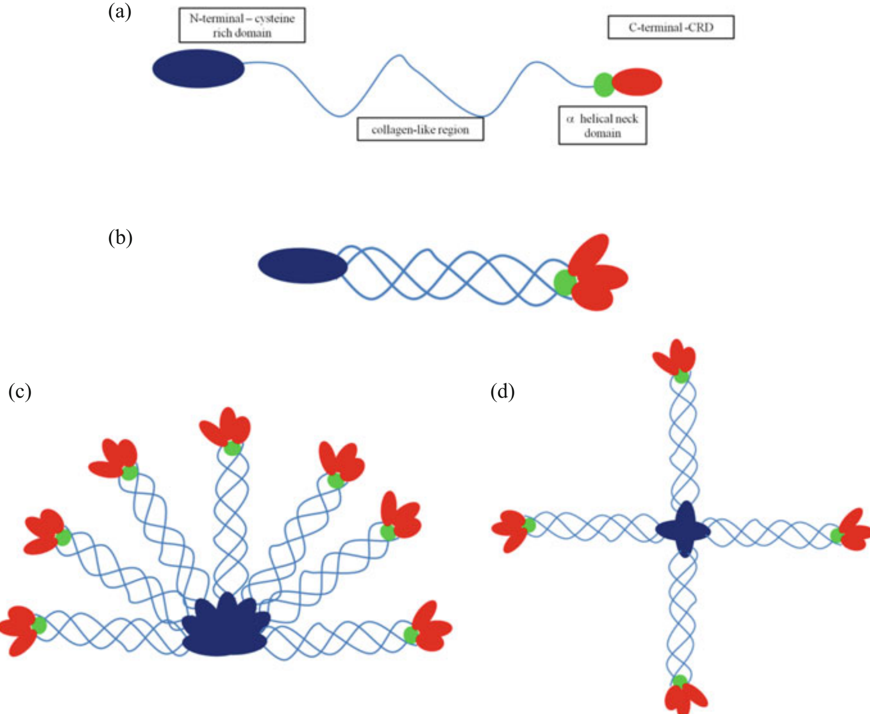


Fig. 2.4 Structure of collectin: (**a**) single polypeptide, showing different regions in monomer, (**b**) trimeric subunit, (**c**) bouquet form, (**d**) cruciform

of a four trimeric subunit that forms dodecamer, reminding a "cruciform-like" structure (Fig. 2.4c, d).

In different families of carbohydrate-binding proteins, oligomerization is a common feature, perhaps because individual CRDs have relatively low binding affinity for carbohydrate ligands, and oligomers can promote binding avidity for multivalent or clustered carbohydrate molecules.

2.5.1.1 MBL

MBL binds to a wide variety of sugar residues, including mannose, fucose, glucose, N-acetylglucosamine, and N-acetylmannosamine. As a result, lectins can bind a variety of viruses, bacteria, yeasts, fungi, and protozoa (De Schutter and Van Damme 2015). Different mechanisms have been played by MBL as defense molecule.

The structure of MBL consists of two α helices, 6 β sheets, 2 disulfide bonds that make the common motif. The functional MBL requires divalent calcium ions, located in loop regions. It is important to note that the MBL structure contains two

calcium ions, one of which preserves the structure, the other co-ordinates with the ligand (mostly mannose in this case).

For ligand binding, asparagine and glutamic acid residues must interact with third and fourth hydroxyl group of the sugar, as well as with one of the two bound Ca^{2+} ions. In addition, 4-OH and C-6 of mannose form van der Waals contacts between the imidazole ring of His189 and also with Ile207, respectively, these interactions play a significant role in binding affinity towards ligand. These residues could be effective for different ligands also including N-acetyl glucosamine, galactose/N-acetyl galactosamine, mannose, and fucose. An orientation of similar binding sites within a ligand facilitates this action (Iobst et al. 1994; Ng et al. 2002).

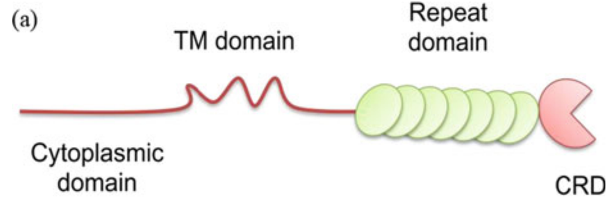
MBL binding with ligand triggers immune response against the respective pathogen. There are different mechanisms involved in functioning of MBL.

- Activation of lectin pathway: Collagenous N-terminal domain of MBL interacts with the proenzyme form of mannose-binding lectin-associated serine proteases (MASPs). This MBL-MASP complex becomes active once it binds with pathogen surfaces rich in mannose. Further it activates complement pathway through cleavage of C4 and C2 (Eddie Ip et al. 2009).
- Phagocytosis: The MBL C-terminal recognition portion binds to carbohydrates on pathogen surfaces, which activates macrophages and facilitates phagocytosis (Jack et al. 2005).
- Anti-viral property: MBL-mediated viral elimination can be achieved by either blocking the recognition of viruses and receptors or by directly neutralizing the viruses (Liu et al. 2015). However, specific recognition of viral particles by MBL is a crucial event during the lectin-mediated complement cascade.

2.5.2 DC-SIGN (Dendritic Cell-Specific ICAM-3-Grabbing Non-integrin)

DC-SIGN and DC-SIGNR (L-SIGN) come from the C-type lectin type II family. In these proteins, N-terminal cytoplasmic domain is followed by a transmembrane (TM) domain and a C-terminus large extracellular region that bears a functional CRD for binding to ligands. The extracellular domain comprises a neck region between the TM and CRD. DC-SIGN and DC-SIGNR are tetrameric proteins, with their C-terminal domains separated from the membrane by a helical extension. A coiled coil forms within the neck (repetitive) region that promotes oligomer formation (Fig. 2.5a, b). As a result of protein oligomerization, cells are more likely to become avid to glycans presented at high density on the wall. Based on the different length of the neck regions encoded, DC-SIGNR might be influenced by its specific neck structure in terms of how it functions on the cell surface (Mitchell et al. 2001).

Studies have shown that the ligands specific for these receptors are high-mannose oligosaccharides such as Man9GlcNAc2 oligosaccharide and they bind more tightly



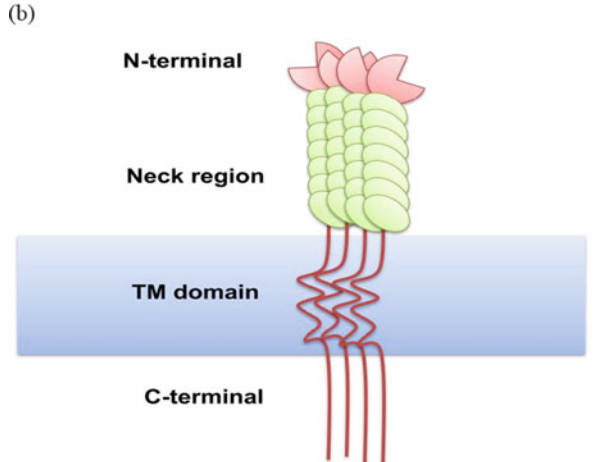


Fig. 2.5 Structure of DC-SIGN: (**a**) monomer of DC-SIGN with N-terminal cytoplasmic domain, a transmembrane domain, extracellular C-terminus domain with CRD. (**b**) Tetramer of DC-SIGN

than mannose (Mitchell et al. 2001). An interesting finding is that the DC-SIGN is highly specific for glycoprotein ligands with multiple high-mannose oligosaccharides arranged at appropriate distances on the surface thereof (Guo et al. 2004).

The ligand specificity of these receptors has been studied using a variety of oligosaccharides. It has been found that 14 different glycans can recognize DC-SIGN, in which the glycans contain terminal fucose residues, but differ in linkage with sugar residue of oligosaccharides. For example, the ligand with terminal fucose linked with galactose and N-acetyl galactosamine can bind DC-SIGN. A linear oligosaccharide containing fucose attached to N-acetyl glucosamine has been found to bind DC-SIGN, and N-acetyl glucosamine increases DC-SIGN binding.

The conserved calcium ion located in CRD of DC-SIGN forms coordination bond with hydroxyl group of pyranose ring. The ligand binding study with different oligosaccharides shows that the hydroxyl group (3-OH and 4-OH) of pyranose ring forms van der Waals interaction with the amino acid residues, including Gly 361, Val 351, and hydrogen bond with Lys 368, Glu 358. Thus studies show both DC-SIGN and DC-SIGNR bind affinity for high-mannose oligosaccharides on enveloped viruses (Guo et al. 2004), especially, binds directly to membrane bound receptor gp-120 of HIV without involving in a CD4 (Curtis et al. 1992). However, DC-SIGN on dendritic cells binds with HIV-1 through T cells rather than endocytose it.

DC-SIGN is involved in endocytosis, trafficking, and releasing ligand at low pH, whereas DC-SIGNR is not involved in endocytosis. In conclusion, DC-SIGN has dual properties to bind ligands and functions in adhesion and endocytosis of pathogens, while DC-SIGNR binds to specific set of ligands and has adhesion receptor properties.

Usually, DC-SIGN plays an important role in cell–cell interactions via, ICAM-3 and ICAM-2, which are molecules expressed on vascular and lymphatic endothelium. By binding DC-SIGN to ICAM-3, DCs are able to cluster with naive T cells and facilitate T cell activation, whereas ICAM-2, which binds to DC-SIGN, promotes DCs to roll and tether under physiological shear flow, as well as the transmigration of DCs across resting and activated endothelium due to chemokines (Geijtenbeek et al. 2000).

One of the key functions of DC-SIGN is clearing glycoproteins from the serum and regulating the production of immunoglobulin E (CD23). Several type 2 receptors remain poorly understood, but some may function as antigen receptors or recognition receptors. In addition, the type 2 receptor may also cause disease by allowing certain pathogens to infect cells: e.g., DC-SIGN signal may increase transmission of viruses, such as HIV-1, ebola virus, dengue virus, herpes simplex virus, Influenza virus A, SARS-CoV, Lassa virus (Geijtenbeek et al. 2002; Yang et al. 2004; de Witte et al. 2006; de Jong et al. 2008; Londrigan et al. 2011; Goncalves et al. 2013).

2.5.3 Galectin

Galectins are relatively small proteins that contain a conserved ~130–140 amino acid residue of carbohydrate recognition domain and it has molecular weight ranging from 14.5 to 38 kDa. The CRD domain contains two antiparallel β sheets, which are sandwiched together in a manner that resembles a closing hand shape, in which the F1 to FX strands are found on the backhand (F-sheet), while the S1 to SY strands form the palm (S-sheet) as the palm. S-sheet side of the sandwich contains binding site for ligand (carbohydrate) and the core motif recognition of β-galactoside is mediated by the S4, S5, and S6 sheets (Fig. 2.6) (Bum-Erdene et al. 2016; Modenutti et al. 2019).

Mammals have been identified with 15 different galectins (Cooper 2002). According to their structure galectin family is composed of three major groups (Fig. 2.7).

1. Prototypes with a single CRD mostly form noncovalent homo-dimers includes, galectin 1, 2, 5, 7, 10, 13, 14, and 15.
2. The tandem repeat with two different CRDs in the N- and C-terminal regions (N-CRD and C-CRD) which are joined by a linker peptide, includes galectin 4, 6, 8, 9, and 12.

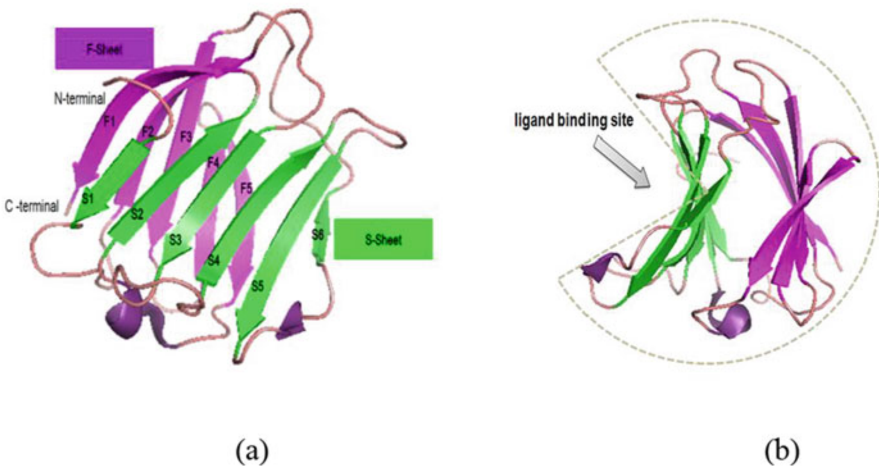


Fig. 2.6 Structure of galectin CRD (pdb: 5DUW): (**a**) monomer showing F-sheets and S-sheets and (**b**) monomer showing ligand binding site

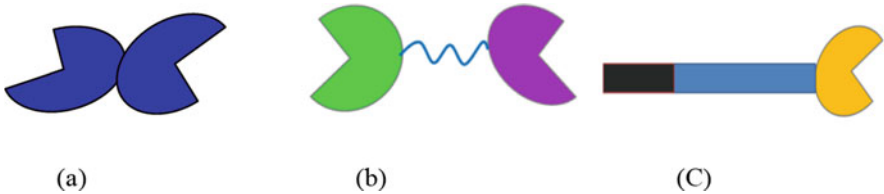


Fig. 2.7 Three types of modular structure of galectin: (**a**) prototype, with single CRD forms noncovalent homo-dimers, (**b**) tandem repeat shows two different CRDs, one in the N-terminal regions and other in C-terminal regions joined by a linker peptide, (**c**) chimera type shows a single CRD and a collagen-like N-terminal domain

3. The chimera-types, including galectin-3, have a single CRD and a non-lectin (collagen-like domain) N-terminal domain, which may group to form an oligomer.

Galectins can bind to both divalent and multivalent glycans. Galectins play a significant role in the extracellular interaction of cells and tissues by crosslinking oligosaccharides in the extracellular space (Kamitori 2018).

Galectin CRDs have different specificities for oligosaccharides. For instance, bovine spleen galectin 1 and human spleen galectin 2 contain three common amino acids (histidine, asparagine, and arginine) at CRD which, respectively, complex with N-acetyllactosamine and lactose (Sharon and Lis 2002). The principal amino acid present in sugar binging domain of galectin has been found to be amino acid with carboxylic acid (Asp,Glu), carboxamide (Asn), basic (His, Arg), hydroxyl (Thr), and aromatic side chain (His, Glu) (Sharon and Lis 2002).

However, in case of tandem repeat galectins-8, the N-terminal CRD shows a strong affinity for α(2–3)-sialylated oligosaccharides, but the C-terminal CRD does not. Similarly, the N-terminal CRD of galectin-9 has high affinity for oligolactosamines with a linear structure, but the C-terminal CRD does not. Still, both N and C terminal CRD of galectin-9 have affinities for N-glycan-type branched oligosaccharides (biantennary oligosaccharides) (Hirabayashi et al. 2002).

Several studies have shown that most of the galectins bind with β-galactose (1–4)-N-acetyl glucosamine and the three hydroxyl groups (3-OH, 4-OH, and 6-OH) in lactose or N-acetyl-lactosamine. The hydroxyl groups form hydrogen bonds with side chains of hydrophilic amino acid residues from galectins. Moreover, these amino acids form hydrogen bond with the hydroxyl group of the terminal sugar and also conserved tryptophan residue has been stacked against the sugar ring at non-polar hydrogen in sugar ring (Dings et al. 2018).

It is important to note that galectin, like other lectin families, is prone to homodimerize and oligomerize, an important characteristic that increases their affinity for glycans and interaction with multiple glycol-conjugates.

Galectin has been found in cytosol, nucleus, and outside the cell. This shows that galectin has its role intracellular too (Yang et al. 2008). The nuclear galectin-3 acts as ribonucleoprotein protein (hnRNPA), in which it modulates mRNA export and splicing (Coppin et al. 2018; Fritsch et al. 2016).

IFN-γ stimulates endothelial cells to make galectin-9, which is expressed in the endothelium of human inflammation lesions. However, galectin-9 has two carbohydrate-binding domains, which mediates the interactions of eosinophils with the endothelium. Thus, the bivalency of galectin-9 facilitates the eosinophil chemotactic activity (Imaizumi et al. 2002). Furthermore, galectins can crosslink glycans on neighboring cells when they bind to them, leading to cell adhesion. Apart from forming lattices at the cell surface, galectins also regulate cell endocytosis, interaction between host and pathogen, and activates immune cells. (Vasta 2009).

Most of the intracellular signaling pathway through protein–protein interaction with cytoplasmic and nuclear proteins influences various cellular functions, including immune and inflammatory response, tumor development and progression, neural degeneration, obesity, atherosclerosis, diabetes and wound repair (Li et al. 2020). Thus, galectin may be a promising therapeutic target for diseases such as cancer, inflammatory diseases, and several other diseases (Yang et al. 2008).

2.6 Biosynthesis and Posttranslational Modifications

Eukaryotes have organelles that specialize in synthesizing protein complexes, while prokaryotes lack such organelles. In prokaryotes, the proteins are synthesized by free ribosomes in the cytoplasm, except those in mitochondria and chloroplasts. In eukaryotic cells, the protein may carry "signal peptide" which is recognized by a signal recognition particle (SRP). Translation is terminated by the SRP, which then excretes the ribosome/protein complex from the endoplasmic reticulum (ER). The

SRP receptor recognizes this complex, which provides a channel for the protein to enter into ER. This process directs the protein synthesis towards the ER lumen and resumes protein synthesis (Cooper and Hausman 2000; Alberts et al. 2002). However, most of the C-type lectins carry "signal peptide" and the protein synthesis occurs in ER lumen. In case of DC-SIGN, the hydrophobic transmembrane segments act as signal sequence, which direct the proteins to the endoplasmic reticulum (Parent et al. 2002). In contrast, galectin lacks signal peptide and synthesized on free polysomes in the cytoplasm. There is a great difference between the lectins synthesized in the cytoplasm and those synthesized in the ER lumen. Recombinant expression of protein acts as a best model system for study of lectin synthesis, processing, post-translational modifications, and differentiation (Martínez-Alarcón et al. 2018).

2.6.1 MBL Synthesis and Processing

MBL is synthesized in rough endoplasmic reticulum and undergoes posttranslational modifications at Golgi (Colley and Baenziger 1987). In hepatoma cell lines, human S-MBL and L-MBL have been expressed using the vaccine virus expression system. Study findings show that S-MBL is released into the medium, while L-MBL resides within the cells. There are some differences between serum and liver MBL, for example, serum could activate complement via lectin, while liver MBL could not (Ma et al. 1997). The translated MBL has single polypeptide chain with four parts; a short cysteine-rich N-terminal domain followed by a collagen-like region, a α-helical neck domain, and a C-terminal CRD. The native protein carries signal peptide which directs the protein into Golgi for post-translational modification. Three distinct post-translational modifications are:

1. Removal of signal peptide: 20 amino acids of signal peptide have been removed from N-terminal by proteolytic cleavage (Ma et al. 1997).
2. Oligomerization of monomer: Synthesized monomers have been oligomerized into high molecular weight multimeric complexes through inter-chain disulfide bonds between subunits, MBL forms in human serum are mostly trimers and tetramers (Teillet et al. 2005).
3. Hydroxylation and glycosylation: Lysine and proline amino acid residues in collagen-like sequences have been hydroxylated and similarly, glucosylgalactosylhydroxylysine and galactosylhydroxylysine have been formed by glycosylation of hydroxylysine. The hydroxylation of S-MBL is required for the complexes to form, which have molecular sizes that range from 200 to 1300 kDa.

Recently, a post-translational modification has been identified as Cys216 / Cys202 which will be modified into trace amount of dehydroalanine. The carbohydrate recognition domain is impaired in this way and thus MBL will lose its activity (Jensen et al. 2007).

2.6.2 Galectin Synthesis and Processing

Lectins with signal peptide can use the well-characterized endoplasmic reticulum/ Golgi network for synthesis and processing. However, lectins like the galectin family, lack signal peptide, appear to be synthesized by free polysomes in the cytoplasm rather than using a classical signal sequence and it accumulates under the plasma membrane in patches prior to secretion (Wilson et al. 1989). Because galectins are synthesized in the cytoplasm, an extremely reducing environment, many of them contain free reduced cysteine residues (Esko and Sharon 2009). It is important to note that galectins do not move directly through secretory apparatuses during their export from cells. In fact, several cytosolic proteins have now been demonstrated to secrete by unconventional transport pathways/non-classical export processes. The non-classical exports may any of the following category (Popa et al. 2018), namely

- Type I secretion: Direct translocation, which may be facilitated by ABC transporters or passive mode.
- Type III secretion: Export via lysosomes or endosomes, which may be facilitated by extracellular vesicles like exosomes or microvesicles.

Galectin-3 requires N-terminal domain for secretion and in addition to that oligomerization is necessary. Interestingly, unlike other animal lectin, galectin is also found in the nucleus and cytoplasm. The study on HeLa cells supports the fact that galectin-3 accumulates at the cell surface; upon addition of lactose galectins are redirected into the cytoplasm and nucleus. Hence, it is evident that secretory machineries vary in their subcellular localization based on each galectin with characteristic domains and sugar binding preferences (Delacour et al. 2009).

2.7 Lectin Gene and Its Transcript

The genes have been detected from different tissues through cDNA library or by direct genome library. According to HUGO Gene Nomenclature Committee (HGNC) C-type lectin genes are divided into 89 groups. C-type lectin receptors contain at least one CTLD, either transmembrane or soluble. They are capable of recognizing both endogenous and exogenous ligands, in which collectins have major role in the innate immunity. The best studied serum collectin is human mannose-binding lectin (MBL).

2.7.1 Mannose-Binding Lectin

MBL gene was first isolated from liver cells. The human MBL2 gene and MBL1 pseudogene 1 (MBL1P1) are located on chromosome 10q11.2-q21 and 10q22.2-q22.3, respectively. MBLP has been reported as nonfunctional gene, due to premature transcriptional termination (Guo et al. 1998). This shows that expression of functionally active protein depends on factors like promoter activation, mRNA stability, post-translational modification.

The gene transcript carries both introns and exons. Number of exon and genomic structure varies with structure of lectin. For example, the soluble lectins like MBL2 gene consist of four exons (Sastry et al. 1989), galectin has six exons (Coppin et al. 2018), ficolin contains eight exons (Matsushita 2010), and transmembrane lectins porcine DC-SIGN has seven exons (Parent et al. 2002) dectin-1 has 6 exons (Gavino et al. 2005; Zhou et al. 2010). After splicing of introns, the functional mRNA has been translated into specific protein.

Exons give rise to different domain structure. For instance, in case of MBL2,

- Exon 1 has 251 base pairs, which encodes 5′UTR, the signal peptide and Cys-rich domain and seven Gly-Xaa-Yaa repeats of the collagen region.
- Exon 2 has 117 base pairs, which encodes the remainder of the collagen-like region.
- Exon 3 has 69 base pairs, which encodes the neck region.
- Exon 4 has 310 base pairs, which encodes the CRD and 3′UTR.

It has been determined that three introns are 600, 1350, and 800 base pair in size (Fig. 2.8a) (Garred et al. 2006). As it is evident, rat S-MBL and L-MBL are encoded by different mRNAs and have distinct amino acid sequences. In contrast, human S-MBL and L-MBL are encoded by single mRNA. This is because of MBL2 gene has been regulated by two alternative promoters (named 0 and 1) in human. As a result, two MBL mRNAs with different sized 5′-noncoding regions have been detected, one with longer transcript begins at exon 0 and the other shorter transcript begins at exon 1 (Naito et al. 1999). A low amount of MBL2 mRNA is present in small intestine and testis tissue, predominantly via promoter 1 transcripts (Seyfarth et al. 2006).

Lectin genes and its polymorphisms play a vital role in disease resistance. The collagenous structure of MBL has greater importance in defense function. Mutations in exon 1 (Arg at codon 52 to Cys; Gly at codon 54 to Asp and Gly at codon 57 to Glu) of the human MBL2 gene disrupt the collagenous structure of the protein, resulting in reduced levels of functional serum MBL (Turner 2000). Similarly, the expression of MBL2 gene has been affected by the promoter polymorphism such as H/L polymorphism (−550), X/Y polymorphism (−221), P/Q polymorphisms (+4) (Garred et al. 2006; Swale et al. 2014).

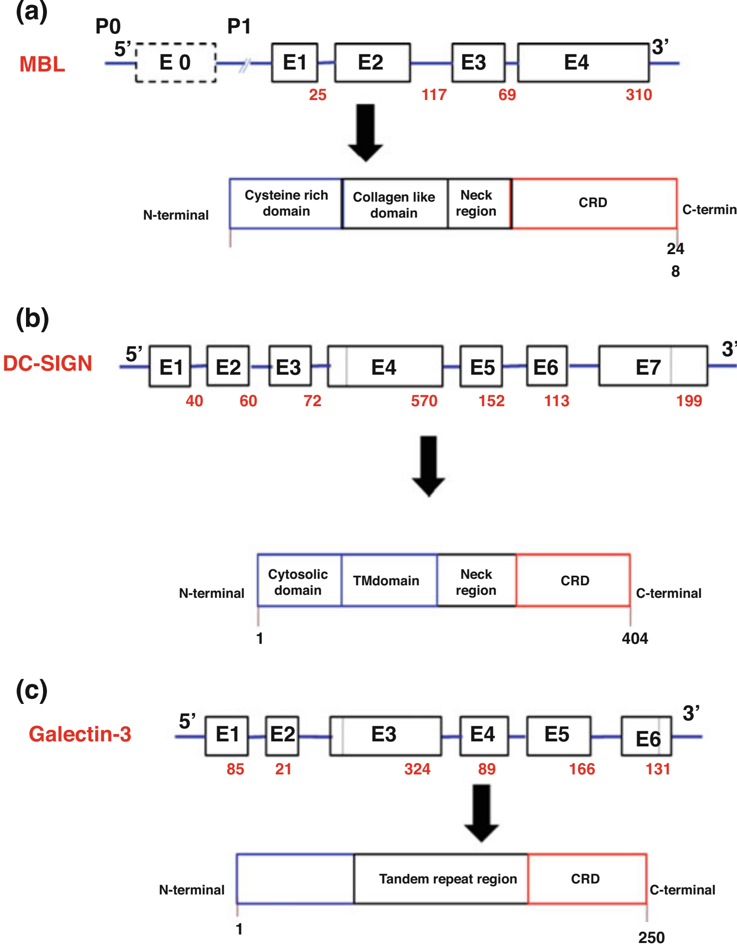


Fig. 2.8 pre-mRNA structure and translated protein domain of (**a**) MBL, showing two promoters P0 and P1; (**b**) DC-SIGN; (**c**) galectin-3, E-exon

2.7.2 DC-Sign

The transmembrane C-type lectin is grouped into two types, type I and type differ in its orientation of the N-terminal end relative to the inside or outside of the cell, respectively. The identified C-type lectins have single carbohydrate-binding domains at the extracellular carboxyl terminus of type II transmembrane C-type lectins (Figdor et al. 2002). Chromosomes 12p12.3-p13.2 and 19p13.3 contain the majority of the genes to code for type II C-type lectins (Soilleux et al. 2000; Liu et al. 2004; Trowsdale et al. 2001).

DC-SIGN has been expressed by dendritic cells and by some subsets of macrophages and DC-SIGNR has been expressed by human sinusoids of liver and lymph node and also by endothelial cells of placenta. In humans and mice, genes labeled "DC-SIGN" are not unique orthologues, despite the fact that they have similar functions and are expressed on dendritic cells (Liu et al. 2004). DC-SIGN, DC-SIGNR or L-SIGN and CD209L2 have been coded by CD209 family of genes. Chromosome 19p13.3 contains these homologous genes (Ortiz et al. 2008).

The DC-SIGN protein consists of a short cytoplasmic domain at N-terminus, a transmembrane region, a stalk domain and extracellular ligand-binding CTLD at C-terminal end for ligand binding. It is coded by mRNA transcript carries 7 exons and 6 introns (Fig. 2.8b).

- Exon 1–2 encodes N-terminal cytoplasmic domain.
- Exon 3 encodes transmembrane region.
- Exon 4 encodes a neck region repeats.
- Exon 5–7 encodes C-terminal extracellular ligand-binding CTLD.

The most conserved feature of C-type lectins is the presence of six cysteines in the domain of external ligand binding (Sattler et al. 2012). The stalk domain, commonly referred to as the neck region, contains similar numbers and lengths of exons among the various types of transmembrane receptors. This provides a linkage between the transmembrane and ligand-binding domain. This neck region is highly conserved and contains a long exon (570 bp) with seven repeats of 23 amino acids in sequence (Liu et al. 2015).

DC-SIGN promoter polymorphisms, SNPs (−139, −871, −939) have been found to be associated with hepatitis C Virus susceptibility in MSM (men who have sex with men) (Steba et al. 2018). The mutations in promoter region alter the binding ability of transcription factors, hence loss or reduction in transcription activity. Similarly, alternative splicing generate different isoforms of type II surface lectin family, including CD23, dectin-1, DC-SIGNR. For instance, exon 3 has been deleted by alternative splicing, which results in lacking of the transmembrane domain, hence the membrane bound receptor has been converted into soluble C-type lectin receptor. Study shows that repeat region of DC-SIGNR (CD209L) polymorphisms and alternative splicing mechanisms are associated with HIV-1 susceptibility (Liu and Zhu 2005). To conclude, increase frequency of both structural mutations and the promoter polymorphisms of lectin gene may lead to loss of functional lectin or failure in synthesis, respectively.

2.7.3 Galectin

In mammals, the LGALS genes encode 15 galectins. These genes are numbered sequentially. Galectin is widely expressed in human cancers. The gene transcript of galectins differs in splicing and generates different isoforms. For example, human galectin-8 forms at least seven different mRNAs through alternate splicing. These

isoforms may be differently expressed in different tissues (Varki et al. 2015). However, it is clear that exons of galectin transcript will undergo translation to give a specific structural and functional domain. According to structural features, mammalian galectins are classified into three types, namely prototype, chimera, and tandem repeats.

Galectin-3 pre-m RNA is composed of six exons and five introns (Fig. 2.8c).

- Exon 1 has two transcription initiation sites located at 52 and 50 nucleotides at upstream.
- Exon 2 possess translation start site.
- Exon 3 and 4 has ribonucleoprotein-like N-terminal domain, containing the proline–glycine–alanine–tyrosine (PGAY) repeat motif.
- Exon 5 encodes carbohydrate recognition sequence.

2.8 Regulation of Gene Expression

A major human lectin gene expression is found in the activated macrophages, peripheral blood leukocytes, dendritic cells, epithelial cells, endothelial cells, and organs like liver, lung, brain, pancreas, spleen, bone marrow, stomach, colon, kidney, and placenta (Gabius 1997).

The expression of eukaryotic lectin genes is important during pathophysiological responses, cellular differentiation, or stress response. Increased expression of most of the lectins involved in immunity has been found during infection and inflammatory response. Lectin gene expression is upregulated by various proinflammatory cytokines.

A range of cytokines and hormones have been shown to induce MBL2 gene expression, including IL-6, thyroid hormones, growth hormone, progesterone, and peroxisome proliferator-activated receptor α and γ. MBL2 mRNA has been found in organs like small intestine, testis, ovary, vagina, bone marrow. The MBL2 gene is overexpressed in ovarian cancer, intestinal biopsies from coeliacs, and gastric biopsies from chronic gastritis patients (Barnum and Schein 2017).

Similarly, galectin-9 gene has been induced by cytokines like interferon-γ and interleukin-4 (Imaizumi et al. 2002). They have been found upregulated during following infections hepatitis C virus, HIV-1, dengue virus (Mengshol et al. 2010; Tandon et al. 2014; Hsu et al. 2015). Galectin-1 and galectin-3 genes are induced by glucocorticoids (steroids), retinoids, and other factors like tumor promoters (phorbol esters), transcription factors (AP-1, AP-2).

Galectin functions have been studied using genetically engineered "knockout" mice. The genetically engineered "knockout" mice lacking galectins 1 and 3 did not exhibit any phenotypic abnormalities. As a result, several galectins are expressed differently in tumor cells than in normal cells (Perillo et al. 1998). However, it is found that the human LGALS3 (galectin 3) promoters have been constitutively expressed by Sp1 transcription factor, hence LGALS3 called housekeeping genes

(Kadrofske et al. 1998) In addition, promoter methylation seems to be an important mechanism regulating expression of galectin-1 (Cooper 2002).

2.9 Conclusion

Lectins are carbohydrates-binding proteins that are constitutively synthesized by liver and epithelial cells in the intestines and lungs. However, activated macrophages, dendritic cells, and neutrophils also express lectins during an inflammatory response. Numerous cancerous cells carry the gene for lectin synthesis, indicating its importance for apoptosis in addition to immune response and cell–cell communication. The mannose-binding lectins and DC-SIGN are synthesized in the endoplasmic reticulum by using signal peptide, whereas the galectin lectin lacks signal peptide and is made by non-classical pathways and unconventional transport. During post-translational modification, monomer lectins are oligomerized to form an active lectin with disulfide bonds. The active oligomeric lectin binds to multivalent glycan ligands on target cell surfaces. The synthesis of lectin has been induced by various cytokines and hormones during infectious conditions and cancer cell development. The expression of functionally active lectin depends on factors like promoter activation, mRNA stability, and post-translational modification. Lectin genes and their polymorphisms contribute to disease resistance. To conclude, increased frequency of both structural mutations and the promoter polymorphisms of lectin gene may lead to loss of functional lectin or failure in synthesis, respectively. Hence, the researchers focus on developing drugs and vaccines for dreadful infectious diseases by targeting lectin biosynthesis. This chapter may provide insight into the gene that is responsible for synthesis and mRNA level during induced expression of lectin genes.

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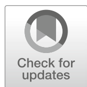
Chapter 3
Classification of Lectins



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Abstract Recent progress in biochemistry and structural biology has identified enormous proteins which specifically bind with carbohydrates and mediate various significant physiological processes. Such proteins termed as lectins can be classified into various categories based on their molecular structure, carbohydrate recognition domain, binding specificity, source of synthesis, and evolutionary relationship. Lectins are widely present in plants, animals, and microbial organisms and are classified into several types based on their evolutionary and structural similarity. Based on their molecular and structural aspects lectins are classified into simple, mosaic, and macromolecular complexes. Also mashed on the carbohydrate recognition domain, lectins could be classified into hololectins, merolectins, chimera lectins, and superlectins. All these categories of lectins can be correlated with their specificity towards glycoconjugate moieties of which the most specific ones being mannose, galactose/N-acetylgalactosamine, fucose, and sialic acid specific lectins. This classification enables successful identification and characterization of novel lectins with significant biological applications.

Keywords Carbohydrate-binding protein · Carbohydrate recognition domain · Legume · Galectins · C-lectins · Haemagglutinin

Abbreviations

ASGPR	Asialoglycoprotein receptor
CBD	Carbohydrate-binding domain
CD-MPR	Cation-dependent mannose 6-phosphate receptor
ConA	Concanavalin A
CRD	Carbohydrate recognition domain

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CRP	C-reactive protein
CTLD	C-type lectin-like domains
ER	Endoplasmic reticulum
ERGIC-53	ER-Golgi intermediate compartment 53 kDa protein.
ERGL	ERGIC-53-like
FP	Pentraxin family
FTLD	Fucose recognition lectin domain
Gal	Galactose
GalNac	N-acetylgalactosamine
GIT	Gastrointestinal tract
IGF-II/MPR	Insulin-like growth factor II/mannose 6-phosphate receptor
LacNac	N-Acetyllactosamine
Man	Mannose
M6P	Mannose 6 phosphate
MMBL	Monocot mannose-binding lectin
MPR	Mannose 6-phosphate receptor
MRH	Mannose 6-phosphate receptor homology
Neu5Ac	N-glycolylneuraminic acid
PTX3	Long pentraxin 3
RCA	*Ricinus communis* agglutinin
RIP	Ribosome-inactivating protein
SAP	Serum amyloid protein
SBA	Soybean
WGA	Wheat germ agglutinin

3.1 Introduction

Lectins are a type of glycoprotein abundant in nature that binds to monosaccharide and oligosaccharide residues with specific structure and configuration. In general, all lectin molecules possess two or more carbohydrate-binding sites, which is required for them to agglutinate cells or glycoconjugates (Goldstein and Hayes 1978). Lectins are a structurally diverse class of proteins distinguished by their ability to specifically bind carbohydrate moieties of cell surface glycoproteins. Lectin can be obtained from plants, microbials, or animals and can be soluble or adherent to membranes. In 1888, the characteristic feature of castor bean seed (*Ricinus communis* L.) extract to agglutinate animal red blood cells led to the discovery of lectins primarily in plants. Following that, similar "agglutinins" were discovered in the seeds of several plants, most commonly in leguminous plants and were renamed lectins as they could differentiate human ABO blood types, significant for blood transfusions (Tsaneva and Van Damme 2020). The asialoglycoprotein receptor (ASGPR), discovered by Anatol Morell and Gilbert Ashwell in the late 1960s while analyzing the transition of a serum glycoprotein called ceruloplasmin, was the first animal lectin found (Hudgin

et al. 1974). Bacterial surface lectins were discovered in the 1970s by Nathan Sharon and colleagues. Along with their haemagglutinating action, the major role of microbial lectins is to provide adhesion to host cells, which is essential for colonization and pathogenicity (Sharon and Lis 2004).

Lectins attach to simple or complex carbohydrate conjugates in a reversible and noncovalent manner, whether free in solution or on cell surfaces. The glycoconjugate-containing surface will only function as a lectin receptor. The specificity of lectins is typically determined using a hapten inhibition test, in which different sugars are evaluated for their ability to block erythrocyte hemagglutination. All lectin molecules have two or more carbohydrate-binding sites, which is required for them to agglutinate cells or react with complex carbohydrates. These molecules will bind hydrophobically, although electrostatic forces are rarely involved. Lectins have been isolated from crude aqueous solutions or saline buffers of diverse tissues using conventional protein extraction techniques. These include the utilization of ammonium sulfate or ethanol precipitation, as well as affinity chromatography (Helliwell 1998). The structural diversity aided upon by their specific carbohydrate-binding properties provides them with a wide range of biological functions in plants, animals, and microbes. Numerous investigations have been done to demonstrate such biological effects of lectins. One such demonstration is the in vitro analysis of the action of lectins on lymphocyte mitogenesis, both activation and inhibition, with lymphocytes from the gastrointestinal tract (GIT) being the most sensitive. It was also found that lectins can agglutinate immunoglobulins, activate the alternative complement system, limit fungal development, and stimulate histamine release from immune cells.

Lectins are resistant to both heat (at 70 °C for more than 30 min) and digestion. Some lectins are extremely resistant to gastrointestinal acids and enzymes (Shah and Rocca 2004). Also, lectins are essential components of biological processes such as cell signal transduction, cell–cell communication, and host–pathogen recognition. Host cell receptors which attach to pathogens are frequently glycan-recognizing complexes like lectins, and the interactions between such lectins and carbohydrate epitopes on the pathogens initiate infection. Hence identification and classification of diverse types of lectins that aid in these processes are therefore critical for effective development of diagnosis and treatments. For example, lectins can act as potential biomarkers in cancer tissues, assisting in the early diagnosis of cancer (Wu et al. 2012). Lectins can be classified into various groups based on their molecular structure, carbohydrate specificity, variance in carbohydrate-binding domain, and their localization within different forms of life including plants, animals, and microbes which imparts their specific biological functions. Lectins are often categorized using carbohydrates as the sensor, with the identification principle relying on the strength of interactions between a carbohydrate ligand and its binding lectin. Lectins are generally classified into five categories based on their affinity towards the monosaccharide substrates such as mannose, galactose/N-acetylgalactosamine, fucose, N-acetylglucosamine, and N-acetylneuraminic acid. The abundance of such monosaccharides as components of typical glycans seen on the surface of eukaryotic cells in nature supports the relevant biological activities of lectins.

Hundreds of lectins have been identified and categorized using affinity chromatography and recombinant DNA methods on immobilized carbohydrates (Hamid et al. 2013).

The characterization of the amino acid sequences of dozens of lectins, along with the identification of around 30 3D structures, has permitted a classification based on shared molecular and structural characteristics. On that basis, the majority of lectins are classified into three types: (1) simple, (2) mosaic (or multidomain), and (3) macromolecular complexes (Van Damme et al. 1998). Lectin proteins possess at least one carbohydrate-binding domain which involves in specific glycoconjugate binding and on that basis, lectins could also be classified as merolectins (single carbohydrate-binding domain—monovalent), hololectins (at least two binding domain that binds structurally same sugar), chimerolectins (one or more C-binding domain with a specific enzymatic activity), and superlectins (binds structurally unrelated sugars) (Damme et al. 1998). In the aspect of species classification aided by structural and binding characteristics, the vast majority of known plant lectins may be divided into seven structurally and evolutionarily associated protein families such as the amaranthin family (amaranthins), the chitin-binding enzymes lectins with the vein domains, phloem lectins from Cucurbitaceae, jacalin-related lectins, legume lectins, monocot mannose-binding lectins (MMBL), and ribosome-inactivating proteins of type 2 (type 2 RIP) (Peumans et al. 2001). Animal lectins are classified into several families, depending on their diverse cellular localization and the binding specificities of their carbohydrate recognition domain (CRD) modules. Earlier characterization of animal lectins is classified them into two principal structural families, the C-type (Ca^{2+} dependent binding) and S-type-galectins (sulfhydryl dependent binding) lectins. The C-type lectin family has become a highly significant group, wherein about 17 classes of proteins have been identified with their structural and genomic analysis (Drickamer and Fadden 2002). In case of microbes, the lectin interactions are utilized to identify bacteria, fungi, and protozoa. Bacterial lectins are similar to carbohydrate-binding characteristics and relative stability of plant lectins (Chesterton 1987). The first lectins on the bacterial surface were reported in the 1970s by Nathan Sharon and his colleagues. The main function of these lectins in microbes is to promote the adhesion or adherence of the bacteria to the host cells. Bacterial lectins are hence commonly referred to as adhesions, which attach corresponding glycan receptors on the host cell surface via carbohydrate recognition domains (CRDs). The ability of these microbial lectins to aggregate or cause the hemagglutination of red blood cells was the basis for their discovery and is termed haemagglutinin. Bacteria may also synthesize soluble toxins, which rely on glycoconjugate-binding subunits to interact with membrane glycoconjugates and transport the functionally active toxic component through the membrane. Many microbial agglutinins, adhesins, and toxins have been identified, cloned, and characterized in the last 30 years (Nizet et al. 2015). The classification of these diverse lectins in terms of various aspects makes it easier to analyze over a wide range of potential applications dependent on their molecular and evolutionary characteristics.

3.2 Classification of Lectins Based on Molecular Structure

Lectins can be categorized into structurally and evolutionarily related protein families based on their amino acid sequence and molecular structure. And in that aspect, most, but not all, members of that particular lectin family are composed of monomers with a homologous basic structure and overall three-dimensional orientation. The basic structure of lectins is influenced not only by the structure of monomers, but also by the degree of polymerization and, in certain circumstances, the post-translational modification also matters (Damme et al. 1998). The three-dimensional topologies of known lectin structures vary widely. The simultaneous functions of subunit location and multivalency, however, provide a paradigm for understanding the structural basis of lectin–carbohydrate interactions (Rini 1995). The majority of lectins fall into one of the three categories: simple, mosaic or multidomain, and macromolecular complexes on the basis of their structure.

3.2.1 Simple Lectins

Simple lectins are composed of a limited number of subunits, all of which are not exactly identical and have a molecular weight of less than 40 k Da. A carbohydrate-binding site is present in each monomeric unit. Almost all known plant lectins and most members of the galectin family of animal lectins, a group of β-galactoside specific animal lectins, fall into this category. Major types of lectins classified under simple lectins include legume, cereal, Amaryllidaceae and related families, Moraceae, Euphorbiaceae, Galectins, and Pentraxins (Lis and Sharon 1998).

3.2.1.1 Legume

Leguminous lectins are a wide group of carbohydrate-binding proteins that are primarily found in legumes. The discovery of *Phaseolus vulgaris* (bean), *Lens culinaris* (lentil), *Vicia sativa* (vetch), *Pisum sativum* (pea) in legumes by Landsteiner and Raubitzchek proved that non-toxic lectins exist among the period with toxic lectins (Landsteiner and Raubitschek 1907). Seed lectins make up a large portion of legume lectins. Some are also present as vegetative tissues such as leaves and bark. Such seeds or vegetative tissues contain two or more distinct lectins. Legume lectins are composed of 30 kDa protomers generated from homologous primary translation components containing about 250 amino acid residues. Most legume lectin protomers are composed of a single polypeptide chain of around 250–300 amino acid residues. Legume lectins were essentially utilized in the fields of plant lectin biochemistry, physiology, and molecular biology research. Legume lectins have the ability to interact with both simple and complex carbohydrates. The carbohydrate-binding specificity of the various lectins in the legume lectin family is extremely

diverse. Hence, legume lectins cover a far larger spectrum of binding specificities than any other lectin family (Young and Oomen 1992). Concanavalin A was the first plant lectin to be isolated, crystallized, and subjected to X-ray diffraction analysis. The soybean seed lectin was the first plant lectin to be cloned, which is a legume lectin (Hardman and Ainsworth 1972).

3.2.1.2 Amaranthin

The lectins known as "amaranthin" are discovered in the seeds of Amaranthus species. The family is called "amaranthin" after the substantive name of the first member of this family that was isolated from *Amaranthus caudatus* seeds. Other species with this lectin involve *A. spinosus, A. caudatus, A. leucocarpus, and A. cruentus*. Amaranthins are defined by their tiny protomers of size about 12–33 kDa, along with three-fold internal repeats built upon 36 amino acids, characterized by the lack of metal action, and poor affinity for the carbohydrate ligand (Chervenak and Toone 1995). Such agglutinin protomers are made up of two domains (the N- and C-domains) connected by a short helix (Transue et al. 1997). Amaranthin is often thought to be a GalNAc-specific lectin although it has a far greater affinity to the GaIB(1,3) GaINAc T-antigen disaccharide. This selectivity implies that amaranthins are intended to also encounter the common animal glycoconjugates (Rinderle et al. 1989). Recent research also proves the potential applications of amaranthin lectin for their antiproliferative activity through exerting a cytotoxic effect that would promote apoptosis (Quiroga et al. 2015).

3.2.1.3 Cereal–Wheat Germ Agglutinin

A variety of lectins can be found in high quantities in dietary staples, such as cereal grains. Lectin activity has been reported in wheat, rice, barley, oats, and corn but WGA is the cereal grain lectin that has received the most attention. Wheat germ agglutinin (WGA) is a lectin found in wheat germ that are of great significance (Cordain 1999). This lectin is a homodimer composed of monomeric subunits. Each protomeric unit of wheat germ agglutinin is composed of four structurally similar domains that share a high degree of amino acid sequence identity. Such domains possess four interconnecting disulfide linkages, within each, that enables a compact protein structure (Goldstein et al. 1997). WGA attaches to the sialic acid present mostly in humans, allowing it to cling to cell surfaces such as the gut epithelial layer (Shaw et al. 1991). The binding of WGA to Neu5Ac in the glycocalyx of human cells and pathogens that produce Neu5Ac further leads to cell invasion and perhaps disrupting immunological tolerance by eliciting pro-inflammatory immune stimuli (Varki 2009).

3.2.1.4 Moraceae–Jacalin

Jacalin lectins were identified, in fact, solely in *Artocarpus* and *Maclura pomifera* seeds and some vegetative tissues (de Azevedo Moreira and Ainouz 1981). On the basis of lectin specificity, they are distinguished into the tiny GalNAc-specific Moraceae lectins and the extended mannose-specific jacalin lectins. These lectins possess mannose-binding specificity and, however, are extensively spread among higher plants. Each Moraceae lectin galactose-specific has four identical, large α-chain and short β-chain protomers that consist of a single sugar-binding site. All known jacalin-related mannose lectins are made up of extremely identical protomers containing around 120–150 residues of amino acid (Sankaranarayanan et al. 1996). The significance of the finding of these novel lectins became obvious when it was discovered that these novel lectins also selectively bind GalB(1,3)GalNAc-residues. Jacalin was widely utilized as a potent immunological tool when its particular IgA-binding activity and mitogenicity were also reported (Skea et al. 1988).

3.2.1.5 Euphorbiaceae–Chitin-Binding Lectins

The chitin-binding lectins are a large and diverse family of proteins that include all proteins with at least one hevein domain (43-amino acid protein that contains a highly integrated chitin-binding site). Chitin-binding lectins with hevein domains are quite common in plants. For example, single hevein domain proteins were purified from *Hevea brasiliensis* (Euphorbiaceae) (Walujono et al. 1975). Other lectins of the family involve beans of the castor tree (*Ricinus communis*) that comprise two closely related lectins, ricin and *Ricinus communis* agglutinin, RCA. Ricin is a 60 kDa heterodimeric protein composed of two S-S linked chains, A and B. B chain possesses galactose-specific carbohydrate-binding sites whereas cytotoxic action is found in the A chain (Macholz 1988). By affinity chromatography on cross-linked arabinogalactan, a N-acetylgalactosamine-specific lectin was isolated from Euphorbia heterophylla seeds. Its distribution over the seed is normal in the regard that it is mostly limited to the main axes (Nsimba-Lubaki et al. 1983).

3.2.1.6 Galectins

Galectins or S-type lectins are soluble β-galactosidase binding lectins that bind to β-galactosidase independent of $Ca^{2+.}$ It functions based on conserved amino acid residues that are similar to those found in the carbohydrate-binding domain (CRD) (Barondes et al. 1994). These proteins were formerly known as S-type proteins because they required sulfhydryl groups, but were eventually substituted by galectins after site-directed mutagenesis that revealed the existence of some soluble protein groups without sulfhydryl groups (Hirabayashi 1996). About 15 galectins have been characterized by primary structural research analysis up to date, which

vary also in their cellular position, binding affinities, carbohydrate-binding domain, and expression. Further galectins have been classified into three groups such as proto galectins, chimera galectins, and tandem repeat type galectins (Hirabayashi and Kasai 1993). Galectins of the prototype, such as galectin-1,2,7,10,13,14 are characterized by only one CRD that exists as dimers. Whereas the chimera galectin which includes galectin-3 possesses a CRD region at COOH terminal and a non-CRD region at NH2 terminal. Galectins of the tandem repeat type, such as galectin-4,8,9,12, have two CRDs bound by a short linker peptide. Galectin-1 seems to have a strong affinity for complex-type N-glycans, while galectin-3 has a strong affinity for the LacNAc repeats (Nio-Kobayashi 2017).

3.2.1.7 Pentraxins

The pentraxins are a group of simple plasma proteins that play a role in invertebrate and vertebrate innate immunity. They have L-type lectin structures and glycoconjugate ligand binding requires Ca^{2+} ions. C-reactive protein (CRP), serum amyloid protein (SAP), and female protein are three of the most important members of the pentraxin family (FP). Pentraxins are composed of five identical noncovalently bound subunits arrayed in a circular pentameric disc structure. Pentraxins are classified into two categories based on the main structure of the subunit: short pentraxins and long pentraxins (Gupta 2012a). The pentraxins (C-reactive protein, CRP; serum-amyloid P component, SAP; long pentraxin 3, PTX3) are effectively involved in complement activation and amplification via association with other complement factors (Ma and Garred 2018).

3.2.2 Mosaic Lectin

Mosaic lectins are multidomain lectins that possess a wide range of molecular weights and are made up of multiple diverse protein modules or domains, only one of which has a carbohydrate-binding domain (CBD). Virus haemagglutinins and some of the animal lectins such as C-, P-, and I-types are the lectins that come under mosaic lectins. These diverse characteristics impart diverse functionality in their applications.

3.2.2.1 Viral Haemagglutinin

Viruses express a huge range of glycan-binding proteins, which resembles lectins. Initially many of the microbial lectins were recognized based on their capacity to aggregate or cause red blood cell hemagglutination (erythrocytes). Alfred Gottschalk in 1950 reported the first microbial haemagglutinin discovered from the influenza virus that binds erythrocytes and other cells was linked via the sialic acid component

of the host cellular glycoconjugates. This binding promotes the infection of the host and hence contributes to the viral pathogenicity (Wiley and Skehel 1987). Viral haemagglutinin comprises two polypeptides, HA1 and HA2, each of molecular mass 36 kDa and 26 kDa, respectively, which is covalently connected by a single disulfide bond (Nizet et al. 2015). Other such haemagglutinin is found in a non-enveloped, icosahedral symmetrical murine polyoma virus, which possesses a circular, double-stranded DNA genome. The capsid of the virion possesses around 360 copies of the viral protein VP1 (with two antiparallel β sheets) of 42 kDa positioned as pentamers (Stehle and Harrison 1997).

3.2.2.2 C-Lectin

C-type lectins are those which possess a carbohydrate recognition domain (CRD) that links sugars by binding to Ca^{2+} in most cases, making the sugar-binding activity Ca^{2+}-dependent (Weis et al. 1998). As a greater number of proteins were identified, it became evident that not every protein with C-type CRDs would bind glycans and Ca^{2+}. To address the discrepancy, the term “C-type lectin-like domains” (CTLD) was coined for such domains. CRD is often used to refer to the short amino-acid motifs found in CTLDs that interact specifically with Ca^{2+} and carbohydrates (Rivkin et al. 2000). C-Type lectin family has become a highly significant group, wherein about 17 classes of proteins have been identified with their structural and genomic analysis. It is also worth noting that the overall layout of a lectin is dependent on how a CRD interacts with other domains, reflecting the multivalent binding of lectins (Drickamer and Taylor 2015). C-type lectin involves various endocytic receptors, collectins, and selectins which have diverse glycoconjugate specificity. Selectins are a Ca^{2+} dependent receptor family that has been discovered to mediate important cell–cell interactions in a variety of processes such as leukocyte trafficking, inflammation, thrombosis, tissue injury, etc. (Rosen and Bertozzi 1994). Collectins are soluble oligomeric proteins with carboxylic terminal upholding the carbohydrate recognition domain (CRD) and a collagen-like domain with a short cysteine-rich N-terminus which together aids in effective functioning (Drickamer et al. 1986).

3.2.2.3 P-Lectin

The carbohydrate recognition domain (CRD) of these proteins has a high affinity for mannose 6-phosphate, therefore, the name “P-type” lectin family (M6P). The cation-dependent mannose 6-phosphate receptor (CD-MPR) and the insulin-like growth factor II/mannose 6-phosphate receptor (IGF-II/MPR) are two molecules that differentiate the P-type lectin family from others by their capacity to recognize phosphorylated mannose residues (Dahms 2002). Mannose 6-phosphate receptors

(MPRs) are the most used term for them. MPRs serve an important role in the targeting of lysosomal enzymes in vertebrates. On binding affinity analysis, it was found that a single MRH domain in CD-MPR exists as dimers which bind with diverse glycans. Such binding of diverse ligand molecules even non-glycans makes P-Lectins a highly efficient protein involved in various physiological functions such as cell signaling, as biomarkers, etc. (Munro 2001).

3.2.2.4 I-Lectin

I-type lectins are glycan-binding proteins that belong to the immunoglobulin (Ig) superfamily and are classified according to the conserved amino acid residues in the CRD region (Powell and Varki 1995). With their conserved CRD domain I-type lectins mostly bind sialic acid on the cell surface and are termed "Siglecs" which is the most characterized I-lectin. Siglecs consist of an N-terminal variable-set Ig domain with a sialic acid binding site, followed by a constant region Ig domain (Crocker and Varki 2001) and also a conserved arginine residue on the F-strand on V-region is a criterion for ligand binding. In humans, 11 main Siglecs and one Siglec-like molecule have been identified. The CD33-related Siglecs have 4 C-set domains and cytoplasmic tyrosine-residues that are implicated in signaling and endocytosis. It was also demonstrated that Siglec binding specificity may be used to create cell-based glycan arrays that could be beneficial in therapeutic targeting against autoimmune disorders and cancer (Crocker 2002).

3.2.3 Macromolecular Complex

Macromolecular assembly of proteins involves lectin organization with multivalent binding and thus imparts significant functions. Bacteria possess a lot of macromolecular assemblies of lectins that are filamentous organelles made up of helical subunits (pilins) and are assembled in a certain sequence (Ofek and Doyle 1994a). These proteins aid in bacterial adhesion followed by invasion and infection. These lectins are heteropolymeric filamentous organelles. The majority of the lectin is composed of a structural polymer and only a small portion contains the carbohydrate-binding site (Ting et al. 2010). Furthermore, along with their large size and complexity, polysaccharide-lectin complexes may be used as model systems to study inter-polysaccharide and protein-polysaccharide interactions. The macromolecular assemblies of complex polysaccharides with galectin-3, a major lectin, and their synergistic effects on function were described in a study (Zhang et al. 2017).

3.3 Classification of Lectins Based on Glycoconjugate Specificity

Lectins are a type of protein that binds carbohydrates in a specific (Table 3.1) and reversible manner. The wide array of applications they perform is in turn the effect of this specific monosaccharide binding and this varies upon different types of lectins (Sharon and Lis 2007). Lectins can be categorized into different groups based on the monosaccharide for which they have the specificity to bind including mannose, galactose/N-acetylgalactosamine, fucose, and sialic acid specific residues. These monosaccharides are typical glycan components found on the surfaces of eukaryotic cells. Lectins with the same glycoconjugate specificity show varied affinity for oligosaccharides and can only show affinity for oligosaccharide derivatives corresponding to monosaccharides (Sharon and Lis 2013).

3.3.1 Mannose-Specific Lectins

Mannose-specific lectins are extensively distributed throughout higher plants, algae, and fungi and are thought to have a role in the detection of microorganisms and plant predators through their high mannose glycans (Barre et al. 2019). The mannose-binding specificity of lectins is mediated by different structural scaffolds, according to structural analysis. These lectins are made up of several structural scaffold components that contain one or more carbohydrate-binding sites and are important in the recognition of mannose-containing glycans. The mannose-binding site possesses a small carbohydrate-binding region responsible for wider sugar-binding specificity towards mannose molecule, surrounded by a larger binding area that is responsible for the specific recognition of larger mannose-containing N-glycan chains (Barre et al. 2001). In animals, pathogenic species and potentially toxic glycoconjugates are cleared by the macrophage mannose receptor which resembles exogeneous type 1 transmembrane receptor proteins. These receptors are efficient proteins that clear glycoconjugates with terminal mannose residues by selectively binding to them. Mannose-specific binding and internalization lead to lysosomal

Table 3.1 Various lectins and their specific sugar residues

Sugar residue	Lectin	References
Mannose/glucose	ConA Macrophage mannose receptor	Ramkumar et al. (2002)
N-acetyl D-glucosamine	Wheat germ agglutinin Endocytic receptors	Levy (1979)
Fucose	Ulex europaeus agglutinin 1 F-box lectin	Chetty et al. (2016)
Sialic acid	Wheat germ agglutinin	Ryva et al. (2019)
N-acetyl D-galactosamine	Jacalin, Amaranthaceae	Zeng et al. (2019)

destruction, resulting in the elimination of foreign pathogens by the innate immune system (Stahl 1990).

3.3.2 Galactose/N-acetylgalactosamine Specific Lectins

Many C-type animal lectins recognize galactose- or N-acetylgalactosamine oligosaccharides. The best-known Gal-binding C-type lectins are the mammalian hepatocyte asialoglycoprotein receptors, which play a role in serum glycoprotein homeostasis. C-type lectins with a high affinity for glycoconjugates containing terminal galactose and N-acetylgalactosamine residues have also been discovered on the surfaces of macrophages and Kupffer cells and seem to mediate tumor cell recognition. N-acetyl-D-galactosamine lectins are significant in determining sugar moiety in blood group in animals (Kolatkar and Weis 1996). Liener and Pallansch were the first to purify soybean lectin (SBA) specific to galactose and N-acetyl-D-galactosamine in plants. Later, Sharon and colleagues isolated the same lectin using affinity chromatography with a column of 6-aminocaproyl-D-galactosylamine linked to Sepharose. Later various plant lectins such as red kidney bean lectin, horseshoe lectin, Concanavalin A legume lectin were identified with galactose and N-glycan specificity (Yosizawa and Miki 1963).

3.3.3 Fucose Specific Lectins

F-type lectins are fucose-binding proteins that are found in a wide range of taxonomic groups, from viruses, plants, and vertebrates. They possess a fucose recognition lectin domain (FTLD) with a novel fold termed F-type fold consisting of a barrel structure with specific fucose- and calcium-binding motif. Although FTLs can have a single FTLD, which is often coupled with more than one diverse domain in single polypeptide, members of this lectin family can also have a variable number of tandemly distributed FTLDs which mediate the process of binding (Vasta et al. 2017).

3.3.4 Sialic Acid Specific Lectins

Sialic acid binding lectins are those which bind to sialic acids selectively and have the potential to be beneficial in the detection, purification, quantification, and characterization of numerous biomolecules containing sialic acids residues, such as glycoconjugates, gangliosides, and polysaccharides. Such lectins can be utilized as particular probes for specific sialic acid derivatives that act as molecular markers in enormous physiological and biochemical processes (Schauer 1983). Limulin is a

significant sialic acid binding lectin isolated from the American horseshoe crab Limulus polyphemus. Agglutinins that bind to sialic acid are also found in the Orthomyxoviridae viral family, which includes influenza viruses, Papoviridae, Reoviridae, and Adenoviridae (Weis et al. 1988).

3.4 Classification Based on Source (Plants, Animal, Microbes)

3.4.1 Plant Lectins

Plants contain lectins that bind specifically to mono- or oligosaccharides with particular properties. These carbohydrate-binding plant proteins, also known as lectins, agglutinins, or haemagglutinins, are a large collection of proteins with diverse applications. Approximately 500 distinct plant lectins have been identified and described in some detail, according to recent research analysis (Van Damme et al. 1998). All these lectins exhibit significant applications on the basis of their specific carbohydrate-binding through carbohydrate recognition domain. Plant lectins are classified into merolectins, hololectins, chimerolectins, and superlectins (Table 3.2) based on their carbohydrate recognition domain (Fig 3.1). Merolectins are proteins that are made up entirely of a single carbohydrate-binding domain, e.g., small chitin-binding lectins. Merolectins are monovalent by definition and so cannot precipitate glycoconjugates or cause agglutination of cells.

Hololectins are made up entirely of carbohydrate into a special category called superlectins. They are made up of at least two carbohydrate-binding domains that are not identical or comparable, but recognize structure binding domains, although at least two of them are identical or highly similar. Hololectins can agglutinate because they are divalent or multivalent (Damme et al. 1998).

Plant lectins are also classified into families based on some shared features as legume lectins, type II ribosome-inactivating proteins, monocot mannose-binding lectins, and other lectins (Lam and Ng 2011). The most well-known kind of lectin is legume lectin. Leguminous plants' seeds have a greater lectin content than their bark, leaves, roots, and stems. The lectins of the Gramineae (cereals, such as wheat germ)

Table 3.2 Plant lectins classification

Carbohydrate-binding site	Structure and evolution	Ligand specificity
1. Merolectins	1. Amaranthin	1. Glucose
2. Hololectins	2. Chitin-binding lectin	2. Galactose
3. Chimera lectins	3. Cucurbitaceae lectin	3. N-acetyl D-glucosamine
4. Superolectins	4. Jacalin	4. Fucose
	5. Legume lectin	5. Sialic acid
	6. Mannose-binding lectin	6. N-acetyl D-galactosamine
	7. Type 2 ribose inactivating lectin	

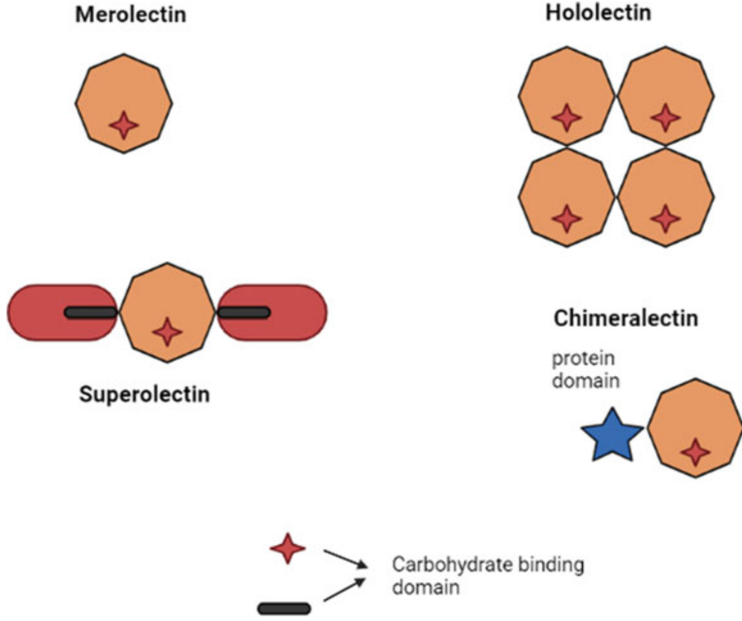

Fig. 3.1 Classification of plant lectins based on carbohydrate-binding domain. (Created with biorender.com)

and Solanaceae plant families have also been discovered (potatoes and tomatoes). Monocot-binding lectins are made up of 1, 2, 3, or 4 12 kDa subunits with a specific affinity for mannose, whereas chitin-binding lectins are built up of hevein domains (Damme et al. 1998). Type 2 ribosome-inactivating proteins are chimerolectins composed of a polynucleotide: adenosine glycosidase domain (also known as the A chain) and a carbohydrate-binding domain positioned in parallel (the so-called B chain). Both chains are produced on the same precursor molecule, which is subsequently processed post-translationally by eliminating a linker between the A and B chains (Barbieri et al. 1993). Major plant lectins include concanavalin A, wheat germ agglutinin, soybean agglutinin, Limba bean, wax bean agglutinin, and Red bean agglutinin.

3.4.2 Animal Lectins

The first animal lectin discovered was the asialoglycoprotein receptor in mammalian cells, which was useful in determining how animal lectins differ in glycoconjugate binding. Depending on their cellular location and the binding specificities of their carbohydrate recognition domain (CRD) modules, animal lectins are divided into numerous groups (Fig 3.2). Animal lectins were formerly divided into two structural

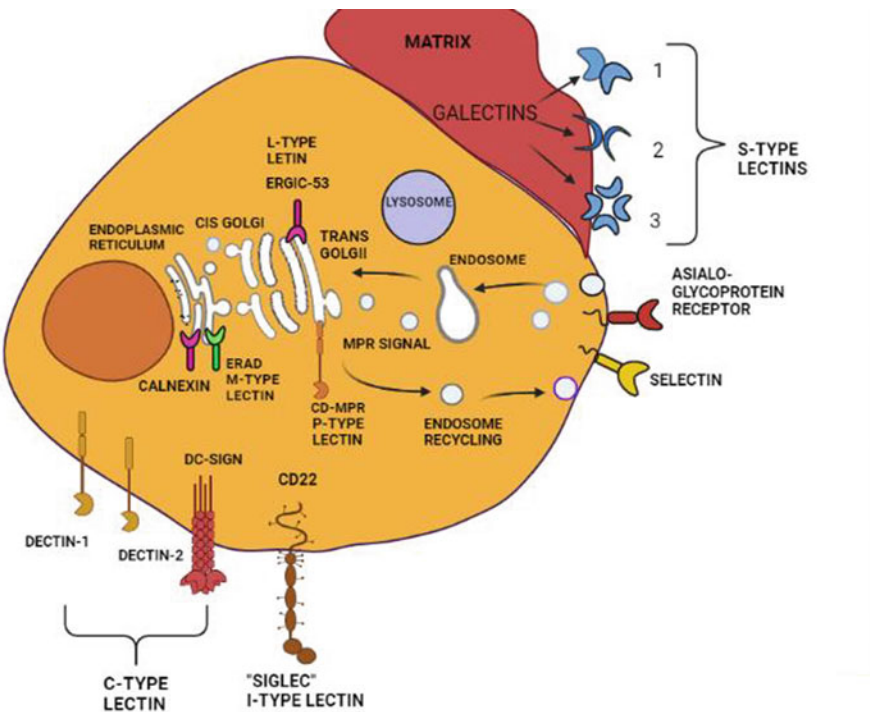

Fig. 3.2 Different types of animal lectins and their cellular location (Created with biorender.com)

families: C-type (Ca^{2+} dependent binding) and S-type-galectins (sulfhydryl dependent binding) (Drickamer 1988). The most important animal lectins, such as endocytic receptors, mannose receptors, selectins, and collectins, belong to the C-type lectin family.

Recent research has identified more than 100 animal lectins and classified them into different families based on the complexity of carbohydrate ligands, metabolic processes they perform, expression levels, and their reliance on divalent cations. These families include calnexin, F-lectin, intelectin, chitinase like lectin, F-box lectin (Table 3.3), and others (Cummings and McEver 2009). C-type lectins are Ca^{2+}-dependent lectins that are found in the extracellular matrix, serum, and membrane and have a conservative domain known as the carbohydrate recognition domain. The distinctive feature of the carbohydrate recognition domain is the direct interaction of Ca^{2+} with the bound sugar via coordination bonds (CRD) (Drickamer 1993).

C-type lectin includes endocytic receptors such as asialoglycoprotein receptors, macrophage mannose receptors, natural killer cell receptors, kupffer cell receptors along with other molecules such as collectin and selectin (cell adhesion molecules) (Cummings and McEver 2009). Another type of animal lectin is S-type lectins that are soluble β-galactosidase binding lectins which bind glycoconjugate through a

Table 3.3 Classification of animal lectins and their ligands

Lectin family	Ligand	References
C-lectin – Asialoglycoprotein receptor – Macrophage mannose Receptor – Natural killer cell receptor – Kupffer cell receptor – Selectins – Collectins	Gal, GalNac, mannose, heparin, fucose glycan, GalNAc, β-Fuc-containing glycans, fucose, sialic acid, sulfated glycans, Man, Fuc, GlcNAc	Cummings and McEver (2009)
S-lectin/galectin	β-Galactosidase	Wada and Kanwar (1997)
M-lectin	Mannose	Tong and Kornfeld (1989)
P-lectin	M6P	Gupta (2012b)
L-lectin	Various	Etzler et al. (2009)
I-lectin	Sialic acid	Bertok et al. (2013)
R-lectin	Diverse lectins	Gupta and Gupta (2012)
Other class of lectins Calnexin F-box lectin Intelectin F-lectin Chitinase like lectin	Glc Man9, Glc Nac2, Gal, Galactofuranose, pentoses, fucose ending glycans, chito-oligosaccharides	Matsuo et al. (2003), Gupta (2012c), Khalil (2015), Vasta et al. (2012), Fusetti et al. (2002)

Ca^{2+} independent manner. It functions based on the conservation of a group of amino acid residues that resemble the characteristics of carbohydrate-binding domain (CRD) (Barondes et al. 1994). L-type lectins are proteins discovered first in the seeds of leguminous plants. For example, ERGL has been identified, where it lacks some basic residues for glycan-binding but, like ERGIC, plays an important role in the secretion of various glycoproteins in specific tissues (Yerushalmi et al. 2001). The term "P-type" lectin family denotes the binding affinity of carbohydrate recognition domain (CRD) in these proteins towards mannose 6-phosphate (M6P). MPRs play a major role in targeting the lysosomal enzymes (Dahms 2002). Other significant lectins include calnexin, calreticulin, calmegin, and calreticulin 2 that serves as the prototype for a small group of ER-resident chaperone proteins (Thomsen et al. 2011), F-box lectins have been identified in the murine F-box protein Fbs1, which functions similarly to the carbohydrate recognition domain (CRD) (Yoshida et al. 2003), and Ficolins which are soluble oligomeric proteins composed of trimeric collagen-like domains that stimulate the complement system (Thomsen et al. 2011).

3.4.3 Microbial Lectins

Lectins derived from fungus, bacteria, protozoa, and viruses make up microbial lectins. Since the 1970s, only a few lectins have been identified nevertheless, the

relevance of microbial lectins has been recognized as a result of ongoing study in this subject, which has led to substantial investigations on lectins from microbes (Slifkin and Doyle 1990). Alfred Gottschalk was the first to discover a microbial lectin in the early 1950s. The influenza virus was used to isolate this lectin, which was shown to be of viral origin. In the 1970s, Sharon et al. were the first to investigate bacterial lectins (Wiederschain 2009). The major function of microbial lectins is to bind to host cells, which is required for infection to occur. Microbes benefit from lectins because they help them attach to the cell surface. Such interactions that aid in microbial growth must be critically analyzed to essentially prevent pathogenic infections and diseases in humans (Ofek and Doyle 1994b). Major microbial lectins include haemagglutinin, adhesins, and bacterial toxins. The influenza virus haemagglutinin, which binds to sialic acid–containing glycans, is a significant viral glycan-binding protein. The specificity of this interaction, like that of other glycan-binding proteins with their glycosyl ligands, is modest, because the haemagglutinin oligomerizes into trimers and the host cell has a large density of glycan receptors, the sensitivity for cell membranes rises (Rott et al. 1996). Bacterial lectins are mostly of fimbriae (hairs) or pili (threads), which are elongated, submicroscopic protein structures that bind with glycoconjugate receptors on the host cells. The mannose-specific fimbriae, the galactose-specific fimbriae, and the N-acetyl galactose-specific fimbriae are identified to be effective cell adhesion molecules (Ofek and Doyle 1994b). Other forms of microbial lectins include bacterial toxins. Bacteria produce lectins termed toxins to prevent other bacteria from colonizing, allowing them to gain an advantage in the struggle for resources and space. For example, A lectin called bacteriocin with two b-lectin domains produced by a gram-negative bacteria proteobacteria was reported to eliminate other bacteria through contact dependent inhibition (Ghequire et al. 2018). A variety of parasites, in addition to viruses and bacteria, employ glycans as adhesion receptors. *Entamoeba histolytica* produces a heterodimeric lectin that specifically binds to galactose/N-acetylgalactosamine residues on the host cell (Wiederschain 2009).

3.5 Conclusion

Carbohydrates are found on the surface of all living cells as a component of glycoconjugates and are involved in diverse physiological functions such as cellular communication, antimicrobial activity, mitogenesis, tumor biomarkers, therapeutic strategies, etc. Such characteristics of lectins make it an inquisitive area for researchers to analyze more lectins of natural origin with beneficial effects. Such inventions of lectins could be significant only if it is classified according to their characteristics such that they could be easily accessed for further analysis. As mentioned above lectins could be classified in several ways according to their source, glycoconjugate specificity, and binding patterns. Further recent research also focuses on the classification of lectins on the basis of evolutionary relationship and gene analysis using bioinformatics tools. One such report is the utilization of

Glyco Bioinformatics databases and tools that aid in classifying lectins upon their functional differences and versatility in addition to origin and specificity. For example, Unilectin3D, a portal dedicated to lectin 3D structures and lectin/glycan complexes was launched in 2018, with 1740 structures encompassing 428 distinct lectins and 765 references. UniLectin3D has been validated as the primary source of data on lectin 3D structures and their glycoconjugate interaction (Bonnardel et al. 2021).

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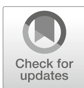
Chapter 4
Molecular Basis of Lectin–Carbohydrate Interaction



Yashika Pusam, Mohamed Jaabir, and Sivakamavalli Jeyachandran

Abstract The ability to bind with carbohydrate molecules has been considered as a significant characteristic of lectins, and lot of such investigations have been conducted from the past two decades. That information shows that it has unique interaction with the small molecules which are predominantly hydrophobic in nature. Also the recent reports show that surface lectins are easier to be expressed with many cells, which as a result are used as recognition molecules, by interacting with the carbohydrates that are present in the opposing cells. The lectins that are extracted from the various sources like plants, animals, and bacteria are responsible for the interaction of sugar onto the respective cell surface. In medicine, there is a lot of room for mediation of animal receptors and bacterial toxic proteins (toxins) mediated through various mechanisms such as pathogen neutralization, glycol-conjugate absorption, cell to cell contacts, and pathogen neutralization. Plant poisons and mitogens are some of the examples that are formed due to lectin–carbohydrate interaction. A recent example of this interaction between lectin and carbohydrate is during the blood clotting and inflammation, the cell adhesion molecules mediate the adhesion of leukocytes to carbohydrates that are present on the endothelium. The structural features of subsite and subunit multi-valency confer context-specific functional properties in some lectin types which are usually seen as X-ray crystal structures. Hence in this chapter we are discussing the interaction between lectin and carbohydrate along with an elaborated explanation of its physical and functional properties and its application in various fields such as drug delivery system, cancer biology, hydrogen bonding, metal coordination bonds, and so on.

Keywords Carbohydrate · Lectin · Molecular interaction · Galactose/*N*-acetylgalactosamine · Agglutinin

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Abbreviation

Arg	Arginine
ASGPR	Asialoglycoprotein receptor
Asn	Asparagine
CDRs	Carbohydrate recognition domain
Con A	Concanavalin A
ECorL	*Erythrina corallodendron lectin*
Fuc	Fucose
Gal	Galactose/*N*-acetylgalactosamine
GalNAc	*N*-acetylneuraminic acid
GlcNAc	*N*-acetylglucosamine
Glu	Glutamic acid
His	Histidine
LBL	Lima bean lectin
Man	Mannose
PHA	*Phytohaemagglutinin*
PNA	Peanut agglutinin
SBA	*Soybean agglutinin*
Tant	T-antigenic
UEA	*Ulex europaeus*

4.1 Introduction

Lectins are basically proteins or non-immune proteins which usually bind with sugar molecules based on several factors such as binding sites (at least two), agglutination of animal or plant cells, precipitated polysaccharides, glycolipids, and even glycoproteins (Kennedy et al. 1995). As far the information and data from the past decade show that lectins have the ability to bind with carbohydrates in a unique manner along with some biological process of cells and proteins. In order to determine the specific protein and carbohydrate groups, X-ray crystallography technique is used by understanding the morphological structure in a crystalline form, and also it is used to study bond formation. The first ever lectin to be crystallized is Concanavalin A (Con A) and this crystallized lectin shows a lesser agglutination activity with the sugar groups, especially with sucrose (Liener 1976).

The denaturation of protein is estimated by ΔCP and ΔH values based on the protein–protein interaction, binding of oligopeptide-protein, surface area of participating molecules with water accessibility (both polar and apolar) (Chervenak and Toone 1995). Lectins are capable of regulating diverse varieties of biological processes via interaction with carbohydrates in specific manner (Sharon and Lis 1989) The structural studies of lectins unveil the recognition between the carbohydrates and lectins. By the quantitative understanding of the molecular basis with the

Fig. 4.1 Structures of monosaccharide ligand

affinity of the binding systems of lectins, it is possible to use it in the drug delivery system (Toone 1994). Many vital biological processes depend on recognition of these binding sites which include clearance of glycoprotein from system, control glycoprotein, immune system, as well as cancer biology. Detailed study of molecular level binding of lectin–carbohydrate interaction can lead to drug designing for treating a wide range of diseases which also include cancer inflammation (Loris 2002). Based on the bond formation abilities the interaction between lectin and carbohydrate is done, the bond formation ability is specific to that of Van der Waals interaction, hydrogen bonds, hydrophobic interaction, and even metal coordination bond. For example, the cross-linking and precipitation of blood cells lead to surface sugar interaction of erythrocytes, so from these phenomena it is understood that the lectin has multiple binding sites, which is basically called as cell agglutination (Sharon and Lis 1972). Cell agglutination is found in various wide range organisms such as invertebrates, vertebrates, bacteria, fungi, and viruses. Based on the structural binding of the receptor and its ligand, the lectin has sugar specificity and also the carbohydrates and lectin interaction is a highly specific reversible reaction. According to Sharon et al. the lectins are classified into 5 groups based on high affinity to the monosaccharide ligands. They are mannose, galactose/*N*-acetylgalactosamine, *N*-acetylglucosamine, fucose, *N*-acetylneuraminic acid, *N*-acetyl galactose-4-sulfate and mannose-6-phosphate (Ambrosi et al. 2005) (Fig. 4.1).

4.2 Types of Lectin and Its Interaction with Other Molecules

4.2.1 Plant Lectins

4.2.1.1 Legume Lectin

Legumes lectins are specifically family of proteins that bind with the sugar groups and these are the most studied class of protein family over more than 100 of other family members, these legume lectins are isolated from the cortical region of seeds, roots, and bark regions of the family *Fabaceae* (Sharon and Lis 1990). The main function of these naturally occurring lectins is that they are released against the predators as a defensive mechanism. *Concanavalin* A, *phytohaemagglutinin* (PHA), *soybean agglutinin* (SBA*), peanut agglutinin* (PNA), and coral tree or *Erythrina corallodendron lectin* (ECorL) are some of the examples of legume lectins (Sumner and Howell 1936). So this helps in understanding the whole mechanism behind the lectin–carbohydrate interaction, because it is easily purifiable and has higher sugar specificity (Sharon and Lis 1990). The legume lectin has the ability to interact with many quaternary structures; because of this these lectins are used as model systems for understanding the protein–carbohydrate interaction (Rojo et al. 2002). Legume lectin has the ability to differentiate between various carbohydrates that are similar, like galactose and glucose are closely related. This phenomenon is possible by the slight change or configuration of a single hydroxyl group (4-OH) (Bourne et al. 1994). They consist of 2–4 subunits that are slightly heterogeneous in nature and with a similarity of 25–30 kDa. For binding an atom is present strongly attached to calcium and manganese ions. Ca^{++} and Mg++ are located very close to carbohydrate binding sites. Many amino acids play an important role in the interaction by forming hydrogen bond with monosaccharide at the binding site. In case of legume lectins four amino acids plays major role, i.e. glycine, leucine, aspartic acid, asparagines. Sometimes amino acid can differ within the species like in PNAleucine is replaced by tyrosine (Sharon and Lis 2001) (Table 4.1).

Hydrogen bond and hydrophobic interaction causes binding of lectin to carbohydrates, which is represented by T-antigenic disaccharide in PNA, which shows Galβ(1→3) -GalNAc (Tant) binding. Overall interaction is done by Van der Waals

Table 4.1 Example of Legume lectin with ligand

Legume lectins	Ligand	Reference
Concanavalin A (Jack bean)	Man/Glc	Gupta et al. (1996)
Phaseolus vulgaris (French bean)	–	Ye et al. (2001)
Peanut agglutinin (Peanut)	Gal, Galβ3GalNAcα	Ambrosi et al. (2005)
Soybean agglutinin (Soybean)	Gal/GalNAc	Rao et al. (1998)
Pisum sativum (Pea)	Man/Glc	Davey and Dudman (1979)
Lens culinaris (Lentil)	Man/Glc	Pavelka and Ellinger (1989)
Dolichos biflorus (Horse gram)	GalNAcα3GalNAc, GalNAc	Ambrosi et al. (2005)

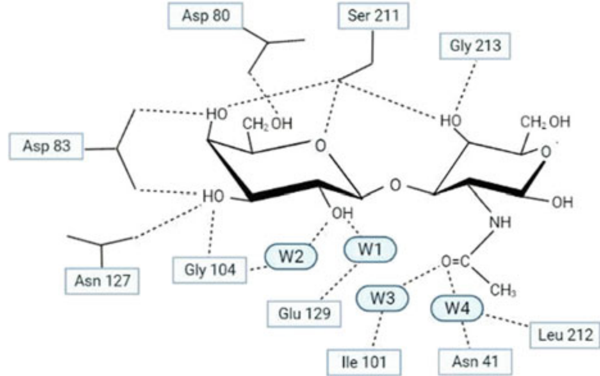

Fig. 4.2 Protein–carbohydrate interaction PAN-Tant complex (created with biorender.com)

force. Hydrophobic patches on the galactosyl surface are created by the steric effect of the hydroxyl group of carbohydrates, which can interact with protein hydrophobic regions (Van Den Hamer et al. 1970).· A water molecule plays an important role in ligand–protein contact as well as (Quiocho 1989) recognition of carbohydrates. Apart from T-antigen (Tant), some other ligand of PNA is present like methyl-*b*-galactoside (MeGal), *N*-acetyllactosamine, (LacNAc44), and lactose (Lac) which are connected with water bridges and have four water complexes W1, W2, W3, and W4 (Davey and Dudman 1979) (Fig. 4.2).

4.2.1.2 Ricin Lectin

Ricin is a cytotoxic glycoprotein discovered in the seeds of the castor plant that is heterodimeric (AB), also known as R-type lectin. Carbohydrate-binding sites (specific for galactose and *N*-acetylgalactosamine) are present in the heavy B-chain (Rutenber and Robertus 1991). The B-chain can attach to cell surface glycolipids and glycoproteins with beta-1,4-linked galactose residues, allowing the poisonous protein (ricin's A-chain) to enter the cell more easily. The A-chain functions as an enzyme (Rutenber et al. 1991). It has the ability to catalytically disrupt protein synthesis and is extremely hazardous that a single molecule is all that is required to destroy a cell (Endo and Tsurugi 1987).

The R-type lectin family is represented in both mammals and plants (Ricin-B family in plants). In mammals, fibroblast growth factors and the cysteine-rich domain of the mannose receptor have structurally similar domains (Pohleven et al. 2009). Although both ricin and the mannose receptor can bind galactose-containing glycan complexes, their carbohydrate binding sites are significantly different. Lactose binding is only seen at two of the six possible sites in the ricin–lactose complex's X-ray crystal structure. Galactose C3 and C4 hydroxyl groups donate hydrogen bonds to an aspartic acid residue's side-chain carboxyl oxygen atoms at both positions, galactose C3 and C4 hydroxyl groups donate hydrogen bonds to an

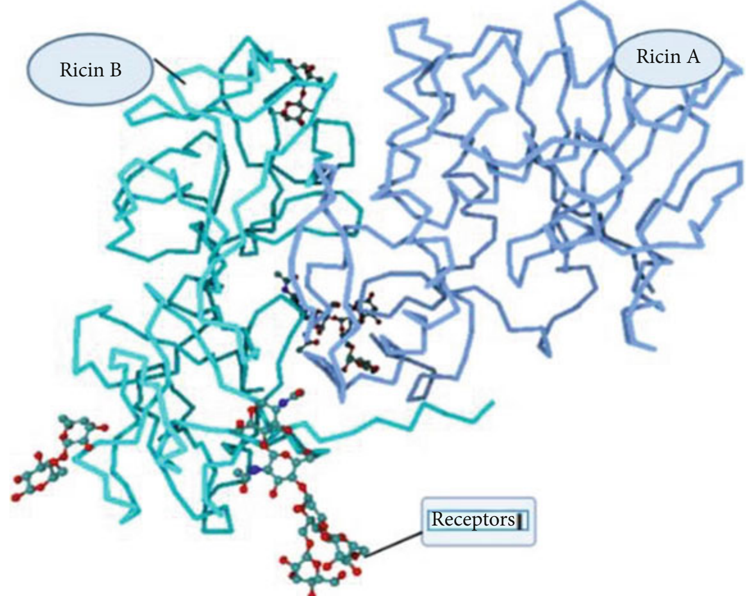

Fig. 4.3 Ricin–lactose complex with heterodimeric chain and mannose receptors (Created with biorender.com)

aspartic acid residue's side-chain carboxyl oxygen atoms at both positions (Lord et al. 2003) (Fig. 4.3).

4.2.2 Animal Lectin

Various types of animal lectin present which can be classified into the basis of their solubility or specificity, i.e. S-lectin and C-lectin are among the animal lectins which are studied first.

4.2.2.1 S-Lectins

Maximum of S-lectin is β-galactose specific and soluble in nature whereas C-lectin is ion dependent like Ca^{++}. S-lectin has protein subunit of 14–35 kD. As per the studies S-lectin in tissue organization, cell adhesion, differentiation, and morphogenesis is possible due to the binding of poly-*N*-acetyllactosamine with protein laminin membrane. S-lectin is commonly called as galectin they share primary structural homology with carbohydrate recognition domain (CDRs). The branching pattern or structure of galectin is Galβ1–3/4GlcNaC which is very specific (Barondes et al. 1994a).

The binding affinity of S-lectin is very low, in the millimolar range. Although all galectins appear to bind terminal β-galactosides, their identification of galactosyl residues within oligosaccharides varies significantly. The C4 and C6 hydroxyls of galactose, as well as the C3 hydroxyl of GlcNAc, were found to be tightly recognized when galectins were co-crystallized with simple galactoside-containing disaccharides. Galectin-1 has been found to have a substantially higher affinity for bigger glycans with repeated galactosyl residues, such as those seen in type-2 polylactosamine sequences represented by the structure -3Galβ1–4GlcNAcβ1–3-Galβ1–4GlcNAcβ1–3Galβ1–4GlcNAcβ1-R (Kilpatrick 2002). Each galectin has somewhat different oligosaccharide binding selectivity and macromolecular ligand affinity. Because galectin-1 only binds to a small number of glycoconjugates, it appears that the presence of galactose residues in glycoconjugates is insufficient to induce high-affinity binding to this lectin. These lectins have been proposed to have both intracellular and extracellular functions, including a role in modulating cell–cell and cell–matrix interactions. The C4 hydroxyl group of galactose is hydrogen bonded to His45, Asn47, and Arg49 in the carbohydrate-binding site, residues that are completely conserved across all galectin sequences (Sharon and Lis 2013).

4.2.2.2 C-Lectin

This lectin belongs to the collectin family, which is a group of soluble lectins that belong to the Ca^{2+}-dependent C-type lectin superfamily (Weis et al. 1998). Each of these proteins has one or more carbohydrate-recognition domains (CRDs), as well as domains that are responsible for the molecule's other functions. Ca++ is required for carbohydrate binding by all C-type lectins, though they have different specificities. The function of the metal ions (Ca^{++} and Mn^{++}) in the legume lectins, as explained earlier, differs fundamentally from the function of the Ca^{++} as a direct sugar ligand in the mannose-binding protein A. The carboxy-terminal amino acid residues that ligate both the Ca^{++} and the mannose are found there (Drickamer and Fadden 2002). The mannose 3-OH is hydrogen bound to one carbonyl of Glu185 and an amide of Asn187, whereas the 4-OH is hydrogen bonded to a carbonyl of Glu193 and an amide of Asn205. Both hydroxyls bind directly with Ca^{++}, which is then coordinated with four carbonyl groups, one from each of the amino acids' side chains. The wide diversity of carbohydrate structures recognized by this lectin, as well as the strong binding of multivalent ligands, could be explained by the presence of numerous binding sites with different affinities for oligosaccharides (Kolatkar and Weis 1996). The binding selectivity of C-type lectins is diverse. Although the relative affinities for sugars in this group vary, several of these lectins bind derivatives of Man and GlcNAc (Man-type ligands) (Fig. 4.4). They form a network of hydrogen bonds with the side chains of amino acids, which also serve as equatorial ligands for this divalent cation (Chervenak and Toone 1995) (Fig. 4.5).

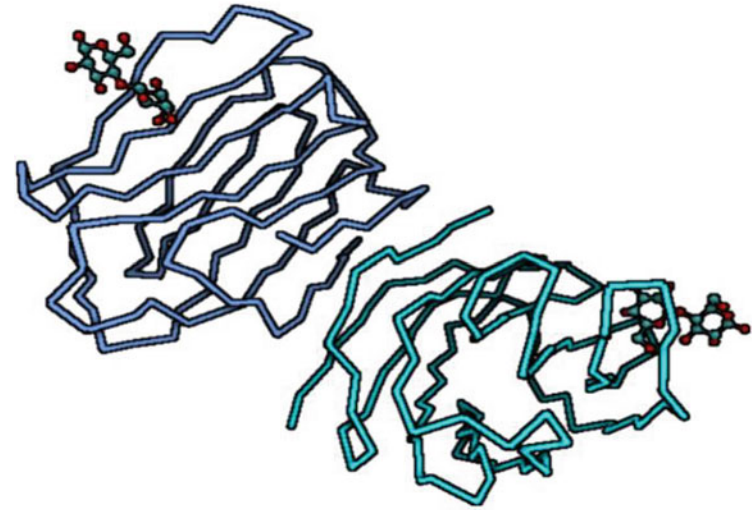

Fig. 4.4 The bound carbohydrate and amino acid side chains in the binding site of the human galectin-2-lactose complex are shown in this diagram

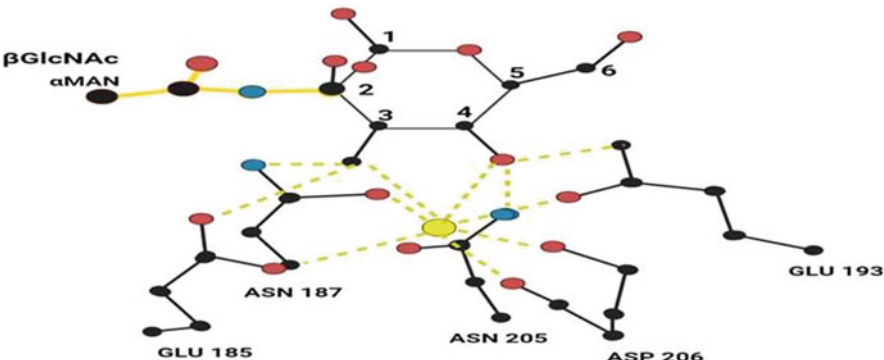

Fig. 4.5 Superimposition of β-*N*-acetyl-D-glucosamine on α-*D*-mannose; numbers on ring carbon atoms are those of both mannose and *N*-acetylglucosamine. (Created with biorender.com)

4.2.3 Bacterial Lectin

Lectins, also known as adhesins, are produced by many bacteria and play a key role in the initiation of infections by mediating adherence to host cells (Qun and Cummings 1993). Other lectins, including those made by bacteria, bind readily to saccharide sequences of cell-surface glycoproteins and glycolipids binding frequently involve not just terminal but also interior carbohydrate sequences (Ofek and Sharon 1988). Bacteria that express surface lectins rapidly bind to phagocytic cells (Sharon 1987). In pathogenic bacteria, lectins are commonly involved in host

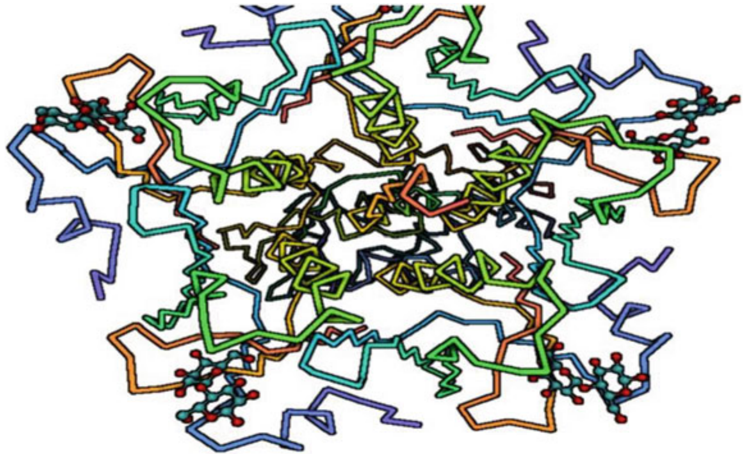

Fig. 4.6 The B-pentamer of the LT-lactose complex, displaying the bound carbohydrate. (Created with biorender.com)

identification and tissue attachment. The importance of carbohydrate-mediated bacterial adhesion in infection has prompted research into bacterial lectin carbohydrate specificity (Perret et al. 2005).

The relevance of carbohydrate-mediated bacterial adhesion in infection has prompted study into the carbohydrate specificity of bacterial lectins, and this study has led to the concept of using carbohydrate antagonists of natural lectin ligands to inhibit bacterial adherence. Site-directed mutagenesis of the carbohydrate-binding component of the sialic acid S-microbial lectin of *Escherichia coli* revealed that replacing Lysl16 with threonine or Argl18 with serine rendered the lectin inactive, implying that ionic interactions are important for the lectin's ability to bind its ligand (Lis and Sharon 1991). The bacterial lectin surface seems to be playing a role of adhesive as seen in infections of the gastrointestinal and urinary tracts. The mannose-specific type-1 fimbriae, galabiose-specific P fimbriae, and *N*-acetylglucosamine-binding F-17 fimbriae, which are produced by distinct strains of Escherichia coli, are the most well-characterized bacterial lectins (Barnes et al. 1999) (Fig. 4.6).

4.3 Molecular Basis of Carbohydrate Interaction

On the basis of mono- and oligosaccharides binding to lectin we divided them into five types which are as follows.

4.3.1 Mannose/Glucose-Binding Lectins

The mannose/glucose-binding lectins are a family of agglutinins found in the *Leguminosae* plant family. The mannose/glucose-specific lectins can be divided into two groups based on their molecular structure: those with four identical subunits (so far only concanavalin A) and those with two light (α) and two heavy (β) chains with the general composition of α2β2, e.g. (Kaku and Goldstein 1992).

A. *Lathyrus tingitanus* (Tangier Pea): This family of lectin binds mannose and glucose. It occurs in α2β2 structure (Bourne et al. 1990). The light and heavy subunits' *N*-terminal amino acids are valine and threonine, respectively. Acidic and alkaline hydroxylic amino acids are found in abundance, but only traces of sulfur-containing amino acids (Rougé and Chabert 1983).
B. *Pisum sativum* (Pea): Van Wauwe and colleagues investigated the carbohydrate-binding specificity of pea lectins in depth. These researchers discovered that configurationally similar monosaccharides, such as mannose, glucose, fructose, and L-sorbose, bind to the lectin by hapten suppression of the precipitation reaction between the *Pisum sativum* lectin and *Pichia pinus* O-phosphonomannan (Van Wauwe et al. 1975).

4.3.2 N-*Acetylglucosamine-Binding Lectins*

N-acetylglucosamine-binding lectins are a broad category of proteins that bind to *N*-acetylglucosamine. With a monosaccharide-specific primary specificity (chitin oligosaccharides) and/or its β-(l→4)-linked oligomers, glucosamine is used in various cases.

A. *Brachypodium sylvaticum* (False Brome Grass): Embryos from Brachypodium sylvaticum contain a blood group-nonspecific lectin with physicochemical features similar to wheat germ agglutinin and other cereal lectins. The *B. sylvaticum* lectin is a dimer ($M_r = 36{,}000$) made up of identical subunits with $M_r = 18{,}000$. It was isolated on a column of immobilized *N*-acetylglucosamine. Only *N*-acetylglucosamine reduced the hemagglutination reaction with erythrocytes among the monosaccharides examined; chitin oligomers were far more efficient (Peumans et al. 1982).
B. *Phytolacca americana* (Pokeweed, Pigeon Berry): Hemagglutination inhibition assay and quantitative inhibition of purified glycoproteins were used to investigate the carbohydrate-binding specificity of pokeweed mitogens. The data show that the pokeweed mitogen isolectins bind to →3Galβ1,4GlcNAcβ1 > or →4GlcNAcβ1,4GlcNAcβ1 (Irimura and Nicolson 1983).

4.3.3 N-*Acetylgalactosamine/Galactose-Binding Lectins*

N-acetylgalactosamine/galactose-binding lectins are the first plant haemagglutinins to be examined, the first to be demonstrated to exhibit human blood group specificity and the most diversified in terms of taxonomic classification. Because the *N*-acetylgalactosamine/galactose-binding lectins have such a wide range of binding activities, we have decided to break down the individual lectins in this group by their monosaccharide specificities (Cherayil et al. 1990). The *N*-acetylgalactosamine-specific lectins will be presented first, followed by proteins that have no preference for either monosaccharide, and finally, proteins that have no preference for either monosaccharide. The high degree of sequence homology among *N*-acetylgalactosamine/galactose-binding lectins indicates that they have structural similarities (Liener 2012).

A. *Phaseolus lunatus syn. Limensis* (Lima Bean): The lima bean agglutinin is the first lectin to be found to have blood group specificity (Boyd and Reguera 1949). It agglutinates type A erythrocytes. The integrity of the free sulfhydryl groups is critical for the haemagglutinating activity of lima bean lectins. *N*-Acetylgalactosamine, a particular lectin inhibitor, protected the lectin from sulfhydryl reagent inactivation (Gould and Scheinberg 1970). The carbohydrate binding site of the lima bean lectin is highly selective. The nature of the interaction between LBL (Lima bean lectin) and the C-2 position of galactose and *N*-acetylgalactosamine using the sulfhydryl group protection assay was also observed. The existence of a hydrophobic area complementary to this aromatic group in the LBL binding site. The lima bean lectin is the most specific of the characterized GalNAc binding lectins for type A blood group material that contains L-fucose: The L-fucose-containing oligosaccharide is only slightly preferred (20%) by the Dolichos biflorus lectin (Etzler and Kabat 1970).
B. *Vicia villosa* (Hairy Vetch): *N*-Acetylgalactosamine is found to be 200 times more effective than galactose as a monosaccharide inhibitor of B4 lectin binding to T. erythrocytes (Tollefsen and Kornfeld 1983). An axial C-4 hydroxyl group and an equatorial *N*-acetyl group at C-2 were also required, as a preference for the α-anomer. The disaccharide GalNAcal,3Gal was shown to be the most effective inhibitor of the precipitin reaction between a mixture of V. villosaisolectins and hog blood mucin, being twice as active as methyl *N*-acetyl-*a*-galactosaminide and twice as active as the α-(l→6)-linked disaccharide (Datta and Basu 1983). The capacity of a succession of glycopeptides to block the binding of Tn erythrocytes, those containing one or two *V. villosa* B4 isolectin terminal *N*-cetyl-galactosamine groups connected to serine or glutamine. The amino acids threonine and arginine were good inhibitors. It is likely that the number and size of the group will grow. The units of *N*-acetylgalactosamine ’ positions along the peptide backbone for binding to the B4 isolectin, are crucial.

Fucose specific lectins: Although several fucose-specific legume lectins have been identified, only two have been thoroughly studied in terms of carbohydrate specificity mechanisms: lectin I from gorse *Ulex europaeus*—UEA-I and the lectin from the *Lotus tetragonolobus* (Pace et al. 1999). Binding tests using deoxy-derivatives of L-Fuc α1–2-D-Galβ1–4-DGlcNAc, the highest affinity, provide some evidence. All three fucose hydroxyls were found to be involved in important polar interactions; however, the effect of removing the O2 hydroxyl is less striking. To obtain precise structural information on the molecules, reactivity patterns of fucoglycoconjugates with a panel of fucose-binding lectins were used.

A. *Aleuria aurantia* (Orange Peel Fungus): The haemagglutinin's composition is characterized by high quantities of serine and glycine, as well as the absence of sulfur-containing amino acids and carbohydrates. Each subunit appears to contain one binding site for L-fucose.
B. *Lotus tetragonolobus* (Asparagus Pea): With the multivalent fucose-binding protein, this trivalent dye can produce a three-dimensional lattice precipitate. Ion-exchange chromatography and dialysis were used to remove the dye and monosaccharide after dissolving the precipitate with L-fucose. Fucoglycopeptides having N-linked glycan units can also be bound by Lotus tetragonolobus lectins. Susz and Dawson (1979) found that 6-*O*-*α*-L-fucosyl-*N*-acetylglucosamine is twice as effective as 2′-O-*α*-L-fucosyllactose as a lectin inhibitor, and that an asparagine-linked glycopeptide of this strong disaccharide binds very effectively to a lectin-sepharose adsorbent. Another research (Debray et al. 1981) conducted a comprehensive investigation on the potential of N-linked glycopeptides to suppress lectin-induced hemagglutination, confirming previous findings and emphasizing that glycans with numerous nonreducing terminal a-L-fucosyl units interact with the lectin the best.

4.4 Importance of Lectin–Carbohydrate Interaction

Protein–carbohydrate interactions are crucial in a wide range of biological entities, and many processes rely on them, including infection, fertilization, inflammation, and cellular recognition. The galactose- and GalNAc-terminated oligosaccharides that arise after desialylation of senescent complex-type glycoconjugates are specific for the C-type lectin ASGPR (asialoglycoprotein receptor). The ASGPR trimer binds to triantennary N-linked glycans with great affinity, causing their endocytosis. ASGPR appears to play a role in the clearance of serum glycoproteins in general (Meier et al. 2000). Long-distance feed-back loops connect cell signaling receptors that regulate cell growth and motility. Long-distance loops are part of a complicated regulatory network that includes several glycoprotein glycoforms and molecular communication pathways mediated by lectin–carbohydrate interactions (Zelensky and Gready 2005).

Lectin–carbohydrate interaction plays an important role in receptor-mediated endocytosis of glycoproteins, whereas others, such as the selectins, play a role in cellular recognition and adhesion. Many animal lectins have biological functions that are well understood. A number of mammalian lectins, for example, are engaged in glycoprotein receptor-mediated endocytosis, whereas others, such as the selectins, are involved in cellular recognition and adhesion. The galectin family has been linked to a variety of diseases. Controlling cell growth, activating inflammatory cells, and regulating apoptosis are all aspects of metastasis (Barondes et al. 1994b).

When lectins connect to cells, particular glycoprotein and glycolipid receptors are frequently cross-linked and aggregated. The mitogenic activities of lectins, the activation of mating events in fungi, and the induction of apoptosis in activated human T lymphocytes are all examples of cross-linking interactions that are linked to signal transduction effects. These molecular interactions aid in analyzing diverse range of lectins and their carbohydrate ligands, thereby investigating the corresponding metabolic pathways and their role in various diseases and diagnostics.

4.5 Conclusion

In conclusion we evaluate the interaction between protein-side chains and pyranose forms of the most prevalent monosaccharides discovered among all high-resolution protein-carbon complex structures. Collecting 3D carbohydrate–protein complex structure information offers the basis for biomolecular engineering, design and molecular recognition process modulation of pharmaceuticals. When binding to several lectins, the carbohydrate chains assume various configurations. In interactions with proteins such conformational multiplicities and lower affinities are unique yet difficult to deal with in both practical and theoretical ways in carbon–protein interaction systems. This combination of experimental and theory methods provides fundamental insight into the molecular processes involved in carbon-protein interactions and, at the same time, carbohydrate–carbohydrate interactions, resulting in new concepts for the development of drugs that targeted neurodegenerative and currently incurable disorders.

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Conflict of Interest The authors have no conflict of interest to declare.

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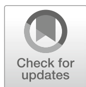
Chapter 5
Animal Lectin



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Abstract Animal lectins bind to soluble carbohydrates on cell surfaces. The majority of animal lectins are nonenzymatic in activity and usually precipitate glycoconjugates in specific animal cells. It controls protein levels in the blood, modulates cell adhesion to glycoprotein production, and binds soluble extracellular and intracellular glycoproteins. Carbohydrates seen in pathogens that are not recognized by immune system host cells are identified by lectins. Animal lectins have a jelly-like consistency. Animal lectins have a jelly-roll tertiary structure with quaternary connections that vary. P type lectins, C type lectins, I type lectins, Chi lectins, and others are the 15 structural families of animal lectins.

Keywords Lectin · Glycan interaction · Carbohydrate binding · Molecular recognition

Abbreviations

AAA	*Anguilla anguilla* agglutinin
CD-MPR	Cation-dependent mannose 6-phosphate receptor
CI-MPR	Cation-independent mannose 6-phosphate receptor
CLRD	C type lectin-like receptor domain
CRD	Carbohydrate recognition domain
ERAD	Endoplasmic reticulum-associated degradation
FTLD	F type lectin domain
IGF2R	Insulin-like growth factor 2 receptor
LMAN2	Lectin, mannose binding 2

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LRR	Leucine-rich repeats
MAG	Myelin-associated glycoprotein
MCFD2	Multicoagulation-factor deficiency 2 protein
MDCK	Madin-Darby canine kidney
SBD	Sugar binding domain
TGN	Trans-Golgi network
TIM	Triose-phosphate isomerase
VIP-36	Vesicular integral membrane protein-36
XCGL	Xenopus oocyte cortical granule lectin
XEEL	Xenopus embryonic cuticular glycoprotein
zINTLs	Zebrafish intelectins

5.1 Introduction

Animal lectins are a variety of non-immune origin proteins that attach to carbohydrates and operate as cell recognition mediators in biological systems (Gupta 2012). The first animal lectin was found in 1899 by M.L Camus from the snail *Helix pomatia*. Various lectins have been identified from invertebrates, including protozoa (Brown et al. 2007), insects (Ourth et al. 2005), molluscs (Takahashi et al. 2008), crustaceans (Sanchez-Salgado et al. 2014), sea cucumbers (Gowda et al. 2008). Lectins have been discovered in vertebrates, including fish (Cammarata et al. 2014) and snakes (Aranda-Souza et al. 2014). There are many well-known lectins of various tissues and cells in humans, including lungs (Sorensen et al. 2007), serum (Wallis 2007), and dendrites (Sorensen et al. 2007).

Through their binding sites, animal lectins preferentially detect and attach to carbohydrates and glycans reversibly. Originally identified for their binding with carbohydrates and are now identified as a more diverse set of proteins, with some of them interacting with other proteins, lipids, and nucleic acids.

Endocytosis, apoptotic processes (Liu et al. 2012), intracellular translocation of glycoproteins, binding to glycoconjugates, defense against pathogens, controlling cell adhesion and migration, and bacterial attachment to epithelium are all thought to be mediated by animal lectins.

5.1.1 Basic Structure of Animal Lectin

Animal lectins have a jelly-roll tertiary structure with quaternary connections that vary. All of them have a visible ConA (concanavalin-Alike) jelly-roll fold structure. Three pairs of antiparallel sheets make up the jelly-roll motif. There are three sheets in the molecule: first is a six-stranded sheet referred to as “back” sheet. It is flat. Second is the “front” sheet which is seven stranded and curved. And the last one is a comparatively shorter one. It is a five member sheet referred to as the “top” sheet. These sheets are interconnected by loops of different lengths (Gupta 2012) (Fig. 5.1).

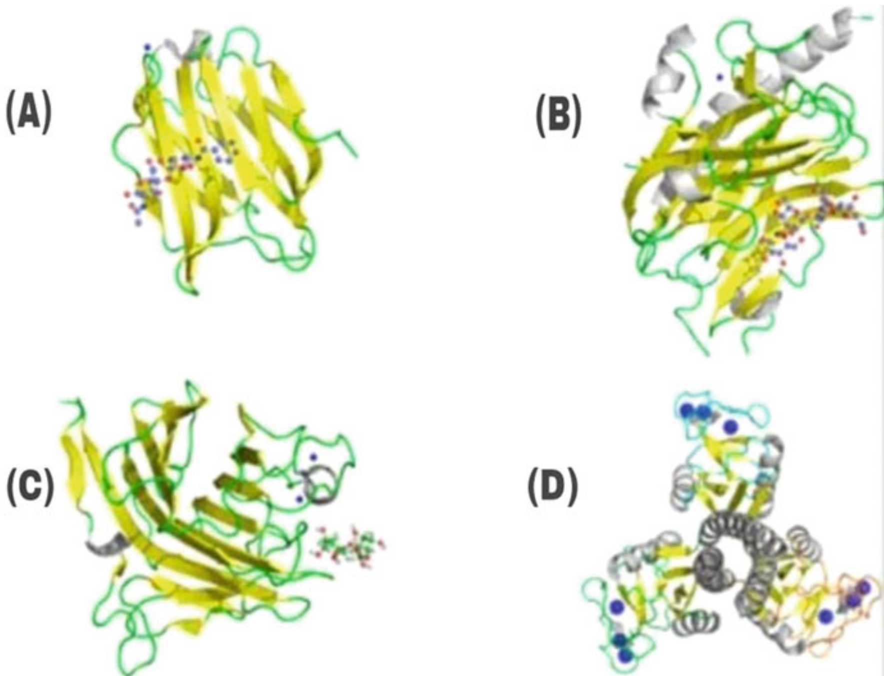

Fig. 5.1 Tridimensional structures of lectins in (**a**) galectin-3 from Homo sapiens; (**b**) calreticulin from Mus musculus; (**c**) L type of humans (*Homo sapiens*); and (**d**) C type of humans. White and yellow represent α-helices in lectins and secondary structures of β-sheet, respectively. The blue color represents the Ca2+ ion and the carbohydrates are represented by ball and stick (Adapted with permission from Renata De Oliveira Dias et al. 2015 #Insights into Animal and Plant Lectins with Antimicrobial Activities)

5.1.2 Classification of Animal Lectin

There were various classifications of animal lectins, the most famous of which was Gabius' classification, which classified animal lectins into five main classes based on structural characteristics: C type lectins, I type lectins, pentraxins, galectin, and P type lectins. Currently, a minimum of 15 structural families are known, and numerous other lectins have structures that appear to be unique among proteins that bind to carbohydrates (Gupta 2012) (Table 5.1).

5.2 Calnexin and Calreticulin

Calreticulin is a Ca2+ buffering ER protein that aids in the wrapping of newly generated proteins and glycoproteins (Trombetta 2003). The "calreticulin/calnexin cycle" is made up of calnexin, ERp57, and calreticulin, where calnexin is an

Table 5.1 Classification of lectin family

Sl. No.	Lectin groups	Specificity	Location
1.	Calnexin and calreticulin	$Glc1Man_9$	ER
2.	M type lectins	Man_8	ER
3.	L type lectins	Variable	ER, ERGIC, Golgi
4.	P type lectins	Man6-phosphate, phosphomannosyl receptors	Secretory pathway
5.	C type lectins	Variable	Cell membrane, extracellular
6.	S type lectins	β-Galactosides	Cytoplasm, extracellular
7.	I type lectins	Sialic acid and other glycosaminoglycans	Cell membrane
8.	R type lectins	Variable	Golgi, cell membrane
9.	F box lectins	$GlcNAc_2$	Cytoplasm
10.	Fibrinogen-type lectins	GlcNAc, GalNAc	Cell membrane, extracellular
11.	Chi-lectins	Chito-oligosaccharides	Extracellular
12.	F type lectins	Fuc-terminating oligosaccharides	Extracellular
13.	Intelectins	Gal, galactofuranose, pentoses	Extracellular/cell membrane

endoplasmic reticulum integral membrane chaperone like calreticulin and ERp57 is an endoplasmic reticulum resident PDI-like protein. This cycle is very important for controlling the quality and folding of freshly generated (glyco)proteins (Trombetta 2003).

5.2.1 Structure of Calnexin and Calreticulin

Calnexin has a ß-sheet globular domain with an extension of 140-hairpin arm that is formed by repetitive motifs (Schrag et al. 2001). The extended arm is curved, providing an aperture that will most likely hold specific substrates. Calnexin's glucose-binding site is placed on the globular domain's surface (Schrag et al. 2001). Globular domain of calnexin has a disulfide bond and a calcium binding site (Schrag et al. 2001). Another disulfide bond can be found near the expanded arm's tip. Calreticulin's N-domain is globular, with a disulfide bridge glucose-binding site, according to a model of the protein's 3D structure. The calnexin-specific extended arm structure is likewise expected in the P-domain.

5.2.2 Function of Calnexin and Calreticulin

Calnexin and calreticulin are chaperones that have comparable roles, such as Ca2+ binding, identification of misfolded proteins, and lectin-like activity. Calreticulin is a luminal protein that can freely move about the ER lumen, whereas calnexin is an integral membrane protein. Calnexin interacts with its protein-folding intermediates transiently in the ER membrane's stationary phase, whereas calreticulin interacts with the intermediates of protein folding in the lumen's more mobile environment. Cell-to-cell communication and recognition, immunological responses and recognition, and intracellular metabolite processing are just a few of the activities they play.

5.3 M Type Lectin

M type lectins are "type II transmembrane proteins" with relatively short cytoplasmic tails that lack important catalytic residues and a critical disulfide bond required for the activity of enzymes. As a result, they "bind to the high mannose glycans that are linked to the glycoproteins present in the ER" (Hosokawa et al. 2001).

5.3.1 Structure of M Type Lectins

Both alpha-helices and beta-sheets are present in M type CRD, which has a barrel-like structure. In M type CRDs, the ligand binding site contains a deep cleft at one end of the barrel. This lectin's deep binding site allows it to engage with high mannose glycans selectively.

5.3.2 Classification and Functions of M Type Lectins

EDEM-1, EDEM-2, and EDEM-3 are the three M type lectins that are found in mammals. By identifying and eliminating misfolded or unassembled proteins, the ERAD (endoplasmic reticulum-associated degradation) mechanisms ensure that only properly folded proteins are retained in the cell. EDEM-1 binds to non-native proteins and directs them to the endoplasmic reticulum membrane dislocation and ubiquitination complex, which also contains SEL1L, via its mannosidase-like domain (Cormier et al. 2009). EDEM-2 overexpression hastens the misfolded a1-antitrypsin degradation (Mast and Moremen 2006). Overexpression of EDEM3 increases glycoprotein ERAD while also increasing mannose-trimming activity in EDEM1.

5.4 L Type Lectins

L type lectins are found in fungus, plants, and animals. Plant lectins are soluble proteins, whereas in animals L type lectins are ER resident protein found bound to mannose oligosaccharides with Ca2+−.The L type lectin having a globular shape consists of two antiparallel beta-sheets. In a version of the jelly-roll fold, the b-sandwich consists of concave b-sheet (major) and a convex b-sheet (minor) (Satoh et al. 2006). L type lectins in animals are mainly found in four types: ERGIC-53, ERGIC-53 like VIP36 and VIPL. They are primarily involved in sorting of proteins in endoplasmic reticulum and localized in the ER, vesicular-tubular cluster, Golgi complex.

5.4.1 ERGIC-53, ERGL, VIP 36, VIPL

ERGIC-53 is found in mammals that bind to newly synthesized glycoproteins using calcium ions through N-glycans and transport them from ER to Golgi apparatus. It is type I transmembrane proteins that recognize high mannose oligosaccharides (MOS) attached to proteins. Carbohydrate-binding activity of ERGIC-53 is regulated differently in contrast to other L type lectins. They are highly selective to mannose but show low affinity towards glucose and GlcNAc.

ERGL is found in mammals specifically in small number of specific tissues and cell types associated with the production of some specific glycoproteins. ERGL differs from other animal type L lectins which all have the sugar-calcium binding activity.

VIP 36 (vesicular integral membrane protein-36) also known as lectin, mannose binding 2 (LMAN2), intracellular animal lectin closely related to ERGIC 53 localized from ER to cisGolgi which involved transportation of glycoproteins. “The VIP36 whose function is unknown but assumed to act as a cargo receptor between the ER and Golgi” (Hauri et al. 2002). They are specific to MOS without binding to Ca2+ at an optimum pH and temperature of 6.0 and 370C, respectively.

The structure of VIPL is similar to VIP 36. However they differ in their characteristics and are also found in invertebrates. The exact function of VIPL remains unknown though they act as an ER export receptor which aids in the production of glycoprotein (Yamaguchi et al. 2007) and also may regulate ERGIC-53 (Nufer et al. 2003).

5.5 P Type Lectin

The P type lectin family consists of the cation-dependent mannose 6-phosphate receptor (CD-MPR) and the cation-independent mannose 6-phosphate receptor (CI-MPR). These are differentiated on the basis of their ability to identify

phosphorylated mannose residues. P type lectins drive freshly generated lysosomal enzymes containing the mannose 6-phosphate (M6P) signal to lysosomes, which is critical for the development of functioning lysosomes in higher eukaryotes.

5.5.1 Cation-Dependent Mannose-6-Phosphate Receptor (CD-MPR)

One of the two proteins that bind M6P tags on acid hydrolase precursors in the Golgi apparatus intended for transit to the endosomal-lysosomal pathway is the cation-dependent mannose 6-phosphate receptor. They identify lysosomal enzymes' phosphomannosyl recognition marker. CD-MPR homologs can be present in all eukaryotes. CD-MPR is a single transmembrane domain type I transmembrane protein (Gupta 2012).

5.5.2 Cation-Independent Mannose-6-Phosphate Receptor (CI-MPR)

Insulin and the insulin-like growth factors IGF-I (IGF1) and -II (IGF2) are structurally identical peptides. They have diverse biological effects on target cells. The IGF2 receptor on the cell surface also acts as a cation-independent M6PR. Therefore it is also known as cation-independent mannose 6-phosphate receptor (CI-MPR). The CI-MPR/IGF2R glycoprotein is involved in lysosomal enzyme trafficking to the endosomal-lysosomal (EL) system from the trans-Golgi network (TGN) and comprises a single transmembrane domain (Gupta 2012).

5.6 C Type Lectin

C type lectins are those which require calcium for its binding. They are a large superfamily of lectins that have at least one CRD (carbohydrate recognition domain). Some of them do not possess calcium- and carbohydrate-binding structural parts and they are known as C type proteins. They are either secreted as soluble proteins or produced as transmembrane proteins.

5.6.1 Structure of C Type Lectin

The C type lectin-like receptor domain is mainly made of two β-sheets which are antiparallel to each other and one of them is 5 stranded which is more distorted and

far from the trimeric center of the structure, and the other one is 4 stranded which is found near the N-terminal end of the protein. There are some strands which are common and play a significant part in stabilizing the entire complex structure (Gupta 2012).

5.6.2 Classification of C Type Lectins (CLRD/CTLD-Containing Proteins)

C type lectins are consists of 17 subgroups based on their number of calcium binding domains, additional lectins, or structure of the gene, which thereby gives the inference that they are individually derived from the same ancestor which already has CLRDs.

The first seven subgroups are classified based on different types of domains in them: (I) Lecticans or hyalectans, (II) asialoglycoprotein receptors, (III) collectins, (IV) selectins, (V) NK cell receptors, (VI) endocytic receptors, and (VII) lectins as the product of regenerating genes (Reg), McGreal et al. (2004). Later Drickamer and Fadden based on structural organization added seven more subgroups (VIII to XIV). Further, three more were added (XV to XVII) by Zelensky and Gready (2005) and Gupta (2012).

5.6.3 Functions of C Type Lectin

> Despite having a highly conserved domain, these lectins are functionally diverse and have been implicated in various processes including remodeling, platelet activation, cell adhesion, complement activation, endocytosis, pathogen recognition, tissue integration.

5.7 S Type Lectin (Galectins)

Galectins can be found in the extracellular environment of the nucleus and the cytoplasm (Liu et al. 2002). Galectins are secreted via a nonclassical mechanism that requires galectins to interact with glycosylated counterreceptors (Seelenmeyer et al. 2005). Galectins play critical roles in the host–pathogen interactions as well as tumorigenesis (Rabinovich and Gruppi 2005).

5.7.1 Classification and Structure of Galectins

There are currently 15 galectins in mammals. Based on organization of domain galectin subfamilies were classified by Hirabayshi and Kasai as “proto-, tandem-

repeat, and chimera types." "Galectin-1, -2, -5, -7, -10, -11, -13, -14, and -15" are mono-CRDs. However, the tandem-repeat galectins that are galectin -4, -6, -8, -9, and -12 are made of two non-identical CRDs connected by a short linker peptide sequence. Galectin CRD structures all have a b-sandwich fold which has two beta-sheets that are antiparallel. Each subunit folds into a single compact globular domain. Galectin-3 and galectin-4 contain either of these domains, among others. The carbohydrate-binding domain is the name given to the shared domain. Their quaternary structures, however, differ. Through extended beta-sheet interactions, galectin-1and galectin-2 form non-covalently associated homodimers.

5.7.1.1 Chicken Galectins

Chicken galectin 14 and 16 are the two avian galectins with different developmental regulation that are found in the intestine and the liver of chicken, respectively. The findings show that "there are quantitative differences in the developmental regulation of the two avian galectins, with obvious similarities in the cell type pattern but a distinct intracellular localization profile" (Stierstorfer et al. 2000).

5.7.2 Functions of Galectins

Most galectins perform multiple functions, including "receptor crosslinking or lattice formation, cell-extracellular matrix interactions, cell-to-cell interactions, intracellular signaling, and posttranscriptional splicing" (Thijssen et al. 2007). Galectins can control "inflammation, cell proliferation, the cell cycle, transcription processes, cell death" (Rabinovich et al. 2002). They have the ability to separate into multiple intracellular compartments for which the preferences are decided by the cell's state. As a result, localization most likely corresponds to compartmental function. Galectins play critical roles in cancer, where they promote tumor cell survival and metastasis, neoplastic transformation. They have the ability to modulate immune and inflammatory responses and may play a critical role in assisting tumors in evading immune surveillance (Liu and Rabinovich 2005).

5.8 I Type Lectin

I type lectins are type I transmembrane proteins parts of the immunoglobulin superfamily and structurally similar to immunoglobulins. They are also called Siglecs (Sia-recognizing Ig-superfamily lectins). They recognize glycans through their Ig-like domains. NH3+ terminus of siglecs is found in the extracellular space, while the COO-terminus is found in the cytoplasm (Crocker et al. 2007).

5.8.1 Classification and Functions of I Type Lectins

I type lectins are classified into two major groups depending on the sequence similarity. First subgroup contains siglec-1, -2, -4a, -4b and Siglec-15 which are recognized by the similar genes in different species. Sialoadhesin (Sn)/Siglec-1 (CD169) involved in cell adhesion that binds specifically to sialic acids. They are found on the surface of macrophages, contain 17 immunoglobulin (Ig) domains. Whereas CD22/Siglec-2 found on the B cells negatively regulates BCR signaling. MAG or Siglec-4a involved in the myelin sheath synthesis and maintenance which is having five Ig-like domains. Siglec 15 another type I transmembrane protein having two Ig domains linked to osteoclast genesis.

The quickly evolving second subgroup contains CD33 and CD33-related siglecs. The biological function of human CD33 (Siglec-3) remains unknown although it works as marker of myeloid cells. The CD33-related siglecs have a sequence similarity to CD33 and display complicated sialylated glycan recognizing motifs. CD33-related siglecs play a role in leukocyte activity, regulate cytokine production, and programmed cell death (Crocker et al. 2007). On cells involved in nonspecific immunity, there seem to be a plethora of CD33-rSiglecs.

In humans CD33 and siglecs-5, siglecs-6, siglecs-7 (7/p75/AIRM1), siglecs-8, siglecs-9, siglecs-10, siglecs-11, siglecs-14 and siglecs-16. The biological function of human CD33 (Siglec-3) remains unknown although it acts as a marker in myeloid cells. The CD33-related siglecs have a sequence similarity to CD33 and display complicated sialylated glycan recognizing motifs. CD33-related siglecs play a role in leukocyte activity, regulate cytokine production, and programmed cell death (Crocker et al. 2007). On cells that contribute to nonspecific immunity, many CD33-rSiglecs have been discovered.

5.9 R Type Lectins

R type lectins belong to a protein superfamily that includes a CRD which has structure similar to ricin's. Ricin is the prototype lectin in this group because it was the first lectin discovered. They are found in plants, animals, and microbes (Varki et al. 2015–2017). R type lectin domain proteins are found in both animals and microbes, and some of them serve as enzymes. Coagulation factor G of Limulus horseshoe crab, for example, contains a core R type lectin domain (Gupta 2012).

5.9.1 Structure of R Type Lectin

The R type carbohydrate recognition domain has a beta-trefoil structure which has three lobes (alpha, beta, and gamma) organized around a three-fold axis due to an early gene duplication eight b-strands.

5.9.2 The Mannose Receptor Family

The human MR family contains four members, all of which have an R type lectin domain, and one fifth member, FcRY, which is present in birds. The four members are MR, DEC-205/MR6-gp200, the PLA2 receptor, and Endo180/urokinase plasminogen activator receptor-associated protein. They are all large type I transmembrane glycoproteins with a single fibronectin type II domain, several CTLDs, and a cysteine-rich domain at the amino terminus. The MR is a component of the nonspecific immune system that aids in the phagocytosis of microorganisms that are rich in mannose. The MR is particularly unique in that it is the only member of the MR family capable of clathrin-dependent endocytosis as well as phagocytosis of nonopsinized microbes. PLA2s are a broad enzyme family involved in the release of arachidonic, the precursor of fatty acids by the breakdown of phospholipids. DEC-205 helps T lymphocytes recognize and internalize antigens before presenting them to them (Varki et al. 2015–2017). Endo180 is a soluble ligand endocytic receptor that participates in matrix turnover. Furthermore, cells expressing Endo180 have better adherence to matrixes, implying that Endo180 helps in cell adhesion (Varki et al. 2015–2017).

5.10 F Box Lectins

The F box protein family is the largest known protein superfamily, with members varying in number from about 20 in yeasts to several 100 in larger eukaryotes. They have a bipartite structure (Kipreos and Pagano 2000).

5.10.1 Structure of F Box Lectins

Each F box protein has two domains. One is a substrate recognition domain that determines E3 complex target specificity and the other is a conserved F box domain that interacts ubiquitin-ligase E3 subunits Mizushima et al. 2007).

5.10.2 Classification and Function of F Box Lectins

F box proteins are classified into three based on the substrate-specific motifs at the protein's C-terminal (Jin et al. 2004). Of which the first and the second classes are the WD40 and FBW (fbl), respectively. The third is the Fbx (or FBXO) family that constitutes the other C-terminal domains (armadillo and tetratricopeptide repeats and Kelch domains, zinc fingers, and proline-rich domain). The latest introduction of

another subfamily under Fbs which has a sugar binding domain in its C-terminal has been identified from mammals which have significant importance in glycoprotein homeostasis (Jin et al. 2004).

Under the subfamily of Fbs (Sugar binding domain) we have Fbs1 (equivalent to Fbx2 or Neural F Box 42) and Fbs2. Fbs1 recognizes proteins with high mannose oligosaccharides and functions in the elimination of N-glycoproteins in the cytosol (Yoshida et al. 2002). Fbs2 recognizes N-glycan and forms an SCF Fbs2 ubiquitin ligase complex, associates in the ERAD pathway that targets sugar chains in *N*-glycoproteins for ubiquitylation" (Yoshida et al. 2003).

The Fbox protein's purpose is to bind to the protein structure (Kipreos and Pagano 2000). Yet, it is unknown how E3 identifies the target protein with such precision. According to research, phosphorylation of specific proteins is required for SCF complexes to cognize (Kipreos and Pagano 2000). Furthermore, proline hydroxylation of the transcription factor hypoxia-inducing factor 1 (HIF1) acts as a signal for ubiquitination by a SCFlike Cullin2-based VBC ubiquitin ligase. The Fbox portion of the SCF mechanism identifies different sites for ubiquitination. Associations with SCF complicated parts are mediated by the Fbox motif of the Fbox protein, whereas associations with other proteins are regulated by a repetition of TrpAsp (WD) or a secondary interacting protein motif such as leucine-rich repeat (LRR) is linked to phosphorylated substrates. F box proteins govern the processes of a diverse range of cellular activities by regulating protein stability by targeting distinct dispositions. As shown by the modeling organism Drosophila, F box proteins perform significant roles in a large number of biological settings that includes tissue formation, tumor growth, and cell death (Ho et al. 2006).

5.11 Ficolins

Ficolins are the "proteins capable of recognizing pathogens," they serve as a pathogen-recognition molecule in the innate immune defense. Human collectins have a similar structure to the ficolins (M-ficolin, H-ficolin, and L-ficolin).

5.11.1 Classification and Structure of Ficolins

Three ficolins are present in humans (M, L, and H-ficolins). They are also seen in some of the mammalian species. So far "H-ficolin has only been found out in humans" (Endo et al. 2004). Ficolins are made of "homotrimeric subunits" that have lectin-like domain containing three fibrinogen-like (FBG)-domains and a collagen-like triple helix. The C. rynchops venom gland contains two proteins that have homology of sequences to the ficolins. These proteins were given the names ryncolin-1 and -2, and the snake venom protein family was named as "veficolins." They are structurally similar to collectins; however, their "collagen-like stalks are

followed by a domain comparable to the fibrinogen b and g chains" (Matsushita and Fujita 2001).

5.11.2 Functions of Ficolins

Ficolins bind to the MASPs, thereby "activating the lectin-complement pathway upon recognition of neutral carbohydrates and N-acetyl groups on pathogens and damaged cells". The H- and L-ficolins have been shown to "increase macrophage adhesion and late apoptotic cells uptake". M-ficolin functions as a phagocytic receptor and it may also participate in monocyte adhesion. The abnormal expression of ficolins play a critical role in the pathogenesis of human diseases such as "infectious and inflammatory diseases, recurrent respiratory infections, apoptosis, and autoimmune disease; systemic lupus erythematosus; IgA nephropathy; clinical syndrome of preeclampsia". The precise identification of the functions of ficolins will definitely provide a novel insight into the pathogenesis of these diseases, thus providing a nonspecific immune therapeutic options to treat the progression of these diseases.

5.11.3 Tachylectins

Proteins that are related to tachylectin are a fibrinogen-type lectin that play a significant part in the nonspecific immunity of a large number of animals, from ancient sponges to vertebrates.

5.12 Chi-Lectins

The chitinase-like lectins are part of glycoside hydrolase family 18, show sequence homology with mammals. They lack chitinase activity but have active carbohydrate-binding site (Bussink et al. 2006). The domain of chi lectin having a triose-phosphate isomerase (TIM) barrel structure (Houston et al. 2003).

YKL-40 the chitinase 3-like protein 1, found in mammals, expressed on neutrophils endothelial cells of blood vessels, smooth muscle cells, hepatic cells, etc. YKL-40 is a chitinase-deficient protein that binds chitin and chitin derivatives via active site cleft (Fusetti et al. 2003). YKL-40's putative actions is linked to various diseases that include malignancy, arthritis, and cirrhosis. The non-glycosylated CHI3L2 or YKL-39 is involved in the production of chondrocytes in humans. They are present in various internal organs such as lungs, heart, brain, spleen, pancreas (Steck et al. 2002). It is associated with immune response in rheumatoid arthritis (Du et al. 2005) and progression of osteoarthritis (Knorr et al. 2003). The

murine proteins Ym1 and Ym2 homologous to chitinase produced in mastocyte, ACPs, and macrophages. Ym1 shows specificity towards the glycoproteins such as glucosamine and its polymers, galactosamine without binding with Ca 2+ in acidic condition. Ym1 is involved in anti-inflammation, healing of wounds, and fighting parasitic infections. Ym2, similar to Ym1 protein whose function is unknown but they are highly produced in allergic lungs.

5.13 F Type Lectin

The most recently discovered lectins are the F type lectins (FTLs). These are proteins which bind to fucose and has a fucose recognition domain. The “F type” fold is a new structural fold with unique fucose and calcium binding sequence patterns.

5.13.1 Structure of F Type Lectins

The F type lectin structure is comprised of a β-barrel with jelly-roll topology having two β-sheets of three and five antiparallel β-strands, respectively, placed against each other. On the barrel’s “top” face, the connecting-strands from the opposite-sheets create five loops (CDRs 1–5) that encircle the extremely positively charged cleft that connects the alpha L-Fuc. The “bottom” of the barrel is closed by two short antiparallel strands. A substructure with three 310 helices near the side of the barrel firmly connects a cation, such as calcium, which helps to stabilize the structure.

5.13.2 Functions of F Type Lectins

The importance of FTLs from fifnish as opsonins for prospective bacterial pathogens in nonspecific immunity was discovered, as were the Streptococcus FTLs as virulence factors (lectinolysins) and the sperm acrosomal proteins (bindins) of Pacific Oyster (*Crassostrea gigas*) and other wide range of functions, including fertilization.

Using biochemical, molecular, immunological, and structural approaches, only a few examples of FTL biological roles have been investigated experimentally. Most FTLs, on the contrary, have putative activities depending on their binding affinity for cell or tissue localization, endogenous or exogenous (viral, microbial) glycosylated ligands, and cues that influence their gene expression, such as immunological challenge or environmental stresses (Bianchet et al. 2002).

5.13.3 Anguilla anguilla *Agglutinin*

Anguilla anguilla agglutinin (AAA) is a fucolectin. They are found in the European eel. It is used in histochemistry and blood typing as it binds exclusively to terminal fucose residues in antigens of certain blood group. However the physiological function of AAA in the nonspecific immune system is to act as a serum pathogen recognition molecule where it recognizes bacterial lipopolysaccharides.

5.14 Intelectins

Fibrinogen-like domains (FRED) and region specific for intelectin are found in these proteins. Yan et al. (2018) report that the carbohydrate recognition domain (CRD) of this protein has just recently been defined. HITLN1 (human intelectin one) and XEEL (Xenopus embryonic cuticular glycoprotein) molecular structure are now known. This information is crucial for understanding the carbohydrates that these proteins bind and how they function (Wangkanont et al. 2016). It is interesting to note that birds and dogs are the only animals that lack intelectin genes Linda Toh and Christopher Wade (Lindblad-Toh 2005).

5.14.1 Classification of Intelectins

ITLN1-like and ITLN2-like intelectins in mammals are distinguished by their N-terminal main sequence and size (313 versus 325) (Yan et al. 2013). It is the first type of intelectin to be discovered in mouse Paneth cells (Itln1). Itln1-like variables are recognized in several mouse strains, but there are no ITLN2-like factors, while humans only have the ITLN1 gene and an Itln2 gene (Pemberton et al. 2004; Lu et al. 2011). As well as the small and large intestines, ITLN1 is expressed in other tissues, such as fat deposits, for which the term "omentin" is commonly used (Yang et al. 2003; Barrett et al. 2008).

5.14.2 Intelectins in Fish

ZINTLs (zebrafish interlectin-like proteins) were studied by Lin et al. (2009), who described 7 distinct zINTLs and compared them to those from other organisms. In one or more tissue types, zINTL1–3 are highly expressed. Humans and various developmental phases, on the contrary, expressed zINTL4–7 at relatively modest amounts. This has resulted in species-specific super molecule architectures and signaling pathways (Lin et al. 2009).

5.15 Conclusion

Lectinsanimal-derived lectins contain diverse range of chemicals that not only attach to carbs but also bring up novel and useful biotech possibilities. Despite the diversity of animal lectins, it is notable that the capacity to bind saccharides is frequently contained in a separate domain (the CRD) that acts independently of the remainder of the molecule. Lectins are utilized in a multiple areas, including the cell differentiation, cell and tissue histochemistry, separation of glycoproteins, and tracing cell surface pathways. Immunologists are fascinated by these molecules because of their ability to interact with other molecules. Immunologists are intrigued by these compounds due to their ability to associate with lymphocyte and stimulate transformation of blast cells. As we learn more about how lectin-carbohydrate interactions work, we are finding some interesting new targets for drug design and therapeutic targeting.

Conflict of Interest The authors have no conflicts of interest to declare.

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Chapter 6
Plant Lectins

Abdullah Bin Abdul Nazar, Aneetta Skinner, Debarghya Ghosh Dastidar, and Preetham Elumalai

Abstract Lectins are a group of proteins with specific carbohydrate binding proteins that are found abundant in nature, especially within the kingdom Plantae. Several plant lectins are isolated and well characterised within the chemical, physico-chemical and biological properties. Plant lectins have the potential to agglutinate red blood cells with specific carbohydrate entities due to the existence of a minimum of one non-catalytic domain that binds reversibly to specific recognised monosaccharides or oligosaccharides without a change it's moiety. In this chapter, we discuss plant lectins, a wide ranging and well characterised group which were discovered 100 years ago. It includes history, production, purification, classification, function and also the application of plant lectins. Most of the plant lectins show extensive biological responses like the need for anti-insect, anti-viral, anti-fungal and immunomodulatory functions in modern research. Plant lectins have proved their potential in crop improvement and also their wide use in biomedical research.

Keywords Plant lectins · Phytohaemagglutinin · Erythrocytes · Carbohydrate binding · Agglutinin

Abbreviations

AIDS	Acquired Immunodeficiency Syndrome
BMGY	Buffered complex glycerol medium
cDNA	Complementary DNA
ConA	Concanavalin A
E. coli	*Escherichia coli*
GalNAc	*N*-Acetylgalactosamine

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GBL	Galactose-binding lectin
GMP	Good manufacturing practices
HeLa cells	Henrietta Lacks cells
HIV	Human Immunodeficiency Virus
HTLV	Human T-lymphotropic Virus
LPS	Lipopolysaccharides
MCF7	Michigan Cancer Foundation-7
PEG	Polyethylene glycol
RCA	*Ricinus communis* agglutinin
RIP	Ribosome inactivating proteins
SBA	Soybean agglutinin
SBL	Soybean lectin
UEA	*Ulex europaeus*
WGA	Wheat Germ Agglutinin

6.1 Introduction

The term 'lectin' was introduced by Shapleigh and Boyd in 1954 to denote a certain group of proteins with carbohydrate binding properties. The lectin adapted from the Latin word legere which denote to choose or to pick out. The term 'haemagglutinins' is used to denote them when their carbohydrate specificity is not known.

Over the past decade, many definitions were put forward to explain about plant lectins and their functions. The earlier concept focused mainly on the ability of plant lectins to agglutinate erythrocytes, but by chance, a range of proteins was identified with few carbohydrate binding sites which have the potential to link red blood cells via lectin molecules. Some lectins have multiple binding sites, contrarily, few molecules show only a partial cohesive property (Goldstein et al. 1980). Chimeric molecules are a group of protein compounds with a number of protein domains in which one of the binding sites exhibit lectin property. Based on all the above mentioned arguments, Peumans and Van Damme came up with a new definition as 'all plant proteins processing a minimum of one non-catalytic domain which binds reversibly to a particular mono- or oligosaccharide are considered to act as lectin molecules'. Under the new proposal a wide range of proteins or protein domains processing the ability to link carbohydrate via a binding site specifically fall under the category of lectin. Despite our knowledge regarding the sugar-binding property of lectin this definition is widely accepted (Peumans and Van Damme 1995).

Plant lectins are used as a reference to check sugar–protein interactions and a sophistical tool for free morpheme or carbohydrates bound by lipids or proteins. Plant lectins also play a role in transfer of drugs to the site of action due to the ability to bind with carbohydrates specifically. Many studies show the anomalous behaviour of plant lectin in the maintenance of biological systems. In addition plant lectins also show illustrative role in clinical diagnosis and sugar structure and (Sharon and Lis 2004).

6.2 History

Plant lectin, also referred to as phytohaemagglutinin, has become a topic of discussion long back in history. During the nineteenth century certain protein molecules with agglutinating properties were identified. By the end of 1960s certain other proteins were also discovered which can agglutinate other cells also. It is believed that primary lectin was identified by Peter Hermann Stillmark in the year 1888. Around the same year, a toxic protein called a ricin was identified in oilseed (*Ricinus communis*) with some agglutinating properties towards erythrocytes. It was observed that some erythrocytes bind together when treated with this protein extract purified to a certain extent. This provides a basic idea of certain proteins with agglutinating properties. The term agglutinin was used by Elfstrand in 1898 to describe the property of some proteins to assemble erythrocytes (Elfstrand 1898). Meanwhile, it was found that not all proteins with agglutinating properties are toxic Landstainer and Raubitschek put forward non-toxic lectins in legume plants, specifically bean, garden pea and *Viscia sativa*. Since the discovery of lectin molecules a wide range of research has been carried out regarding their functions, structure and its biological role. Only by 1902 the ability of lectins to recognise and bind to the specific carbohydrate was noticed.

In 1902, Karl Landsteiner observed plant lectins bind to specific red blood cells and this paved the way for the discovery of A, B and O blood groups in human (Landsteiner and Raubitschek 1907).

During the 1950s, scientists identified the reason for certain molecules binding to some specific cells. Sumner and Howell could inhibit binding nature of lectins using sugarcane syrup (Sumner and Howell 1936). By the start of 1952, Watkins and Morgan established the connection of agglutinating property and their ability to recognise carbohydrate moiety on the surface of red blood cells, hence proving the specific binding nature of lectins (Watkins and Morgan 1952) (Fig. 6.1).

After the discovery of primary plant lectins, numerous plant lectins have been recorded (Etzler 1986). Most researchers concentrate on legume plants for lectin research due to its abundance in nature (Sharon and Lis 1990). Subsequently, Concanavalin A, one among the primary lectins, was identified from the seeds of a bean *Canavalia ensiformis*. Towards the end of 1980s, researchers shifted towards storage tissues (such as corms, rhizomes and roots) for the identification of plant lectins, this enabled identification and characterisation of more lectins with a wider range of sugar agglutinated property.

Research in 1980 concentrated more on molecular properties of lectins including the nature of composition, peptide sequencing and advancement in their 3-D structure (Etzler 1985). The complete polypeptide sequence of concanavalin A and its 3-D structure was identified for the first time in history. (Edelman et al. 1972) During 1983, soybean lectin was cloned. The advancement in biomedical science made us to know more about the plant lectin and their functions (Hardman and Ainsworth 1972). Due to technological growth, the idea of lectin expands to its molecular level and their specific binding property. The availability of lectin samples made

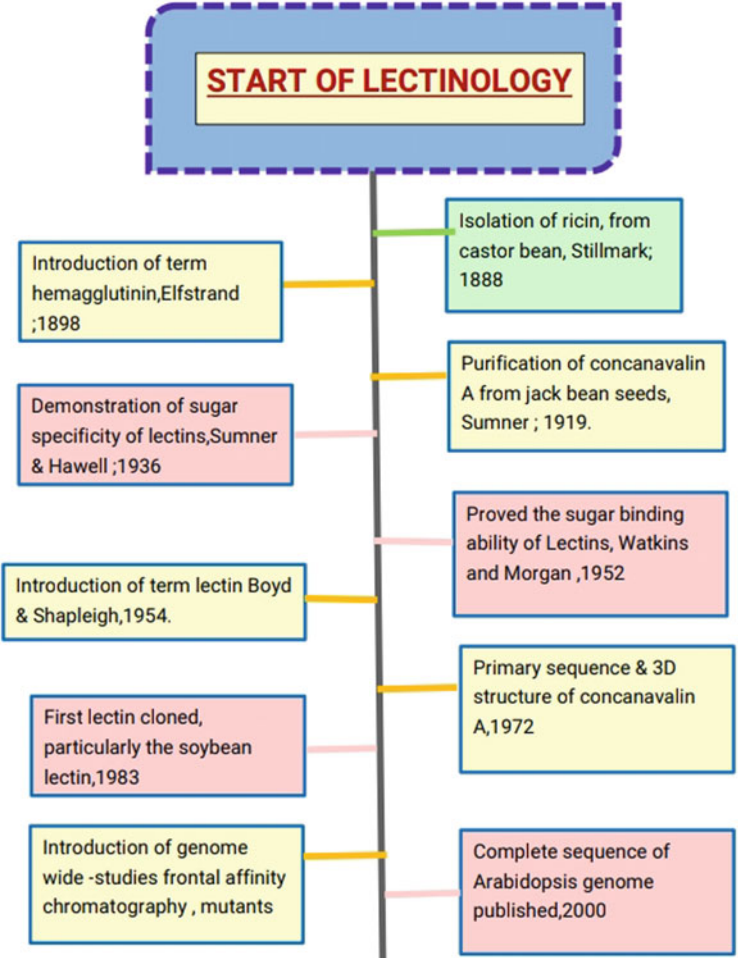

Fig. 6.1 Schematic diagram showing most important hallmarks in lectinology starting from the discovery of ricin. (Adapted and modified with the permission of Mariya Tsaneva, Els J. M. Van Damme (2020): 130 years of plant lectin research, pp 2, review article)

their studies easier both at molecular and physico-chemical levels. (Vodkin et al. 1983) Advancements achieved in the field of biochemistry allowed us to know more about lectins including their chemical properties, polypeptide sequences and molecular cloning of cDNA. (Van Damme et al. 1998).

Achievements made in molecular science helped in sequencing the polypeptide chain of lectins. Genome level studies showed evolutionary establishment of lectins and their mode of expression, hence leading to their physiological role.

6.3 Classification

The group of plant lectins is very diverse which can be analysed by the specificity of different legume lectins on different carbohydrates. The legume lectins, the largest group of lectins, show a wide range of carbohydrate specificity, create the classification process a challenge (Sharon and Lis 1990; Strosberg et al. 1986). In the 1970s and 1980s, studies were conducted to categorise lectins based on their sugar specificity, and categories such as mannose-binding lectins, *N*-acetylglucosamine binding lectins, fucose-binding lectins and so on. This method was later abandoned because it failed to account for molecular and taxonomic links among plant species. Later in 1998, a new system was developed considering the sequences of plant lectins and that system grouped the majority of plant lectins into seven structurally and evolutionarily related protein families (Van Damme et al. 2008). This classification algorithm worked well, although it needed to be updated numerous times as additional sequence data became available. The plant lectin classification is still evolving and an accepted system is not accomplished.

The classification of plant lectins is mainly done on three bases.

1. Based on the number of carbohydrate binding sites.
2. Based on structure and evolution.
3. Based on sugar specificity (Fig. 6.2).

6.3.1 Based on the Number of Carbohydrate Binding Sites

1. Merolectins.
2. Hololectins.
3. Chimerolectins.
4. Superlectins (Fig. 6.3).

6.3.1.1 Merolectins

Merolectins are lectins that have only one sugar-binding domain. They cannot precipitate the agglutinate cells due to their monovalent nature. Only a few merolectins have been identified so far. The chitin-binding hevein isolated from the latex of rubber plant (*Hevea brasiliensis*) has hevein as the carbohydrate binding domain with GlcNAc specificity is an example. The monomeric mannose-binding proteins from orchids are the other example (Van Parijs 1991).

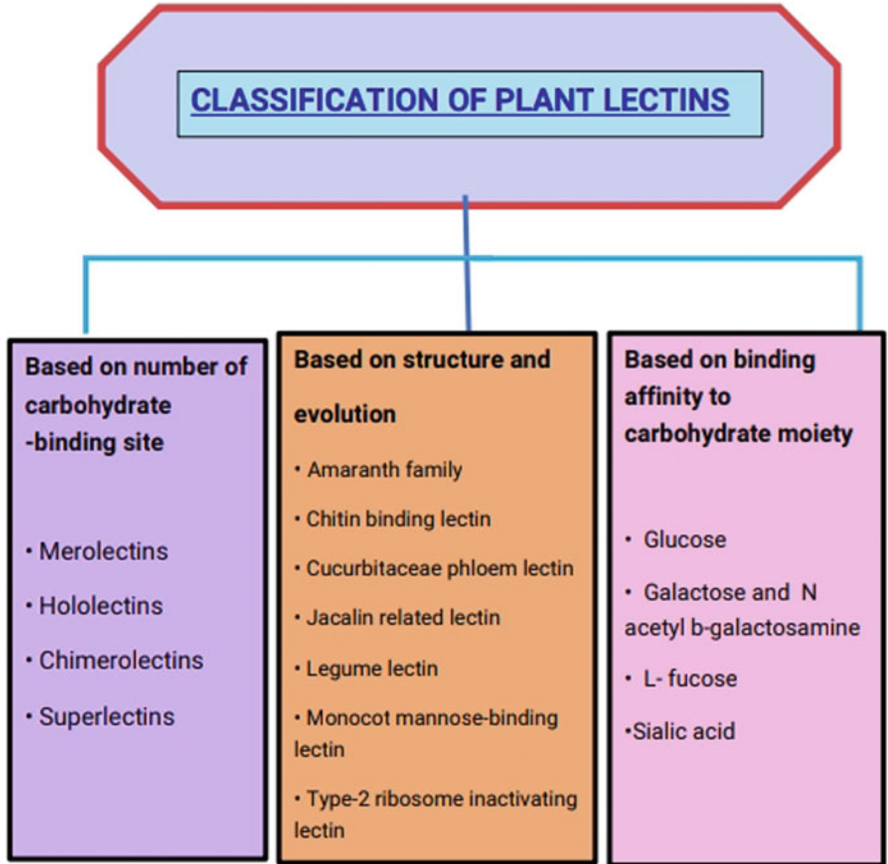

Fig. 6.2 Schematic representation of the classification of plant lectins into three based on number of carbohydrate binding sites, structure and evolution and on sugar specificity. (Rohan Dhinam (2019) Structure-function and application of plant lectins in disease biology and immunity. Published as a part of Food and Chemical Toxicology)

6.3.1.2 Hololectins

Hololectins have multiple binding sites which are nearly identical and bind either the same or structurally similar sugars. Due to multiple binding sites (divalent or multivalent nature), they are capable of precipitating glycoconjugates or agglutinating cells. The majority of plant lectins are grouped as hololectins.

6.3.1.3 Chimerolectins

Chimerolectins are fusion proteins that contain a sugar-binding domain that is attached to another unrelated domain which possess a specific catalytic or enzymatic

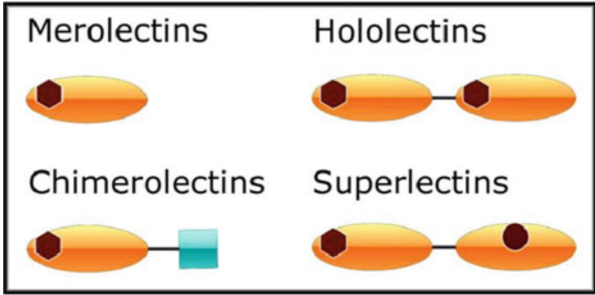

Fig. 6.3 Classification of plant lectins based on the number of carbohydrate binding sites. The orange eclipse represents the lectin domain while the blue square represents another protein domain. The hexagon or circle represents different carbohydrate binding sites in the lectin domain. (Adapted with permission from Mariya Tsaneva, Els J. M. Van Damme et al. (2020): 130 years of plant lectin research, pp 3, review article)

activity and functions independently of the other domain. They can act as merolectins or hololectins depending on the number of carbohydrate binding sites available. Chimerolectins with multiple carbohydrate binding sites behave as hololectins while with single site behaves as merolectins. Type 2 RIP act as hololectins, as they are multivalent and can agglutinate cells. Class 1 plant chitinases are monovalent and do not behave like merolectins (Barbieri et al. 1993; Collinge 1993).

6.3.1.4 Superlectins

Superlectins are a type of hololectin that is also classified as a subgroup of chimerolectins. They are fusion proteins made up of two carbohydrate binding domains which are structurally different and bind structurally dissimilar sugars. The only superlectin that has been described is TxLCI, lectin extracted from the tulip bulbs that contain two dissimilar sugar-binding sites that specifically binds mannose and GalNAc sugar residues (Van Damme et al. 1997).

6.3.2 Based on Structure and Evolution

1. Amaranthin family.
2. Chitin-binding lectins.
3. Cucurbitaceae phloem lectin.
4. Jacalin-related lectin.
5. Legume lectin.
6. Monocot mannose-binding lectin.
7. Type 2 ribosome inactivating lectin.

6.3.2.1 Amaranthin Family

The lectins of this family are solely derived from seeds of the Amaranthus genus (Koeppe and Rupnow 1988; Zenteno and Ochoa 1988). It was first extracted from the seeds of *Amaranthus caudatus* giving the name Amaranthin to its family. Amaranthin domain has homodimer β-trefoil structure with GalNAc specificity showing higher affinity for T-antigen disaccharide Gal β (1,3) GalNAc (Transue 1997; Rinderle 1989). Despite the fact that amaranthins had long been thought to be distinctive to the Amaranthaceae family, amaranthin related sequences have been found in 34 of the 84 examined plants which include ferns, lycophytes and gymnosperms. They are hololectins, but there are reports suggesting the presence of amaranthin domain in chimerolectins tagged with aerolysin domain.

6.3.2.2 Chitin-Binding Lectin

Chitin, a polymer of *β*-1,4-*N*-acetylglucosamine, present in the cell wall of most fungi and the principal component of the exoskeleton of some insects. The chitin-binding lectins contain all the proteins with at least one hevein domain. The 'hevein' domain, a 43 amino acid protein, mostly extracted from the latex of the rubber tree (Hevea brasiliensis), with GlcNAc specificity. The chitin-binding lectin, Wheat Germ Agglutinin (WGA) were first isolated and characterised by Nagata and Levine group (Nagata and Burger 1972; LeVine et al. 1972; Wright 1990). Gramineae lectins, such as those from the embryos of wheat (WGA), barley (BL) and rice (RL), are composed of four domains while the lectins from *Urtica dioica* rhizomes (UDA) are composed of two domains (Beintema and Peumans 1992). The class I chitinase from bean (*Phaseolus vulgaris)* belongs to the chitin-binding lectin not legume lectins.

6.3.2.3 Cucurbitaceae Phloem Lectin

The cucurbitaceae phloem lectins, commonly found in phloem of the cucurbitaceae family, are the sub-family of chitin-binding lectins. It is also known as pumpkin lectins as it was first isolated from pumpkin (*Cucurbita maxima*) (Liu 1996). Later the researches led to the isolation of this lectin from the phloem of different generas of cucurbitaceae such as Cucurbita, Citrullus, Cucumis, Luffa and Coccinia species (Allen 1996; Read and Northcote 1983). The Cucurbitaceae phloem lectins are considered to be defence proteins.

6.3.2.4 Jacalin-Related Lectins

The lectin from the seeds of jack fruit (*Artocarpus integrifolia*) is commonly named as jacalin. At present, this group of lectin is considered as the collective group of all lectins that are structurally and evolutionary associated with the jack fruit lectin. There are two subgroups of lectins in the jacalin family. Firstly, the GalNAc-specific Moraceae seed lectins, which are extremely similar to the jack fruit lectin, and secondly, the Convolvulaceae lectins having sequence similar to that of the Moraceae lectins but maltose/glucose specific (Van Damme 1999). The presence of potent haemagglutinin in the seeds of Maclura pomifera discovered by Jones J. M. Cawley in 1967 was the first evidence of lectins from jacalin family.

6.3.2.5 Legume Lectins

The legume lectins refer to the lectins found in Leguminosae. It should be noted that all the lectins found in legume species do not belong to the legume lectins. It is the largest group containing 100 legume lectins isolated from 70 different species of this family. The first legume lectin was isolated from the bark of the legume tree black locust (*Robinia pseudoacacia*) in 1890 by Power and Cambier (Liener 2012) and the seed lectins from *Abrus precatorius* belongs to the type 2 RIP.

6.3.2.6 Monocot Mannose-Binding Lectin

Monocot mannose-binding lectin, widely seen in the monocotyledonous plants, belongs to the superfamily of mannose specific lectins. It was first isolated from snowdrop bulb (*Galanthus nivalis*) called *Galanthus nivalis* lectin or agglutinin (GNA) (Van Damme et al. 1987; Wright 1990). These lectins are mostly seen in the vegetative tissues such as leaves, flowers, roots, bulbs, tubers, rhizomes (Van Damme et al. 1995) and even in nectar. Monocot mannose-binding lectins have been found in six different monocot families, namely Alliaceae, Amaryllidaceae, Araceae, Bromeliaceae, Liliaceae and Orchidaceae.

6.3.2.7 Type 2 Ribosome Inactivating Lectin (RIP)

Ribosome inactivating proteins (RIP) are proteins that inactivate eukaryotic ribosomes catalytically (Barbieri et al. 1993). There are two types of RIPs: type 1 and type 2. Type 1 RIPs are single chain proteins with adenosine glycosidase (PAG) activity, while Type 2 RIPs are composed of two polypeptide chains that are functionally and structurally distinct A and B chains with enzymatic and carbohydrate binding activities, respectively. Type 2 RIP belongs to the chimerolectin family. Type 1 RIPs are commonly seen in families such as Cucurbitaceae,

Euphorbiaceae and Fabaceae and Type 2 RIPs in families Euphorbiaceae (*Ricinus communis, Croton sp.),* Leguminoseae (*Abrus precatorius*), Viscaceae (*Viscum album* and *Phoradendron californicum*), Lauraceae (*Cinnamonum camphora*), Cucurbitaceae (*Momordica charantia*) and Iridaceae (*Iris sp.).*

6.3.3 Based on Sugar Specificity or Binding Affinity to Carbohydrate Moiety

1. Mannose-binding lectins.
2. Galactose and *N*-acetylgalactosamine binding lectins.
3. *N*-acetylglucosamine binding lectins.
4. *N*-acetylneuraminic acid binding lectins.
5. Fucose-binding lectins.

6.3.3.1 Mannose-Binding Lectins

Mannose-binding lectins have their carbohydrate binding domain found in association with collagenous structures with its range of sugars including glucose, mannose, fucose, *N*-acetyl-glucosamine and so on. Some of the examples include Concanavalin A (ConA) isolated from *Canavalia ensiformis*, Lentil lectin (LCH) from *Lens culinaris* and Snowdrops lectin extracted from *Galanthus nivalis.*

6.3.3.2 Galactose/*N*-Acetyl Galactosamine Binding Lectins

Galactose-binding lectin (GBL) binds the carbohydrate sequence Gal-β (1,3)-GalNac. Champedak fruit (*Artocarpus integer*) is a rich source of CMB (champedak mannose-binding) and CGB (champedak galactose-binding) that binds to mannose and galactose, respectively. Some of the lectins and their sources are as follows.

- Ricin, *Ricinus communis* Agglutinin, RCA 120 from *Ricinus communis*.
- Peanut agglutinin from *Arachis hypogaea.*
- Jacalin isolated from *Artocarpus integrifolia.*
- Hairy vetch lectin extracted from *Vicia villosa*.

6.3.3.3 *N*-Acetyl Glucosamine Binding Lectins

This lectin was first identified in Wheat Germ Agglutinin (WGA) isolated from *Triticum vulgaris* with Neu5Ac (sialic acid) as the ligand motif. It is a disulphide rich domain. Soybean *(Glycine max*) agglutinin (SBA), also known as soybean lectin (SBL), binds *N*-acetyl-D-galactosamine/galactose.

6.3.3.4 Fucose-Binding Lectins

Ulex europaeus lectin (UEA I) binds L-fucose and recognises the Fuc-α-1-2-Gal linkage in the chain. It shows great homology with some other legume lectins. The Ulex europaeus seeds possess two agglutinins: *Ulex europaeus* I (UEA I) and *Ulex europaeus* II (UEA II). *Lotus tetragonolobus* is another example of gucose-binding lectin but it shows no sorts of similarity with UEA I (Wick and Hornick 2011).

6.4 Production of Plant Lectins

Plant lectins, due to their outstanding antitumour properties, are widely used for biomedical applications. Plant lectins have numerous biological activities leading to their complex interaction and protein structure leading to their diverse interaction with carbohydrates. DNA recombinant technology is adapted for the production of newly found plant lectins.

6.4.1 Recombinant Plant Lectins

Most of the plant lectins can be synthesised using recombinant technology. Table 6.1 presents the source and yields of few plant lectins. Plant lectin yields have increased up to several folds due to the advancements in their production methods. For the production of a moderate quantity of lectin, fermentation is carried out in GMP condition. *E. coli* is commonly used for this. A variety of strains such as BL21 (DE3) RIL strain is used in expressing various lectins. It is of utmost importance to carry

Table 6.1 Yields of plant lectins produced by recombinant DNA techniques. (Upadhyay et al. 2010; Oliveira et al. 2008)

Natural source of lectin	Lectin yield (mg/L culture medium)	Genetically modification in cells	Reference
Allium sativum (garlic) leaf	5	cDNA was cloned into Ndel and BamHI restricted plasmid pET19b and expressed in *E. coli* strain BL21 (DE3) cells	Upadhyay et al. (2010)
Artocarpus incise (breadfruit)	16	cDNA was cloned into the pET-25b(+) and expressed in *E. coli*	Oliveira et al. (2009)
Artocarpus incise (breadfruit)	18–20	cDNA was cloned into EcoRI/XbaI restricted plasmid pUC57 and expressed in *E. coli*	Oliveira et al. (2008)
Nicotiana tabacum (tobacco) leaves	6	cDNA was cloned EcoRI/NotI restricted plasmid and expressed in *E. coli* strain top 10F	Lannoo et al. (2007)

out this process in a sterilised condition like Luria-Bertani medium, along with the supply of antibiotics, e.g., ampicillin, to ensure a contamination free environment. Fermentation is usually done under optimised temperature, moisture, PH and inducer concentration. Isopropyl β-D-thiogalactoside is the commonly used inducer. This is followed by centrifugation, resuspension with lysis buffer and sonication for harvesting the lectins from the cells. Additionally, chromatographic methods are adapted for their purification (Tateno et al. 2004).

6.5 Purification of Plant Lectins or Haemagglutinins

Plant lectins serve as a tool for drug recovery, diagnosis and even used as therapeutic drugs against various diseases. All these growing demand for lectin emphasises on the rapid isolation, wide scale manufacture as well as an alternate pathway for their purification. Among the discovered lectins only very few are extracted and purified. This purification is carried out by primitive methods using reagents and solvents. Around 2005, Zhang and his colleague purified ricin B by using PEG-sodium sulphate from ricinus communis. His experiment showed that the quality and strength of ricin B increased when splitting it to the top phase and no degradation of protein was observed in contrast with the same experiment conducted in hairy root culture method, where 60% of protein degradation was reported. Usage of PEG is regarded as the cause behind the extra stability of proteins in top phase purification (Zhang et al. 2005).

One of the outstanding methods developed recently is that of chromatographic techniques, with which a range of lectins can be purified easily. This technique includes various methods for the purification of undefined lectin such as ion-exchanging, gel filtration, hydrophobic interaction and affinity chromatography. Chromatography is carried out in multiple steps. Affinity chromatography involves a single step in the purification of lectin. A slight reduction in the lectin recovery yield is seen with the increase in the purification steps. A steady decline is observed in the recovery period as the number of steps goes on increasing. Precipitation with ammonium sulphate followed by affinity chromatography is commonly used to purify lectins.

Recent advancement in the field of technology and the rise of genetic engineering helped in the production of lectins on a larger scale through DNA recombinant technology. Conventional methods cannot produce lectin on a large scale and are not economically compatible. Modern advancement make use of methods such as:

(a) *Magnetic separation*—It uses the help of nanoparticles with compatible size and their ability to bind with organic molecules. Also, these nanoparticles can be linked with some ligands for their binding with biological moiety. These linked nanoparticles can be separated with the help of magnetic fields enabling them to separate from other solutions.

(b) *Affinity precipitation*—It separates the target molecule or protein with the help of an affinity micro ligand due to it's selective nature. This precipitation is mainly used in the purification of chitin-binding lectin (Naeem et al. 2007).

The amount of lectin varies according to the organism. High amount of lectin indicates their mass production and utilisation. Plant lectins find application as a drug against microbes and also in tumour therapy.

6.6 Functions of Plant Lectins

Recent studies on plant lectins show that they have a wide-spread physiological role in recognition of pathogens, overcoming environmental stress, defence mechanism, out of which two functions are researched in depth: (a) interaction between plants and microbes and (b) protective function of lectins in preventing plant infections.

Lectin acts as a carrier for carbohydrates or as their storage molecule. Lectins have the property of agglutinating cells which is mostly used for the defence mechanism and this property is considered crucial for both symbiotic as well as in microbe–host interactions. Plant lectins are used to search for specific cell receptors on the cell surface of bacteria and other microorganisms. They have the ability to detect glyco-conjugate molecules present in the surface of cells which is useful for their separation and analysing their structure. They are of great importance in cell to cell signalling and interaction (Mirelman et al. 1975).

Furthermore, lectin molecules act as a defence molecule by protecting plants from pathogens and predatory organisms. They help in the symbiotic relationship between nitrogen fixers and host plant species. Lectins on the cell surface of legume plants bind to the target molecules present on the surface of nitrogen fixing bacteria. This recognition is crucial for the synthesis of infection thread which serves as an important link for nitrogen fixation in legume plants (Barkai-Golan et al. 1978).

Plants identify their pathogens based on the particular arrangement of carbohydrate molecules and their peculiar structure present at the cell surface of pathogens using immune receptors at the intracellular level. Plant lectins contain protein domains which has the role of immune receptors in plants. Upon various studies, many nucleocytoplasmic lectin molecules have been identified. This type of lectins is released in response to the changing environmental factors to overcome the stress faced by the plants. (Mishkind et al. 1982).

6.7 Lectin Applications

Lectins exhibit an extensive mode of actions, including pathogen management (antibacterial, antiviral, antifungal and so on), medicinal properties and technological advancements. The main attribute of lectins is their ability to identify specific carbohydrate chains, which makes them unique for use in a variety of applications.

6.7.1 Pathogen Controlling Strategies

6.7.1.1 Insecticidal

Bio-engineering is well used in lectins comprehensively for modifying crops, comprising of potatoes, tobacco, rice and wheat that is seen as one of the potential medium to counter insect menace. The said method can be employed amounting to an overall pest control strategy or to combat caveat pests. By attaching to the membrane protein vesicle of stomach brush boundary, lectin from *Arum maculatum* tuber led *Aphis craccivora Lipaphis erysimi* die (Majumder et al. 2005). Lectin added to artificial diet obtained from the leaf of *Bauhinia monandra* killed *Zabrotes subfasciatus* and *Callosobruchus maculatus*.

6.7.1.2 Fungicidal

Plants produce lectin, which is a key defensive protein. Attachment of glucoconjugates over membranes of fungi by fungal or plant lectins have been negatively impacted due to cell wall barrier. Lectins bonding to carbohydrates over cell wall surface of fungi, on the other hand, may have indirect effects.

Stinging nettle (*Urtica dioica* lectin which is chitinase-free chitin-binding lectin) inhibited fungal growth. This result in reduced chitin deposition, disrupting cell wall synthesis. The consequences that lectin of nettle have fungal cell walls and morphology of hyphae tell us that nettle lectin controls rhizome endomycorrhizal colonisation. The development of *Colletotrichum lindemuthianum*, *Botrytis cinerea, T. viride* spores was dramatically lessened following the precursor gene introduction pertaining to stinging nettle named isolectin I inside tobacco (Does et al. 1999). As a result, lectins could be used to defend plants from fungal infection.

6.7.1.3 Antiviral

Lectins can be utilised as an antiviral medicine because they interfere with the virus's biochemical and structural features. The D-mannose-particular lectin out of *Gerardia savaglia* is first to be shown for protecting H9 cells from HIV-1 infection. Furthermore, by reacting along the side chains of oligosaccharide pertaining to the HIV-1 gp120 envelop molecule (high-mannose oligosaccharides), this lectin inhibited syncytium development in the cell system of HTLV-IIIB/H9 Jurkat and HIV-1/human lymphocyte. As a result, lectins could be used to treat AIDS. In the severe acute respiratory syndrome coronavirus, plant lectins, particularly mannose-binding lectins, showed anti-coronaviral action. They disrupted viral binding during the initial stages in the duplication cycle and inhibited viral manifestation at the climax of the virulent cycle (Keyaerts et al. 2007).

6.7.1.4 Anti-Microbial Activity

Plant lectins are commonly found at potential microbial invasion sites, where their association along glyco-components on the cell membrane surface of microbe inhibits its growth, adhesion and migration. Microorganism agglutination and immobilisation are two methods by which they exercise their microbicidal activity (Lagarda-Diaz et al. 2017).

6.7.1.5 Anti-Parasitic Activity

Plant lectins have been shown to influence parasites that cause illnesses such as *Giardia lamblia, Trypanosoma cruzi*, *Tetrahymena pyriformis* and *Leishmania* spp. among others, due to their potential to function as adjuvants. Plant lectins have an anti-parasitic effect due to their ability to bind to particular sugars found in parasites, interfering with downstream cellular processes (Iordache et al. 2015). Jacalin, a lectin secluded out of the seeds taken from jackfruit, displayed to act as an adjuvant which alters cellular and humoral immunity.

6.7.1.6 Antibacterial Activity

Plant lectins' antibacterial activity is related to their capacity to associate with a range of composite carbohydrates found above bacteria's surfaces. Peptidoglycans, techoic acid and lipopolysaccharides are the most common carbohydrates that engage with the lectin's glycan binding site via hydrogen bonding. In both gram negative and positive bacteria, this interaction causes morphological changes that lead to hole development and cell wall bubbling, respectively (Hamid et al. 2013). Bacterial adhesion and invasion were also inhibited by lectins.

6.7.2 Medicinal Purposes

6.7.2.1 Cancer Treatment

Antitumour Drugs

Lectins have been shown to have antitumour properties. Lin et al. (2008) found that lectin of glossy, small, black soybean inhibited the multiplication of breast cancer and hepatoma cells, namely MCF7 cells, HepG2 cells, respectively (Cheung et al. 2009). Lectin of Del Monte banana inhibited the proliferation of hepatoma (HepG2) and (L1210) cells. Cancer patients can benefit from mistletoe lectin to improve their quality of life. Apoptosis is influenced by lectins in various types of cancer cell. Lectin of Korean mistletoe cured B16-BL6 melanoma cells and human A253 cancer

cells (Choi et al. 2004) and recombinant galectin-9 resistant to protease cured myeloma cells are few examples.

Through Apoptosis

A lectin segregated from *Artocarpus* named Frutalin promoted apoptosis in HeLa cells by inducing stress to cell, which led to tumour cell growth inhibition. A legume lectin named Con A that attaches selectively to glucose or mannose has been shown to cause pro-apoptotic activity in PU5-1.8 cells of murine macrophages via assembly in mitochondria and release of cytochrome c (Li et al. 2008). Another study found that treatment with Con A causes mitochondrial arbitrated apoptosis in cells of human melanoma A375 by causing mitochondrial membrane potential breakdown, which leads to release of cytochrome c and activation of caspase.

Lectin Based Delivery System

After coupling with chemotherapeuticagents, lectins operate as a bearer to target tumour cells preferentially. According to one study, when nano carriers were functionalized with WGA, their binding affinity to urothelial cells increased, resulting in higher anti-neoplastic action. When lectins from seeds of *Pisum sativum* were enclosed in microbeads made of alginate, medication dispatch to hepatocellular carcinoma was improved (El-Aassar et al. 2014).

6.7.2.2 Clinical Diagnosis

To recognise samples, lectins are used because of their carbohydrate specificity. The steroid conjugated lectins hapten digoxigenin allow for immunological recognition of lectin linked structures. The carbohydrate chain is detected using lectins that particularly target the terminal sugars (Sobral et al. 2010).

6.7.2.3 Detection Using ELLA

Enzyme linked lectin assay was employed to identify a particular carbohydrate unit over the surface of unfixed cells. This technique is established along the lines of ELISA (Enzyme linked immunosorbent assay principle). In sandwich ELLA, instead of antibodies, plant-extracted lectins (for e.g. PNA) are utilised as a catching and locating reagent (Hashim et al. 2017). The amount of covered glycol-conjugates is determined by the strength of enzyme conjugated lectin. Plant Lectins are particularly specific for distinct glycan structures, hence they are used in ELLA technology to identify glycan expression profiles in various samples of tissue.

6.7.2.4 Recognition of Different Groups of Blood

Lectins out of *Vicia cracca* seeds bind to human blood group A erythrocytes exclusively. A few lectins can be used as exceptional indicator for anti-A, anti-B, anti-N and other antibodies. *Dolichus biflorus* lectin is utilised for anti-A, *Griffonia simplicifolia* lectin for anti-B. As an anti-N typing reagent, lectin out of *Vicia graminea* has been believed as useful (Khan and Khan 2011). Economic viability, on the other hand, is a major concern.

6.7.2.5 Biosensors

Lectins are used in mass, thermal, optical and electrochemical biosensors for disease diagnosis. The said approach is label-less, and electrochemical biosensors are quick, cheap and simple to use, leading to the development of a variety of methodologies. ConA is utilised in biosensors to detect glucose levels in diabetic patients and glycoproteins from dengue or norovirus patients, as well as serotypisation (Pihíková et al. 2015).

6.7.2.6 Possible Treatment of Diabetes

Diabetes is a metabolic condition in which the body produces less or no insulin and uses it incorrectly, resulting in an increase in blood sugar level. Many researches have indicated that plant lectins have a favourable effect in diabetes patients. For example, it found that treating Con A used in streptozotocin-activated diabetic mice decreased high blood sugar condition (Kolb et al. 1986). *Viscum album* aqueous extract has been illustrated to have an anti-diabetic outcome by stimulating production of insulin in clonal B cells (Gray and Flatt 1999). In alloxan-stimulated diabetic mice, lectin deriving out of *Crateva tapia* bark was discovered having anti-diabetic effect, lowering plasma glucose levels alongwith improving nephritic, hepatic consequences.

6.7.3 Other Applications

6.7.3.1 Chromatographic Type

Lectin bonded chromatography represents a chromatography type that uses gel beads to immobilise lectins. Lectins bond with glycoproteins, which are then eluted with a particular carbohydrate. This result in using it to purify glycoproteins by fractionates method centred on their unique characteristics. Lectin column chromatography (Serial) can also be used to isolate very minute quantities of glycoproteins.

Oligosaccharides can be purified quickly when used in conjunction with other separation processes (Hashim et al. 2017).

6.7.3.2 Lectin Microarray

Because of the linked carbohydrates, glycoprotein structures are challenging to decipher. A solution is provided by lectin microarray. Arrays of lectins are immobilised on a single chip. The array is hybridised with fluorescent-tagged samples. Even if the glycosylation of materials is low, details on carbohydrate configuration can be gleaned by analysing the spots-binding pattern (Pilobello and Mahal 2007). Lately, lectin microarray has been used to differentiate mammalian cells contaminated of *Cryptosporidium parvum*, an internal apicomplexan parasite. Fluorescently tagged lectins were used to probe the cells.

6.7.3.3 Detection of Glycoconjugates in Solution

The identification of glycoproteins segregated on polyacrylamide gels, both immediately or after blotting, radioactively labelled lectins and its conjugates (for e.g. with fluorescent dyes, colloidal gold, biotin or enzymes) act as discrete and subtle reagents. The insulin and epidermal growth factor receptors; glycocalicin, the predominant glycoprotein for the membrane of human platelet; C2, another component of human complement; the sulphated glycoproteins taken from the plasma membrane of calf thyroid; along with the vital glycoprotein of Ehrlich ascites cells' plasma membrane are all recent examples of lectin-purified glycoproteins (Gershoni 1985).

6.7.3.4 Detection of Glycoconjugates on Cells and Organelles

Lectin staining is useful for distinguishing between different cell types. Peanut agglutinin is used to identify immature thymocytes in mice and humans, as well as germinal centre cells and human monocytes. The same lectin may be employed such as histochemical marker for the epidermis in initial frog embryos as well as human granular cells, independent from where they are found. *U. europaeus* lectin I binds selectively to vascular endothelium, making it easier for tumour cells to recognise vascular invasion. Lectins distinguish between high and low metastatic potential cultured tumour cells (Schrevel et al. 1981).

6.7.3.5 Mapping Neurological Pathways

As the conjugates are hooked by neurons and carried inside the axons, lectins attached on horseradish peroxidase, known to act as valuable markers in mapping

central neuronal circuits. Wheat germ agglutinin when carried the couple anterograde along with retrograde, also transneuronal, whereas L-PHA, ricin conjugates are only carried anterograde and retrograde, individually. The lectin conjugates' uptake appears to be governed by receptors (Schrevel et al. 1981).

6.7.3.6 Typing in Bacteria

Currently, it is discovered that lectins may discriminate between microbial species. By agglutinating with wheat germ agglutinin, *Neisseria gonorrhoeae* may be distinguished from other *Neisseriae* and similar bacteria, and *Bacillus anthracis* can be recognised using soybean agglutinin. *Bacillus thuringiensis* serovars were divided into ten groups based on their capacity to associate with different lectins which can distinguish among pathogenic from nonpathogenic strains in respect to *Trypanosoma cruzi*, as well as between distinct morphological stages of *Leishmania donovanii*, such as amastigotes and promastigotes (Doyle et al. 1984).

6.7.3.7 Agricultural Purposes

Plants are frequently exposed to several stresses in their natural environment, including water stress, temperature stress and nutritional stress. Few plants have evolved a sophisticated line-up of physiological reactions to cope beside these environmental changes, leading to plants with improved growth capabilities and bearing. Plant lectins are a type of antigen found in the immune system of plants. Observations were made on the role of capable plant lectins and lectin with receptor-like kinases in various abiotic stress reactions. One such well-researched jacalin-related lectins is Orysata, also known as SaLT (Lannoo and Van Damme 2014).

6.7.3.8 Lectin Blotting

This type of blotting is a variation in relation to western blotting in which lectins bound with carbohydrate modules replace antibodies. Their ability to see small amounts of protein with great specificity and sensitivity is used in glycosyl specific lectin probes to detect a variety of glycan structures. It is also a very practical way to screen complicated protein samples (Lannoo and Van Damme 2014).

6.7.3.9 Flow Cytometry

This is a strong tool for determining the structural characteristics of diverse kinds of cells in a combination. It utilises chemically altered lectins to study the distinct cell surface glycan composition for various cells (Lam and Ng 2011), and this technology can also be availed for cell sorting.

6.8 Conclusion

Lectinology has contributed to the development of immunology and has a good potential for brand new developments and applications in various fields such as agriculture and modern medicine. Methods are developed to use lectin as molecular tools and for biomedical research. Introduction of high stress tolerant crops by classical breeding methods and true recombinant technologies can be like the introduction of lectins.

Lectins differ greatly in their amino acid sequence, sugar specificity, stability, relative molecular mass and number of subunits. In spite of all the differences within the properties of those lectins, most of them exhibit a standard biological function, i.e. they need antitumour, antiviral, anti-insect, antifungal, anti-parasitic and immunomodulatory activities. They were also able to inhibit microbial infections in numerous animal models because of their potential to control immunomodulation. A number of the lectins are used as adjuvants for the event of effective vaccine as they induce Th1 response. These immunotherapeutic studies have also improved our knowledge about the host–pathogen relationship and will be helpful to produce insights for the event of the latest therapeutic strategies. One drawback in the production of plant lectins is the shortage of suitable purification techniques for the production of various lectins in large quantities. We would prevail over these difficulties in the near future to provide aggregates of smaller fragments of lectins which will retain the high target specificity and the easy manipulation with the advancement in protein engineering and drug delivery techniques. Thus, the utilisation of plant lectins as immunomodulatory agents to combat different infections and diseases will grow in the upcoming years which would lay milestones in the healthcare facilities of the world.

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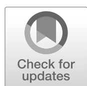
Chapter 7
Microbial Lectins



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Abstract Lectins are group of proteins which specifically binds to carbohydrate on the cell surface. Microbial lectins are the glycoproteins present in microorganisms which assists them to bind to host cell surfaces and the association among themselves. These properties render them to be an important tool in the diverse fields such as immunology, oncology, biotechnology, and microbiology. Lectins are extremely helpful to microbes since it assists their adherence to cell surface. When this function is prevented, it will lead to curtailing of several human microbial diseases. Adhesion property of lectins can be the basis of many upcoming applications and approaches in biomedical sciences. The interactions of lectins with carbohydrate moieties trigger the neutrophils to invade the infection site and it in turn initiates immune responses in humans. In contrast, the microbial lectins generally are incapable of eliciting immune response. There are several classes of microbial lectins based on their location, carbohydrate specificity, structure, and origin with diverse functions and applications.

Keywords Microbial lectins · Agglutination activity · Mitogenic activity · Viral lectin · Fungal lectin

Abbreviations

CLR C-type lectin receptor
CRD Carbohydrate recognition domain
EPS Extracellular polymeric substance
HIV Human immunodeficiency virus

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MR	Mannose receptor
PBL	Probiotic bacterial lectins

7.1 Introduction

Lectins are group of glycoproteins which are capable of interacting specifically with carbohydrate moeities on the cell surfaces. Lectins are ubiquitous in nature. Microbial lectins are the lectins present in or produced by microorganisms including bacteria, fungi, virus, and protozoans. These lectins are instrumental in binding of these microorganisms to host cell surfaces (infection) and the interaction among themselves, such as adhesion and inhibition of other microbes (Singh et al. 2011). These properties render them to be an important tool in the diverse fields such as immunology, oncology, biotechnology, and microbiology. Microbial lectins also have diverse functional applications in various bioprocesses such as bioremediation, bio flocculation, etc.

Till 1970s, only few lectins were isolated. Later, importance of lectins was realized and it led to extensive studies on lectins from microorganisms (Paiva et al. 2010). First identified microbial lectin was isolated from influenza virus by Alfred Gottschalk in 1950 and it was revealed that the primary function of this lectins were to mediate the interaction with host cells which is the initial step to cause infection (Shen et al. 2007). The adherence of the microbes to host cell surface is assisted by the lectins and hence they are important in initiating infections and cell–cell interactions. When this function is prevented, it will lead to curtailing of several human microbial diseases.

This specific binding property of the lectins is exploited in many applications and approaches in biomedical sciences such as drug delivery in cancer therapy, protein purification by affinity chromatography, bioflocculation, bioremediation of heavy metals, etc. (Singh et al. 2019). The interactions of lectins with carbohydrate moieties trigger the neutrophils to invade the infection site and it in turn initiates immune responses in humans.

Only a few among protozoal and fungal lectins are studied in detail. One of the most studied protozoal lectin is the galactose specific lectin in *Entamoeba histolytica* which mediates the adhesion of this parasite to human intestinal mucin glycoproteins and initiates contact dependent cytosis which eventually leads to infection (Abd Alla et al. 2012).

The microbial lectins are incapable of eliciting immune response and in turn they mediate the interaction of microbes with host cell surfaces and also the interaction among themselves to form biofilms. Microbial lectins are of different types, based on their carbohydrate specificity, amino acid sequence, three-dimensional structure, and molecular weight. There are only limited reports available regarding their classification, functional aspects, and structural peculiarities (Santos et al. 2014; Esko and Sharon 2009).

Lectins usually contain a carbohydrate recognition domain (CRD) which recognizes and binds to the carbohydrate moieties. In some lectins there are metal-binding sites to which the metal ions like Ca^{2+} ions binds which mediates the binding of lectins (Shen et al. 2007). Mostly lectins exhibit hemagglutination and antimicrobial activities (Singh et al. 2011). Thus, lectins have a promising role in the field of life sciences, and this has made the lectin research a hot cake among scientific fraternity.

7.2 Microbial Lectins

The first microbial lectin to be isolated was from the influenza virus in the early 1950s by Alfred Gottschalk (Nizet et al. 2017). The bacterial lectins were studied for the first time in 1970s (Esko and Sharon 2009). Later on, extensive research was carried out in this field which unraveled the potential applications of microbial lectins (Nizet et al. 2017; Esko and Sharon 2009; Slifkin and Doyle 1990). The first fungal lectin to be studied on the crystal structure was *Aleuria aurantia* (Wimmerova et al. 2003).

Microbes initiate their attachment to host cells through lectins by specific cell adhesion and hence microbial lectins are also known as adhesins. The adsorption and attachment of the microbes eventually leads to their colonization, pathogenesis, and infection to the host. Hence, disease resistance can be manipulated by modulating the activity of lectins (Ofek and Doyle 1994; Sharon and Ofek 2000). Microbial lectins are non-immunogenic in nature and a very little studies have been reported regarding their structure and classification (Santos et al. 2014). Broadly, the microbial lectins can be classified as lectins from bacteria, fungi, viruses, and protozoa.

7.2.1 Bacterial Lectins

The bacterial lectins present on the bacterial surfaces serve as adhesins to bind to the host cell receptors which in turn may initiate infection. They have the ability to specifically recognize complex carbohydrate moieties present on host cell surfaces and can also inhibit other microbes as well (Imberty and Varrot 2008; Sharon 1996; Springer and Gagneux 2013). Bacteria possess fimbriae and pili, the appendages that help them to attach to the host surfaces, serves as lectins or adhesins which binds to the glycoprotein receptors on the host cells. It was reported that *Escherichia coli* bearing type 1 fimbriae which is specific for mannose could agglutinate erythrocytes (Ofek and Doyle 1994). The other bacterial lectins reported from different strains of *E. coli* are P fimbriae and F-17 fimbriae which specifically binds to galabiose and *N*-acetyl glucosamine, respectively. Type 1 fimbriae can bind to the glycoprotein uroplakin Ia in epithelial cells of urinary bladder and hence it is important in urinary

tract infections (Faris et al. 1980). Type P fimbriae of *E. coli* are specific for Galα4Gal and Type II fimbriae of oral actinomycetes are specific for βgalactosides (Goldstein and Hayes 1978).

A bacterial lectin, LecB was identified in *Pseudomonas aeruginosa* which can bind with L-Fucose in the presence of Ca^{2+} ions (Mitchell et al. 2002). A bacteriocin, L1pA, with a lectin-like property, i.e. with two β-domains, was produced by the gram negative *Proteobacteria* (Ghequire et al. 2018). In a study reported by Kehr et al. (2006), *Microcystic aeruginosa*, a cyanobacteria secreted microvirin, which is a mannan binding lectin that helped them in colonization by attachment (Kehr et al. 2006). The bioluminescent bacteria, *Photorhabdus asymbiotica* was reported to secrete a PHL, a novel fucose binding lectin, which had an antimicrobial activity and phenoloxidase activity (Jančaříková et al. 2017).

The carbohydrate specificity of the bacterial lectins depends on the interaction of lectins with other surface structures of bacteria as well as the primary structure of the lectins. The knowledge on the specificity of lectin binding can help in describing the range of susceptible tissues in the host. Multiple lectins were identified in a wide variety of different species of bacteria with different carbohydrate specificity (Table 7.1).

7.2.2 Fungal Lectins

Fungal lectins are produced by unicellular yeasts as well as multicellular molds and many of them play a pivotal role in human infection. The fungal lectins are isolated from fruiting bodies, spores, conidia, and mycelium. Both filamentous and non-filamentous fungi are reported to possess carbohydrate specific adhesins or lectins that interact with host cell surfaces such as buccal and vaginal epithelium and even in ocular cells as in case of ophthalmic mycosis and several other fungal infections (Ballal and Inamdar 2018).

Aspergillus and *Candida* are the major human fungal pathogens and they cause aspergillosis and candidiasis, respectively. *Aspergillus* is a filamental fungus while candida is a non-filamentous fungus but both are opportunistic pathogens. AFL, a conidial lectin expressed by *Aspergillus fumigatus,* is involved in the pathogenesis during early stage of infection (Houser et al. 2013). A mucin-binding fungal lectin was purified by Singh et al. (2011) from *A. nidulans* by two step purification process of ion exchange and gel filtration chromatography. Another mucin-binding lectin FleA was purified from the conidia of *A. fumigatus* which is responsible for lung infections. But when it binds to the respiratory epithelial cells, the FleA is recognized by the macrophages which in turn elicit immune response in host against the pathogen (Kerr et al. 2016). The fungal lectins secreted by *Aspergillus nidulans, Cephalosporium,* and *R. bataticola* were described to exhibit mitogenic potential (Pujari et al. 2010). A novel GlcNAc-binding lectin, Paracoccin, secreted by *Paracoccidioides brasiliensis* was described to elicit fungal pathogenesis in man

Table 7.1 Bacterial lectins and their specificity

Bacteria	Lectin	Carbohydrate specificity	Role/activity	Reference
Escherichia coli	Type 1 fimbriae P fimbriae S fimbriae G fimbriae K 99 fimbriae Prs	Manα1-3 (Manα6Manα1-6) Man Galα1-4Galβ- Gangliosides GM3, GM2 GlcNAc Gangliosides GM3, Neu5Gcα2-3Galβ1-4Glc Galβ1-4Glcβ	Agglutinate erythrocytes, role in urinary tract infections	Faris et al. (1980), Leffler and Svanborg-Eden (1986), Parkkinen et al. (1986), Smit et al. (1984), Lindstedt et al. (1989, 1991), Stromberg et al. (1990)
Pseudomonas aeruginosa *Pseudomonas sepacia*	Prs LecB Type 1 fimbriae	Galβ1-3GlcNAc GalNAcβ1, 4Gal L-Fucose Galβ1-4GlcNAc L-Fucose Galactose Mannose Thiogalactosides Galβ1-4GlcNAc	Mediate attachment to the host Involved in host cell invasion and cytotoxicity Reduces ciliary beating of airway epithelium	Ramphal et al. (1991), Stromberg et al. (1988), Mitchell et al. (2002), Gunnarson et al. (1984), Gilboa-Garber (1986), Tuomanen et al. (1988), Nilsson et al. (1983), Krivan et al. (1988a, 1988b)
Staphylococcus saprophyticus		Galβ1-4GlcNAc	Mediate attachment to the host	Ramphal et al. (1991)
Streptococcus sanguis *Streptococcus cricetus* *Streptococcus sobrinus* *Streptococcus pneumoniae*	Type S fimbriae	NeuNAcα2, 3Galβ Glcα1,6 GlcNAcβ1, 3Gal	Phagocytosis, initiates the lectin pathway of complement activation	Murray et al. (1982), Drake et al. (1988), Landale and McCabe (1987), Andersson et al. (1983)
Klebsiella pneumonia *Klebsiella aerogenes*	Type 1 fimbriae	Galβ1-4GlcNAc Mannose	First-line immune defense	Krivan et al. (1988a, 1988b), Nilsson et al. (1983), Duguid and Old (1980)
Vibrio cholerae		L-Fucose	Hemagglutination and proteolysis	Jones and Freter (1976)
Propionibacterium spp.		Galβ1-4Glcβ		Karlsson (1989)
Bordetella bronchiseptica	S fimbriae	NeuNAcα2, 3Galβ		Parkkinen et al. (1986)
Campylobacter pylori	S fimbriae Type 2 fimbriae	NeuNAcα2, 3Galβ Gal (SO_3)		Ishikawa and Isayama (1987), Saitoh et al. (1991)
Clostridium spp.		Galβ1–3GalNAc		Hansson et al. (1983)

(continued)

Table 7.1 (continued)

Bacteria	Lectin	Carbohydrate specificity	Role/activity	Reference
Haemophilus influenzae		Galβ1-4GlcNAc		Krivan et al. (1988a, 1988b)
Lactobacillus spp.		Galβ1–3GalNAc	Anti-inflammatory role	Hansson et al. (1983)
Microcystis aeruginosa	Microvirin	Mannan		Kehr et al. (2006)
Photorhabdus asymbiotica	PHL	Fucose		Jančaříková et al. (2017)

Table 7.2 Fungal lectins and their specificity

Fungi	Lectin	Carbohydrate specificity	Reference
Candida albicans *C. glabrata*	Fimbriae EPA 1 EPA 6 EPA 7	βGalNAc(1–4)β-Gal Lactose and N-acetyl lactosamine Galα1–4Gal and Galα1–3Gal Galβ1–3Gal orGalβ1–4Glc	Yu et al. (1994), Cormack et al. (1999), Zupancic et al. (2008)
Aspergillus fumigatus *A. oryzae* *A. spargus*	AFL FleA AOL ASL	α 1–6 Fuc Mucin α 1–6 Fuc, α 1–3 Fuc, α 1–4 Fuc *N*-acetyl-d-galactosamine, and d-galacturonic acid	Houser et al. (2013), Kerr et al. (2016), Matsumura et al. (2009), Singh et al. (2014)
Trichophyton rubrum *T. mentagrophytes*	Conidia	Mannose and galactose	Esquenazi et al. (2004)
Penicillium griseofulvum *P. thomii* *P. duclauxii* *P. proteolyticum*	PGL PTL PDL PPL	Galβ1-3GalNAc, d-xylose, *N*-acetyl galactosamine Galβ1-3GalNAcα *N*-acetyl galactosamine GlcAβ1-3GalNAc6SO-3	Singh et al. (2011), Singh et al. (2014), Singh et al. (2014)
Cryptococcus neoformans	adhesin	Galβl-4Glcβ3-lCer	Jimenez-Lucho et al. (1990)
Paracoccidioides brasiliensis	Paracoccin	GlcNAc	Coltri et al. (2006)

(Coltri et al. 2006). Many lectins have been identified in different microfungi (Table 7.2).

7.2.3 *Viral Lectins*

Only a few viral lectins have been reported till date. The most studied viral lectins are hemagglutinins of influenza viruses and they binds specifically to sialic acid. This

interaction of virus to their host cells initiates viropexis (internalization of the viral particles by endocytosis). Though the affinity of the interaction is low, the adsorption of viral particles is directly proportional to the abundance of the receptors human and avian influenza viruses binds to N-acetylneuraminic acid (Neu5Acα) 2–6Gal- and Neu5Acα2–3Gal- receptors of the host cell, respectively, whereas porcine influenza viruses bind to both type of receptors (Nizet et al. 2017). Lectins with anti-HIV activity were reported from a green algae and the mechanism of its antiviral activity is not yet elucidated. The viral C-type lectins, CpBV produced by a wasp, *Cotesia plutellae* could induce immune suppression in their hosts (Lee et al. 2008). Szymanski et al. (2017) reported that the glycoproteins in the Herpes simplex virus are specific to 3-*O*-sulfated heparan sulfate, while the capsid proteins of entero viruses, gp120 V3 loop of HIV and envelope protein of Dengue viruses are specific for heparan sulfate. Some corona viruses possess lectins with both the hemagglutinin and receptor destroying activity.

7.2.4 Protozoal Lectins

Numerous parasitic protozoa possess lectins which mediate the adhesion of parasites to host cells based on their carbohydrate specificities. This kind of interactions could be used for developing novel therapeutics targeting the adherence and thus it is helpful in preventing the wide spread of various protozoan diseases.

A 260 kDa heterodimeric lectin was isolated from *Entamoeba histolytica*, which could identify and bind to terminal Gal/Gal NAc residues present on the intestinal epithelium of host. The extent of this interaction determines the virulence of the parasite as it mediates the attachment and invasion to host cell surfaces which leads to the development of infection. Moreover it may function in binding of *E. histolytica* to bacteria as a source of food (Abd Alla et al. 2012). The adhesion may also bring out protective immunity and is a potential target to manage the infection caused by *E. histolytica*.

Malaria is developed as a result of interaction of an adhesion, erythrocyte-binding antigen-175 (EBA-175), in *Plasmodium falciparum* merozoites mainly with the Neu5Ac sialic acid residues on the red blood cells (erythrocytes) of the host (Nawrot et al. 2014; Persson et al. 2013). Adhesin-glycan binding triggers the invasion of the merozoites into red blood cells, where they mature into schizonts which then rupture and release newly formed merozoites into the bloodstream, thus facilitating the development of infection.

The C-type lectin receptors constitute a superfamily of more than thousand proteins and it is classified into 17 groups based on their domain organization and phylogeny. Most CLRs possess one or more C-type lectin domains.

Mannose receptor (MR) is a C-type lectin. It is a transmembrane glycoprotein with eight C-type lectin-like domain that is expressed on the surface of various cell types (Takebe et al. 2013). MR mediates the binding and internalization of

mannosylated glycoproteins and participates in the endocytosis of different pathogens having mannose residues on their surface.

7.3 Roles of Microbial Lectins

7.3.1 *Biofilm Formation*

The first and foremost function of microbial lectins is to initiate adhesion which surges with time and leads to the formation of biofilms. The lectins interact with the carbohydrates-glycocalyx on the cell surface and help the bacteria to adhere to the host surface and initiates colonization which eventually leads to the biofilm formation. The biofilm formation is the first step leading to the development of infection.

Biofilms are the aggregation of bacteria at the host surfaces which is characterized by the production of carbohydrate mucous layer that further helps in the attachment of other bacteria and maintaining the coherence of the biofilm (Blaser 2005). The communication of bacteria through lectin-mediated biofilm adhesion is known as quorum sensing (Mack et al. 2008).

7.3.2 *Antimicrobial Activity*

The microbial lectins are reported to have antimicrobial activity which inhibits the colonization of other bacteria. A 30 kDa lectin isolated from *Acinetobacter baumannii* could inhibit both Gram-positive and Gram-negative bacteria. The antibacterial activity of the lectin was instrumental in inhibiting multidrug resistant pathogenic bacteria (Alyousef et al. 2018).

The *Proteobacteria* secreted a lectin, L1pA, could inhibit other bacteria from colonization in a competition for nutrients and space, by contact-dependent killing (Ghequire et al. 2018). The lectin-like bacteriocin microviridin produced by the cyanobacteria, *Microcystic aeruginosa* enhances the bloom formation by facilitating the colonization of *Microcystic aeruginosa* over other species of phytoplanktons (Kehr et al. 2006).

Lectins from various fungi were also found to possess potent antimicrobial and antifungal activities. Lectins from several *Penicillium* species and *A. gorakhpurensis* exhibited antibacterial activity against *Bacillus cereus, Staphylococcus aureus,* and *E. coli*. In addition, *A. gorakhpurensis* was reported to possess anti-fungal activity against *Saccharomyces cerevisiae* (Singh et al. 2014).

7.3.3 Antitumor Activity

Microbial lectins exhibit antiproliferative activity and this property can be exploited for the development of therapeutic agents to treat cancerous growths. The tumor cells express specific altered glycoconjugates at their surfaces which can be recognized by the lectins and bind to them. The binding of lectin with cancer cells triggers various signal transduction pathways which eventually arrests the cellular activities ultimately leading to cell death. The proliferation of HeLa cells was reported to be inhibited by a 30 kDa lectin isolated from *Acinetobacter baumannii* as revealed by the MTT assay (Alyousef et al. 2018).

7.3.4 Mitogenic Activity

Several microbial lectins are reported to exhibit mitogenic potential and trigger the binding of T cell receptor complex with the ligand which in turn initiates the mitosis of the cells by a signal transduction pathway (Kilpatrick 1999). Such mitogenic activity was reported in the lectin produced by the fungus, *Rhizoctonia bataticola.* The mitogenic potential of the fungal lectins secreted by *Aspergillus nidulans, Cephalosporium,* and *R. bataticola* were described to have applications in histochemistry, glycobiology, and oncology (Pujari et al. 2010). A novel mucin-binding microfungal lectin was purified by Singh et al. (2011) from *A. nidulans* was reported to exhibit mitogenic potential which helped in elucidating the biochemical changes of immune cells.

Lectins purified from the fungi *Hericium erinaceus* (Li et al. 2018), *Trametes versicolor* (Singh et al. 2019), and *Hygrophorus russula* (Suzuki et al. 2012) exhibited mitogenic activity in murine splenocytes. Mannose binding lectins produced by *Pseudomonas spp*. induced T cell proliferation and lectin-mediated phagocytosis (Abraham et al. 1988; Sharon 1984). Among bacterial lectins, *Pseudomonas* spp. exhibit lectins that are mostly mitogenic.

7.3.5 Bioflocculation

In microorganisms, role of lectin-mediated aggregation in bio flocculation of activated sludge was revealed by hemagglutination and inhibition assays on extracellular polymeric substances (EPS) derived from several activated sludges. It revealed strong hemagglutination with trypsin-treated human red blood cells and the agglutination was inhibited by several glycoproteins, indicating that glycoprotein specific lectins are present in activated sludge.

7.3.6 *Bioremediation*

Lectins bind the cell together and form cluster of cells. This is because microbial glycol conjugation reacts with their specific lectin. This property serves as an important tool in bioremediation process. There is a lot more to be studied and applied in this field.

7.4 Future Perspectives and Applications

Bacterial lectins are capable of binding specifically to different carbohydrates present on different cells of the human body and this specificity is a causative factor that leads to an infection. This specific adhesion property can be utilized in the synthesis of antiadhesive drugs. However, bacteria and viruses possess different types of lectins and they bind selectively to various carbohydrates for adherence. Therefore, researchers face highly challenging task for developing antiadhesive therapy.

The mitogenic potential of the fungal lectins secreted by *Aspergillus nidulans, Cephalosporium,* and *R. bataticola* were described to have applications in histochemistry, glycobiology, and oncology. Horizontal gene transfer occurs as a result of this amoeba-lectin-mediated internalization thus provides a useful microbiome homeostasis model. These lectins can also be used to identify the infectious organisms that cause tissue damage without using any specific diagnostic tool.

The specific and augmented adhesive property of microbial lectins can also be exploited in the large-scale application in bioremediation. Biosorption of cuB2 from contaminated areas was enhanced by the aggregates of yeast cells which was facilitated by the presence of lectins as the heavy melts could occupy the binding sites of lectin.

A laboratory scale system was set up consisting of an activated sludge. This set up, arranged by Park and Novak (2009) was effective in checking the efficiency and ease of process using lectins. This process revealed that lectins play an important role in bio flocculation and increase the rate of process. In 1998 Murthy observed the lectin-like proteins matrix which in turn results in bio flocculation.

The bactericidal and bacteriostatic effects of various lectins may open a new way for antimicrobial research. Currently, antibiotics are widely used for the treatment of various diseases which may induce allergic responses and nonspecific reactions. The unethical use of antibiotics may lead to several serious threats to the public health including development of new multidrug resistant strains and loss of beneficial intestinal flora. Recent studies of deadly multidrug resistant microbial pathogens reveal that lectin-based medicines could be an invaluable alternative to the antibiotics and may also be used for targeted drug delivery and its implication needs further studies.

Recently, fluorescent staining technique has gained more importance and popularity because it is easy to handle, and has high specificity. Also, this method avoids the long radioactive permission procedure. A particular species can be detected from a mixture of different microbes by using lectin-based fluorescence staining (Fife et al. 2000). A similar technique to this was used by Sizemore et al. (1990) to selectively differentiate moderately thermophilic and acidophilic mining bacteria in mixtures that contain *Thiobacillus ferrooxidans.* This was made possible by binding of wheat germ agglutinin to the n-acetyl glucosamine residue in the peptidoglycan layer of Gram-positive bacteria.

Probiotic Bacterial Lectins (PBL) are lectins produced by certain bacteria such as *Lactobacilli* which is instrumental in maintaining the relationship between the gut microbes and the host. When PBLs were tested against certain clinical pathogens such as *Candida* and *Staphylococcus*, the results showed that PBLs have inhibitory effect on the growth of the various strains along with its proteolysis (Lakhtin et al. 2012).

7.5 Conclusion

Lectins are prevalent and are produced by both prokaryotic and eukaryotic organisms. The tremendous properties of lectins attracted researchers to focus more on lectins and hence carried out enormous studies on lectins from bacteria, fungus, viruses, protozoan which revealed the importance of lectins. With the advancement of molecular biology techniques, more microbial lectins are expected to be studied and analyzed for its biomedical and industrial applications.

Microbial lectins are core factors which involved in the host–pathogen interaction. Lectins are hope of scientists for the discovery of new highly effective drugs which can prevent severe infections in humans. Lectins are proteins that recognize and bind to specific carbohydrate target on host cell therefore structure, specificity, and composition are attributes of lectin–cellular interaction. Studies on lectin–carbohydrate interaction indicate that lectins play a pivotal role and that are discussed in this chapter. Lectins have role in bacterial communication, antimicrobial activity which ensures competitive advantage for nutrition and space, i.e., growth and survival. Cellular interaction mostly ends up in infection of host.

Lectins are specific carbohydrate binding protein, which makes special interest of glycobiologists to concentrate on this topic. The important applications of microbial lectins can be attributed to various fields such as bioremediation, bioflocculation, biomedical applications, fluorescent staining techniques, antiadhesive drug development, and targeted drug delivery (Fig. 7.1). However, further studies in this area will shed light to more potential advanced applications of the microbial lectins in diverse fields.

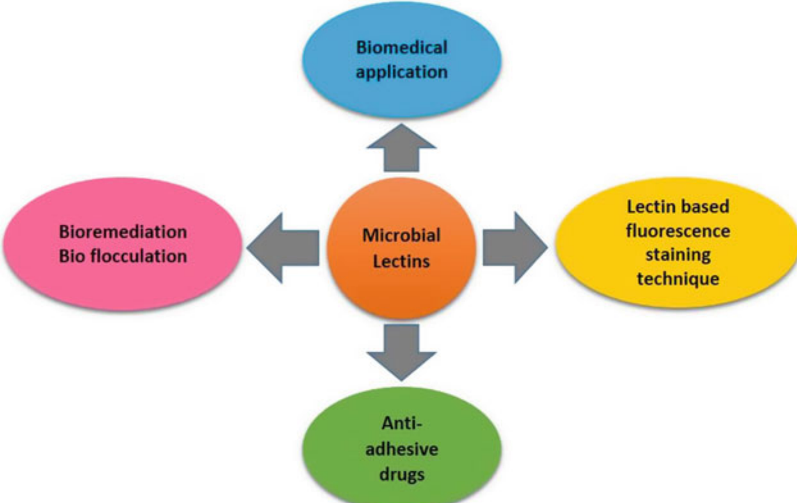

Fig. 7.1 Applications of microbial lectins

Conflict of Interest The authors declare no conflict of interest.

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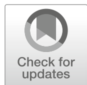
Chapter 8
Regulation of Immune Responses by Lectins



Shamna Naseemashahul and Femi John Fawole

Abstract Lectins are carbohydrate-binding proteins having receptors for recognizing different carbohydrate moieties such as mannose, galactose, rhamnose, fucose, lactose, N-acetyl glucosamine, and N-acetyl galactosamine with specificity. They perform this function with or without the support of divalent cations. Moreover, some lectins can bind erythrocytes and result in agglutination, and, hence called "phytohaemagglutinin." Lectins are structurally diverse and are present in both plant and animal kingdoms. Lectins participate mainly in innate immune defense in animals by identifying carbohydrate and glycoconjugate moieties in cells and biological fluids. Based on the carbohydrate recognition domain (CRD), lectins can be classified into various groups. The plant lectins are very well studied, while among the animal lectins many groups including fish and shellfish lectins are less studied compared to plant lectins, especially the regulation of lectins.

Keywords Fish lectin · Shellfish lectin · Lectin mediated immunity · Carbohydrate recognition domain · Types of lectin

Abbreviations

AGPs	Arabinogalactan proteins
Artin M	Artocarpin mannose binding
CFL	*Cratylia argentea* lectin
CLCP	Charcot Leyden Crystal Proteins
CLP	Collectin like protein

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CLRs	C-type lectin receptors
Con A	Concanavalin A
ConBr	*Canavalia brasiliensis* lectin
CR1	Complement receptor 1
CRD	Carbohydrate recognition domain
CTLs	C-type lectins
DORN1	Does not Respond to Nucleotides 1
FTLs	F-type lectins
GalNAc	N-acetylgalactosamine
GluNAc	N-acetyl glucosamine
IFNγ	interferon-gamma
IL	interleukin
LecRLKs/RLPs	lectin receptor-like kinases/proteins
LOX-1	Lectin like oxidized low-density lipoprotein receptor 1
LysM	Lysin motif domain
MBPs	Mannose-binding proteins
Nictaba	Nicotiana tabacum agglutinin
OniL	*Oreochromis niloticus* Lectin
PAMPs	Pathogen associated molecular patterns
proPO	Prophenoloxidase system
PRRs	Pathogen recognition receptors
RBLs	Rhamnose-binding lectins
RcaL	*Rachycentron canadum* Lectin
Siglecs	Sialic acid-binding immunoglobulin-like lectins
Th1	T1 helper cell
TLR	Toll-like receptors
UAE1	Ulex europaeus agglutinin 1

8.1 Introduction

Lectins are glycoproteins that play important role in non-specific immune system activation and function as receptors for recognizing microbial pathogens via their carbohydrate surface (Ewart et al. 2001a, b; Wang and Wang 2013; Elumalai et al. 2019). One of the most exciting and significant functions of lectins is in the area of immune system activation occasioned by their ability to recognize sugars on the surface of a potential pathogen, and this often incites the lectins-protein to engulf invading microbial pathogens thereby resulting in their destruction by the phagocytic cells (Lu 1997; Ewart et al. 2001a, b; Vasta et al. 2011). Lectins are of different types and differ in their molecular weight, amino acid sequences, and sugar-binding specificity. They are widely found in microbes, yeast, plants, and animals, and play an important biological role in teleost, animal, and plants such as antitumor, immunoregulatory, pathogen recognition and cell adhesion, antimicrobial, cells

agglutination, and cell signalling (McGreal et al. 2004; Wang and Wang 2013; Ng et al. 2015; Brinchmann et al. 2018). A recombinant mannose-binding lectin homologue was reported to agglutinate *Streptococcus agalactiae* and *Aeromonas hydrophila* in vitro (Mu et al. 2017). Furthermore, in vivo challenge with bacterial pathogen showed higher expression of mannose-binding lectin gene in the liver, spleen, and kidney of Nile Tilapia (*Oreochromis niloticus*) (Mu et al. 2017). Several other studies have also documented the role of lectins in host protection against bacterial infection (Arasu et al. 2013; Rajan et al. 2013; Wang et al. 2014; Zhou and Sun 2015; Zhou et al. 2016; Liu et al. 2016; Wang et al. 2017; Huang et al. 2020). This points to the agglutination capability and immune response activation of lectins against bacterial pathogens in fish. Aside from immune system function, some lectin types have been reported to bind endogenous ligands and are involved in different physiological functions such as preventing polyspermia or acting as antifreeze protein (Vasta et al. 2011). Nevertheless, these molecules (i.e. lectins) with potency to fight microbial pathogen in host cells are involved in many intra- and extracellular processes. Hence, this chapter presents information on different types of lectin, role on immune responses in animal and plant, mode of actions, regulation of lectins, and the prospect of lectins in fish health management.

8.2 Type of Lectins

Lectins are produced by a wide variety of living organisms and they can be grouped according to their species of origin, such as animal lectins, fish lectins, fungal lectins, bacterial lectins, and plant lectins (Mishra et al. 2019). Several types of lectins families have been identified and characterized especially in fish based on their specificity for carbohydrate recognition domain, structure, and calcium dependency (Taylor and Drickamer 2003; Elumalai et al. 2019). Among them include C-type lectins, F-type lectins, ricin type, lily type, galectins, rhamnose-binding lectin, fish-egg lectin, interlectin, and tectonin-type lectins (Ogawa et al. 2011; Ng et al. 2015; Brinchmann et al. 2018; Elumalai et al. 2019). In addition to C-type and galectins, other animal-specific families of lectins have also been reported such as I-type lectins, L-type, pentraxins, R-type, F-box lectins, ficolins, P-type lectins, and tachylectins (Ogawa et al. 2011; Wang and Wang 2013). Comprehensive reviews on different lectins family in animals, fish, and plants are available (Weis et al. 1998; Kilpatrick 2002; Zelensky and Gready 2005; Ogawa et al. 2011; Elumalai et al. 2019; Tsaneva and Van Damme 2020), and some important groups are mentioned in Table 8.1.

Table 8.1 Classification, localization, and functions of important groups of lectins

Sources	Lectins family/ domain	Localization	Function	Reference
Animal	C-type lectins, Rhamnose-binding Lectins, Pentraxins, I-type lectins, L-type, R-type, F-box lectins, ficolins, P-type lectins, and tachylectins	Gill, liver, intestine, spleen, muscle, heart, kidney, and mucus	Immune response, opsonization, phagocytosis, and activation of complement pathway	Ogawa et al. (2011), Wang and Wang (2013), Gabius (1997), Lis and Sharon (1986), Huang et al. (2020)
Plant	Agaricus bisporus lectin,	Nucleus, cytosol	Recognize glycoconjugate on cell surface, host–pathogen interaction, cell signalling and cell–cell communication, protection against harmful phytopathogenic microorganisms, insect, and predatory animals	Lannoo and Van Damme (2014), Tsaneva and Van Damme (2020)
	Cyanovirin, Chitinase related agglutinin, Jacalin,	Nucleus Vacuole, membrane bound Nucleus, cytosol, vacuole		
	Legume lectin, LysM,	Vacuole, nucleus, Cytosol or membrane-bound		
	Ricin-B,	Vacuole, nucleus, cytosol		
	Amaranthin	nucleus, cytosol		
Algal	N-glycan specific, High mannose type N-glycan specific		Anti-inflammatory, Anti-tumor, Antiviral	Mishra et al. (2019)
Fungal			Growth, development, morphogenesis, molecular recognition, early-stage infection	Mishra et al. (2019)

8.3 Immune Functions

Glycosylation of proteins can be considered as one of the major posttranslational modifications and many proteins have specificity in recognition to these glycosylated sites. The carbohydrate-binding proteins can recognise and bind different forms of carbohydrate in glycoproteins, proteoglycans, free sugars etc. This carbohydrate-binding property of proteins can be a basis for the initiation of immunity in all kingdoms of life. Generally, there are three receptor groups, galactins, siglecs (Sialic acid-binding immunoglobulin-like lectins) and C-type lectins (CTLs).

In multicellular organisms, various physiological functions like cell migration, homeostasis balancing, and innate immune signalling etc are depending on the capability of cells to distinguish glycoconjugates (glycoproteins and glycolipids) via glycan-binding proteins or lectins. Despite the differences between the immune system of animals and plants, there are some striking similarities like lectin-mediated immune response. Lectins can act as pattern recognition receptors or pathogen recognisers and initiate the immune response. However, lectins are involved in damage associated patterns as well as immune pathology. Various immunological processes such as phagocytosis, opsonisation, cell adhesion, cell activation, migration and differentiation and cell death or apoptosis involve lectin-carbohydrate interactions. Enhanced respiratory burst activity and bactericidal activity were also stimulated by lectins. In cells, the lectin receptors are present on the cell surface or insoluble form (Ni and Tizzard 1996).

8.3.1 Lectin Mediated Immunity in Plants

Plant lectins are well known for their immunomodulatory functions and studies revealed that there are 12 families of lectins having cytokine and other immune mediator production functions (Da Silva and Correia 2014; Coelho et al. 2017). Plant lectins can bind with the bacterial cell as well as with the glycan moiety of immune cells. Hence, their action can be on both microbial cells and immune cells and enhance the signalling and further action of activator mechanism (Van Damme et al. 2008; Souza et al. 2013). Con A, ConBr, CFL, UEA1, Cramoll, Artin M, and Jacalin lectins are widely observed in plants and studied on the immunomodulatory activity in response to various pathogens. Con A when treated in murine macrophages stimulated the toll-like receptors (TLR) to enhance the secretion of cytokines and nitric oxides and promoted various signalling pathways (Sodhi et al. 2007). Moreover, in mice, the ConA enhanced the survival of Klebsiella infection and reduced liver necrosis (Kuo et al. 2007).

8.3.2 Lectin Mediated Immunity in Animals

Similar to plant lectins, lectins obtained from animal tissues are found to be potent immune boosters. Though not identified as lectin, it seems that the first reported animal lectin is Charcot Leyden Crystal Proteins (CLCP). However, the lectin activity was first observed in snakes. But, the biochemical analysis of the eel agglutinin revealed the non-immunoglobulin nature for the first time. The animal lectin has both immune and non-immune functions. The action of lectin is carried out by agglutination, structure-activity divergence, tertiary structure convergence, non-carbohydrate binding, etc. Similar to plant lectins, animal lectins also recognize molecules in the immune system. This function is possible by the direct defense,

suppression or enhancement of the immune system, blocking autoimmunity, and recognition and molecule trafficking. Animal lectins showed an extraordinary ability to bind protein, lipid, and nucleic acid, i.e., the animal lectin binds with non-carbohydrate moieties also. Hence, the animal lectins can be called "bifunctional molecules" (Kilpatrick 2002). The collectin like protein (CLP) cloned from serum and hepatic tissues of humans can promote phagocytosis (Nakamura et al. 2001; Ohtani et al. 2001).

8.3.3 Lectin Mediated Immunity in Fish

Several reports are published on the immune-stimulating effect of lectin and it is depicted that several fish lectins mediate pathogen recognition (Yano 1996; Vasta et al. 2011; Lino 2013; Elumalai et al. 2019). Galectins, C-type lectins (CTLs), F-type lectins (FTLs), and pentraxins are the predominant lectin families observed in cartilaginous and bony fishes (Cammarata et al. 2016). The presence of humoral and membrane-associated lectins which helps in establishing positive interactions with colonizing microbes are also observed in fishes (Vasta et al. 2011). Most of these lectins are C-type and several other functions such as agglutination, immobilization with complement-mediated neutralization are also performed by fish lectins (Table 8.2). Different types of CTLs were isolated from species such as zebrafish, *Silurus asotus*, fugu, Japanese eel, rainbow trout, common carp, etc. (Tasumi et al. 2002; Singha et al. 2008; Zhang et al. 2011). Mucus lectin from the skin surface of *Genypterus blacodes* was the first isolated fish lectin (Oda et al. 1984).

Isoforms are also observed in fish lectins, for example, the F-type lectins from Japanese eel had various forms (Honda et al. 2000). Serum of *Sparus aurata* contains fucose binding lectin (Sau FBP32) and mucus extract of loach is also reported to have lectins having agglutination property (Sun et al. 2019). The smelt lectin classified in type II antifreeze protein belongs to the C-type lectin group. The same category of lectins is isolated from herrings (Ewart and Fletcher 1993). Lectin isolated from the ovaries and roe of grass carp showed mitogenic activities on splenocytes of murine and phagocytic activity in seabreams (Ng et al. 2003). Similarly, a lectin (at 14 μM) with a mannose-binding property, isolated from the ovary of *Rachycentron canadum* also exhibited a mitogenic activity on a mouse (Ngai and Ng 2007). Serum lectin from *Rachycentron canadum* or RcaL is also reported to be an immunomodulator. Furthermore, lectin isolated from grass carp ovaries having specificity towards rhamnose enhanced the expression of interferon-gamma (IFNγ) and interleukin 2 (IL-2) expressions in splenocytes (Lam and Ng 2002). Rhamnose-binding lectins are one of the unique fish lectins present in serum, embryos, and eggs of fish (Watanabe et al. 2009; Cammarata et al. 2014). In vitro T1 helper cell (Th1) was inducted by a mannose-specific serum lectin (OniL) in *Oreochromis niloticus.*

Table 8.2 Immunomodulatory and biological roles of fish lectins

Lectin family	Lectin	Source	Mode of action	Target	References
F-type lectins	GANL (220 kDa)	Bighead carp	Growth inhibition by agglutination	*Vibrio harveyi*	Pan et al. (2010)
Galectins	AJL-1 (30 kDa) PfGAL9 (35.12 kDa) Congerin, LcGal9 Pufflectin	Japanese eel Yellow catfish Conger eel Yellow croaker Pufferfish	Growth inhibition by agglutination Cellular encapsulation Binds parasite	*Streptococcus difficile, E. coli, Aeromonas hydrophila, B. Subtilis*, *B. Megaterium*, and *S. aureus* Bacteria Parasitic nematodes *V. Alginolyticus* and *A. hydrophila* *Heterobothrium Okamotoi*	Tasumi et al. (2004) Wang et al. (2016) Nakamura et al. (2012) Zhang et al. (2016) Suzuki et al. (2003)
Intelectins	SaIntL (35 kDa	Catfish	Growth inhibition by agglutination	*Aeromonas salmonicida*	Tsutsui et al. (2011)
Fish-egg lectins	RbFEL (28.2 kDa) Coho egg lectin zFEL KPL (140 kDa) RBL (30 kDa)	Rock bream Coho salmon Zebrafish Skipjack tuna Chinook salmon	Enhanced lectin activity on challenged with pathogens Binds bacteria Phagocytosis of microbes by macrophages Hemagglutination Anti-proliferative activity	*Edwardsiella tarda*, *Streptococcus iniae* and red sea bream iridovirus (RSIV) *Aeromonas salmonicida* *Aeromonas hydrophila* Blood type A erythrocytes Human breast cancer MCF-7 cells and hepatoma Hep G2 cells	Kim et al. (2011) Yousif et al. (1995) Wang et al. (2016) Jung et al. (2003) Bah et al. (2011)
C-type lectins	HjCL BGL (80 kDa) SauFBP32 LycCTLR OniL (17 kDa)	Japanese bullhead Shark Potcha fish Atlantic salmon Yellow croaker Tilapia fish	Blood coagulation Binds bacteria Activates immune response Exhibit strong cytotoxic effects Binds to bacteria	Erythrocytes (Shark blood) *Aeromonas hydrophila*	Tsutsui et al.(2015) Fock et al. (2001) Ewart et al. (1999) Ao et al. (2015) da Silva et al. (2012) Coriolano et al. (2012) Ngai and Ng (2007)
	RcaL MBL	Cobia fish Cobia fish	Induced higher IFN-γ Production Lower IL-10 as well as Nitrite release Induced production of IL-2 and IL-6 and proliferative responses, Mitogenic activity	Mice *Vibrio anguillarum* and *A. salmonicida* *E. coli* and *B. cereus* Mouse splenocytes	

Adopted with permission from Elumalai. et al. (2019): The Role of Lectins in Finfish: A Review. Reviews in Fisheries Science & Aquaculture, 27(2), 152–169

8.3.4 Lectin Mediated Immunity in Shellfish

The lectins present in the invertebrate hemolymph have a role in neutralizing pathogens and support the phagocytosis in hemocytes. The lectins of C-type are observed in crustaceans and arthropods, while pentraxins are quite common in shellfish. Beta, 1–3 glucan and tachylectins identified from yeasts and bacteria has the capacity to activate the prophenoloxidase system (proPO) in shrimp. The activity of lectins on clotting proteins in crustacean immunity is also well studied (Hall et al. 1999). However, compared to other arthropod groups, the studies on crustacean lectins are less. A lectin isolated from *Limulus polyphemus* (Horseshoe crab) showed a property of broad-spectrum recognition to various monosaccharides (Vasta 1992). Similarly, lectin obtained from horseshoe crab also showed a binding property to sialic acid.

The structurally different lectins obtained from invertebrates act as humoral innate immunity and enhance phagocytosis, melanization, respiratory burst activity, and encapsulation (Zhang et al. 2011; Wang and Wang 2013) (Fig. 8.1). The globular region of lectin having less than 200 amino acids is considered as the sugar-binding site which promotes phagocytosis even in invertebrates. Most of these lectins belong to the C-type family while other groups also show similar action. Furthermore, the pathogen recognition receptor (PRRs) group having CTLs with C-type lectin domain (CTLD) act as the major group in crustaceans which shows the predominant role of C-type lectins in the innate immune response of crustaceans (Zhang et al. 2020a, b). Most of these groups showed agglutination of human erythrocyte or bacteria or yeast and sugar-binding nature (Elumalai et al. 2019; Zhang et al. 2019). Antiviral action was observed in a lectin (PcLec) isolated from crayfish and its action against bacterial pathogens need further investigation (Zhang et al. 2020a, b). In shrimps, lectins with multiple roles and different structure and expression patterns are observed and are of seven groups, viz., galactins, L-types, M-types, C-type, p-type, fibrinogen like, and calnexins (Wang and Wang 2013). The

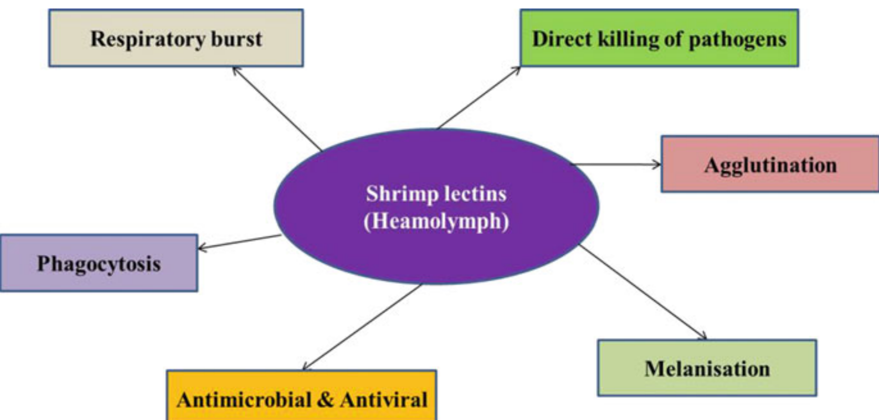

Fig. 8.1 Mode of action of shrimp lectins. Adapted and modified from Wang and Wang (2013)

rhamnose-binding lectins (RBLs) showed specificity towards L- rhamnose is found mainly in fishes and shellfishes (Tateno 2010).

8.4 Regulation in Plants

Recent researches using molecular techniques and omics studies paved the way towards the action and interaction of lectins in plants at the molecular level. Studies on the expression of lectin genes and the pathways triggering immunity by lectins showed the importance of the molecule in innate immunity and cell signalling. The surface localized pathogen recognition receptors (PRRs) that recognize apoplastic elicitors and the intracellular receptors regulate the immune action in cells (Jones and Dangl 2006; Bos et al. 2010). During the entry of pathogen through the cell wall, the cell wall degrading enzymes get activated and AGPs and degradation products of pectin (oligogalacturonides) can be recognized by lectin receptor-like kinases/proteins: LecRLKs/RLPs (Benedetti et al. 2015; Davidsson et al. 2017). In addition to the vacuolar and surface lectins, the cytoplasmic and nuclear lectins also play a major role in lectin mediated immunity in plants.

Cytoplasmic lectins such as Nictaba (Nicotiana tabacum agglutinin) are involved in cell signalling (Van Damme et al. 2004). Nictaba is specific to GlcNAc or GalNAc residues (Lannoo et al. 2006). ConBR from *Canavalia brasiliensis* enhanced the production of Interleukin 2, 6 (IL-2, IL-6) and interferon-gamma (IFN-γ) and decreased the production of IL-10 in the splenocyte murine (de Oliveira Silva et al. 2011). ArtinM interacts with intracellular matrix Mannose by a process called haptotaxis and supports the migration of neutrophils (Souza et al. 2013). In mice, another lectin called Cramoll regulate the level of reactive oxygen species (ROS) and different interleukins such as IL-6, 10, 17, and IFN-γ. Arabidopsis have a lectin designated as DORN1 have recognition to peptides and nucleotides. The universal LysM lectins and LecRLKs have both positive and negative roles in plants as microorganisms having these lectins will suppress plant immunity (Rovenich et al. 2016). Effectors have the capacity to downstream signalling events. Plants have both constitutive and inducible lectins. Constitutive lectins are highly abundant in cells while in response to stress exposure the inducible lectins are expressed at a low base level. Stress inducible lectins are mostly nucleoplasmic in nature (Van Damme et al. 2004). However, the mechanism of action and regulation of nucleocytoplasmic lectins are studied less.

8.5 Regulation in Animals

Lectins are associated with pathogen-associated molecular patterns (PAMP) and they recognize pathogen recognition receptors (PRRs) in a reversible binding and activate the lectin pathway (Vasta et al. 2011). The binding of carbohydrate moieties

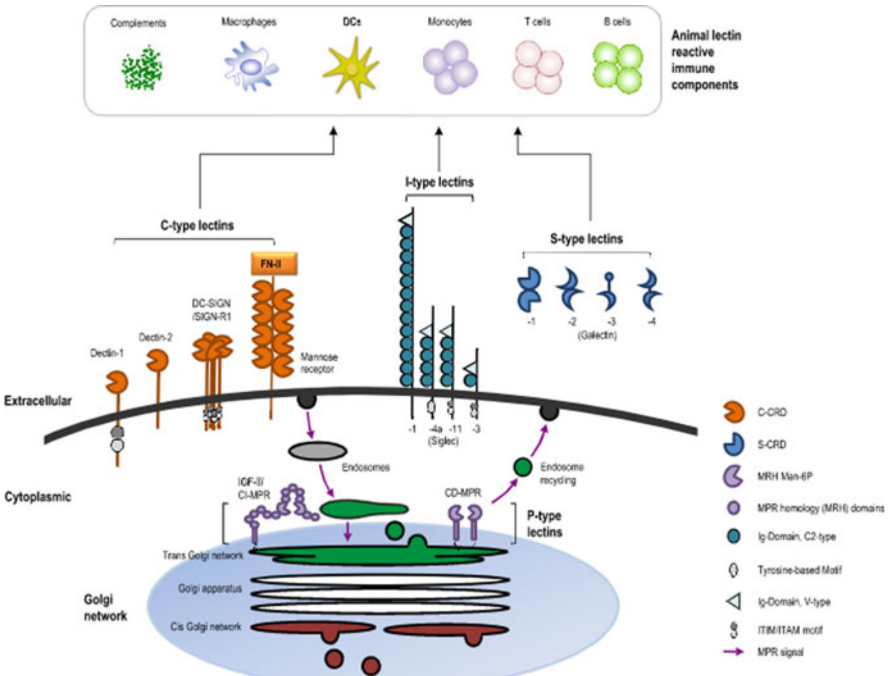

Fig. 8.2 Domain structure models, the location of several representative animal lectins, and the immune cells reacting with animal lectins

Carbohydrate recognition domains (CRDs) of each lectin are depicted in the respective whole structures. S-type lectins are secreted into the extracellular matrix of body fluids. C-type and I-type lectins are localized to the plasma membrane, and P-type lectins are located in luminal compartments of the secretory pathway. CD-MPR, cation-dependent mannose-6-phosphate receptor; CI-MPR, cation-independent mannose-6-phosphate receptor; CRD, carbohydrate recognition domain; DC, dendritic cell; DC-SIGN, DC-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin; IGF, insulin-like growth factor; MRH Man-6P, mannose 6-phosphate receptor homology; ITIM/ITAM motif, immunoreceptor tyrosine-based inhibition/immunoreceptor tyrosine-based activation motif. (modify)

Adapted from Loh et al. (2017): Animal lectins: potential receptors for ginseng polysaccharides. Journal of gingseng 41: 1–9

activates various immune responses such as complement pathway, agglutination, immobilization, and opsonization (Sharon and Lis 2004) (Fig. 8.2). The animal lectin galectin has a carbohydrate recognition domain with two β-sheets folded antiparallel producing a ligand-binding site that is concave in shape. This helps in binding β-galactoside glycans specifically (Diehl et al. 2010). Other important lectins in animals such as Calnexin and Calreticulin have both globular and proline-rich (P) regions and are made of two β-sheets, and hence, look like a β-sandwich (Chouquet et al. 2011). Divalent cations have a role in carbohydrate recognition in some animal lectins, especially in the C-type lectin family. C-type

lectin receptors play a critical role in immune responses and inflammation and recognition of molecular pattern of pathogens. The micro-RNA regulated C-type lectin receptors can distinguish molecular patterns of endogenous and exogenous pathogens and initiate downstream signalling cascade which induce the production of effector immune cells and inflammatory responses, furthermore, this maintain homeostasis in cell (Ganguly et al. 2020).

Lectins with N and O-glycan specificity control regulatory signals of cell homeostasis. They amplify or silence autoimmunity responses or neoplastic disease responses (Rabinovich and Croci 2012). Conformational and allosteric changes on a specific protein as a result of post translational modifications can leads to generation of new epitopes (binding site). This can lead to the formation of signalling complexes in cell. Some cell surface glycans acting as a ligand for lectins that facilitate receptor dynamics (Dennis and Brewer 2013). Hence, density of glycan epitopes has an important role in lectin mediated receptor and transporter regulation in animals (Dam and Brewer 2010). This might be due to the higher regulation of glycan binding sites on cell surface and hence we can derive a conclusion that interaction between surface glycan and lectin and golgi glycosylate transferase helps the regulation of cell surface receptors and transporters to respond to cellular signalling and environmental changes (Dennis and Brewer 2013). In addition, in autoimmune diseases, C-type lectin receptor controls the expression of BIC/MicroRNA 155 and therefore a feedback regulation of T cell development through thymus control is indirectly mediated by CD69, a C-type lectin receptor (Sánchez-Díaz et al. 2017). In humans, lectin like oxidized LDL receptor (LOX-1) plays an important role in cardiovascular diseases and is regulated by several mechanisms like upregulation of receptor expressions, signalling modulation through internalization or proteolysis, and adjusting the activity of surface receptors (Mentrup et al. 2021).

In human serum, five different pathways of lectin are observed and only two pathways (ficolin and collectin 11) are found to be effective on activation of complement pathway during pneumococci infection (Ali et al. 2012). However, the mannan binding lectin which (a group of collectin proteins) pathway activate complement pathway in human serum is upregulated by *Neisseria gonorrhoeae* through activation of complement receptor 1 (CR1) (Gulati et al. 2002).

Several studies are reported in recent years explaining the mode of actions of lectin in fishes (Elumalai et al. 2019). In fishes, the presence of lectins is observed in the intestine, plasma, liver, gills, eggs, embryos, serum, and surface mucus (Mistry et al. 2001a, b; Ng et al. 2015). In response to stress or infection, the concentration of the F-type lectin is observed to be increased in the plasma of teleosts fish (Parisi et al. 2015). The F-type lectins prove to be effective against *Vibrio harveyi* as they agglutinate the bacterial cells (Pan et al. 2010). Similarly, they are found to be effective against heavy metal accumulation in fishes (Guardiola et al. 2015). Functionally analogous lectins in fish, including mannose-binding lectin, have been reported to effectively agglutinate *Streptococcus agalactiae* and *Aeromonas hydrophila* in vitro. Also, in vivo challenge with bacterial pathogen showed higher expression of mannose-binding lectin gene in the liver, spleen, and kidney of Nile

Tilapia *Oreochromis niloticus* (Mu et al. 2017). In Obscure pufferfish *Takifugu obscurus*, the gene expression of two novels CTLs (ToCTL1 and ToCTL2) were found up-regulated after being challenged with both the gram-positive and gram-negative bacteria (Huang et al. 2020). The authors opined that the CRDs inhibited the growth of the bacteria by binding to the sugar on the bacteria surface, thereby establishing the vital role of CTLs in clearing invading foreign agents. In addition to agglutinate cells as well as precipitate carbohydrate moieties, fish lectin can initiate an innate response against pathogens and helps in the establishment of positive interaction with colonizing microbial species (Vasta et al. 2011).

Mucus lectin in the skin of fish is the first line of defense against pathogens and it was confirmed when intelectins got activated in response to *Aeromonas salmonicida* infection. However, in the study, the gene expression of mucus lectin was not inducted in vivo (Tsutsui et al. 2011). Moreover, an egg lectin isolated from coho salmon could not prevent the growth of *Aeromonas salmonicida* indicating multiple roles of lectin other than disease prevention (Yousif et al. 1995). The C-type galactose binding lectins such as eCL-1 and eCL-2 were found to be downregulated in Japanese eel when it was shifted to the marine environment from freshwater (Mistry et al. 2001a, b). Lectin isolated from yellow croaker (galectin) also showed an agglutination property against bacteria (Zhang et al. 2016). Fully developed immune tissues of rock bream had lily type lectin (OfLTL-2 and 3), which play an important role in immunity.

8.6 Prospects of Lectins in Fish Health Management

One of the most exciting and significant functions of lectins is in the area of immune system activation occasioned by their ability to recognize sugars on the surface of a potential pathogen, and this often incites the lectins-protein to engulf invading microbial pathogens thereby resulting in their destruction by the phagocytic cells (Lu 1997; Ewart et al. 2001a, b; Vasta et al. 2011). In the innate part of the immune system, lectins are one of the important agglutinins which help in the agglutination of a foreign body (i.e. non-self). Since fish depends majorly on the innate immune system, and antibiotic resistance is one of the problems confronting the global aquaculture industry, lectins could be useful in fish health management as different lectins have been reported to show cytotoxic effects against the bacterial pathogen (Iordache et al. 2015). Furthermore, the opsonizing lectins present in fish serum could be useful in fish health, both in terms of gaining a better understanding of the immune system and the development of applications for disease prevention. Functionally analogous lectins in fish, including mannose-binding lectin, have been reported to effectively agglutinate bacterial cells. This points to the agglutination capability and immune response activation of lectins against bacterial pathogens in fish. Ottinger et al. (1999) showed that salmon lectins could opsonize *A. salmonicida* and make the bacterial more susceptible to the action of phagocytes. He further reiterated that the binding of the salmon serum lectin to the bacteria prior to

phagocytosis improved the bactericidal activity of macrophages and increased the degradation of internalized bacteria (Ottinger et al. 1999). Other documented literature have also clearly indicated that many of these lectins have the capability to enhance the phagocytic activity of immune cells, however, further research should be directed at understanding the molecular mechanism of the functional aspect of different types of lectins in fish immunity and the regulatory aspects. This will help in the development of fish lectins-based immunotherapeutic agent or nutraceuticals to fight infectious diseases in aquaculture.

8.7 Conclusion

Lectins are a versatile protein distributed widely in various kingdoms with innate immune modulation. Reports on the role and function of lectins in plants, animals especially in aquatic organisms are continuously increasing in recent years. There are several in vitro as well as in vivo studies to understand the mode of action of lectins in various plants and animals. They have biomedical, clinical, antimicrobial, and immune modulating applications. Different types of lectins have different mode of actions and regulations in different tissues. Researches showed that more than pattern recognition, lectins play a major role in immunity by regulating the glycan receptor binding through density levels. Different lectin types will have different mode of actions and regulations. In animals especially humans, the regulation part has been studied in a better way. However, very few studies have reported on the regulation of lectins in fishes and shellfishes. More researches have to be carried out in this regard and various genomic and feedback regulation through signalling has to be studied in detail.

Conflict of Interest The authors have no conflicts of interest to declare.

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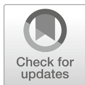
Chapter 9
Lectin–Carbohydrate Interactions in Pathogenesis



Anbazhagan Veerappan and Siva Bala Subramaniyan

Abstract Pathogenesis is a broad discipline that deals with the study of chain of events involved in disease aetiology and progression. An important event in pathogenesis is the interaction between the host and pathogen. Lectins are glycan-binding proteins present in all form of organisms, which has important role in cell–cell recognition. In this chapter, a general overview of some pathogen lectin involved in the recognition of the host cell surface glycans vice versa host lectin recognizing pathogen cell surface glycan was illustrated. An understanding of lectin–glycan interaction is useful to develop reverse lectin approach to tackle pathogenesis was proposed. Current evidences demonstrated that lectins capable of binding to exogenous ligand are helpful in targeting the glycans of pathogens. A detailed study may pave way for new therapeutic strategy based on lectin-mediated bioadhesion.

Keywords Lectin · Pathogenesis · Glycan interaction · Carbohydrate binding · Molecular recognition

Abbreviations

CD	Cluster of Differentiation
CLEC5A	C-type lectin domain family 5 member A
CL-L1	Collectin liver protein
DAP12	DNAX-activating protein of 12
DCIR	Dendritic cell Immunoreceptor
GD	Ganglioside
GT	Trisialoganglioside
HDACs	Histone deacetylases
IL	Interleukin

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JPtNPs	Jacalin capped Platinum Nanoparticles.
MHC	Major Histocompatibility complex
MMR	Macrophage mannose receptor
NK	Natural Killer
NKG2D	Natural Killer Group 2-D
NLRP	NLR family pyrin domain
SIGN	specific intercellular adhesin molecule-3- grabbing non-integrin
SP	Surfactant protein
Th	T helper cell type
TLR	Toll like receptor
TNF	Tumour necrosis factor
Trim5α	Tripartite Motif containing 5 alpha
ULBP1	UL16 binding protein 1

9.1 Introduction

Pathogenesis refers to the origination and development of disease or disorders. The most common types of pathogenesis occur from microbial infection, inflammation, malignancy and tissue breakdown (Casadevall and Pirofski 2000). Pathogens responsible for pathogenesis are categorized into six types such as viruses, bacteria, fungi, protozoa, parasites and prions. Disease can crop up through the toxins secreted by the pathogen, malfunctioning of immune systems or ageing process. The pathogens are capable of infecting a wide range of hosts (Bäumler and Fang 2013). The invasion of the pathogen into the host system is not always an easy step, because pathogens have to cross the host defensive barrier. Generally, hosts have unique mechanisms to elude the pathogen from the system, which differs from host to host. Hence, the study of host–pathogen interaction becomes an integral part of clinical research to understand the mechanism of pathogenesis. Studying pathogen interaction with human model is unsafe and unethical, therefore, *Caenorhabditis elegans*, *Drosophila melanogaster*, *Danio rerio* was popularly used as model organism in clinical biology to study the host–pathogen interaction (Madende et al. 2020). The information obtained from the model organism was useful in extrapolating with human diseases, because these organisms share close genetic similarity with humans.

The interaction between host and pathogens was achieved in four stages. The first stage is the host exposure to pathogens. Once exposed, the pathogens adhere to host cell surfaces or mucosal membranes and proliferate for survival in the second stage. In the third stage, the proliferated pathogens evade host immune response and invade the host to spread infection in the fourth stage. The invasion step is crucial for pathogenesis, thus, each pathogen follows a unique strategy to invade the host. Viral pathogens follow envelope mediated invasion through merging with the host membrane (Cossart and Helenius 2014). Bacteria or parasites invade the host cell through

inducing physiochemical change by secreting toxins (Brito et al. 2019). Fungi use surface hyphae to interact with the host cell to persuade the endocytosis process. The pathogen recognition associated with interaction to the specific host receptor with its conserved ligand to activate the signalling molecules present in the host, which activates the host defence mechanism. The persistent survival nature of pathogens attack the host defence system by changing its conserved sights and vandalizes the defence mechanism by dysregulating or destroying the host defence molecules (Thakur et al. 2019). The surviving pathogens start to adapt to the host condition and proliferate by captivating the host machineries. The process of host–pathogen interaction was decided through ligand–protein interaction or by protein–protein interaction. This includes the host cell surface or secreted protein interaction with pathogen ligand, and pathogen cell surface protein interaction with host secreted protein or ligand. There are several reviews about the involvement of proteins and ligands in pathogenesis, readers can refer to that (Davis et al. 2007). In this chapter, the discussion is limited to the lectins in pathogenesis.

9.2 Pathogen–Lectin and Host–Glycan Interaction in Pathogenesis

Lectins are carbohydrate binding proteins present in all kingdoms of life which includes human, animals, plants, bacteria and viruses (Chen et al. 2021). Lectins present in pathogen cell surface can aid the pathogen to attach the host cell surface glycan receptor (Nizet et al. 2017). Similarly, the lectins present in the host and the glycans of pathogens have a significant role in the host–pathogen interaction. The cell surface glycans present in the form of monosaccharides, polysaccharides or oligosaccharides helps the host or pathogenic lectin to recognize each other to initiate the colonization (Varki 2017). Each microbial lectin adopts a unique approach to interact with the host glycan receptor for its entry, colonization and activation of immune response. The following section briefs about some lectins present in microbes and its interaction with glycans (Fig. 9.1).

9.2.1 Viral Lectins Versus Host Glycans

The outer envelope proteins of viruses interact with the target cell receptor for its cellular entry during pathogenesis. They generally target the host cell surface glycosaminoglycans. The interaction is driven by charge difference where the positively charged outer surface of virion interacts with negatively charged glycosaminoglycans (Shi et al. 2021). For instance, the lectin, haemagglutinin esterase (HE), helps to release the viral progeny from infected cells and shun binding towards non-permissive cells. HE binds specifically binds to host cell surface sialic acid.

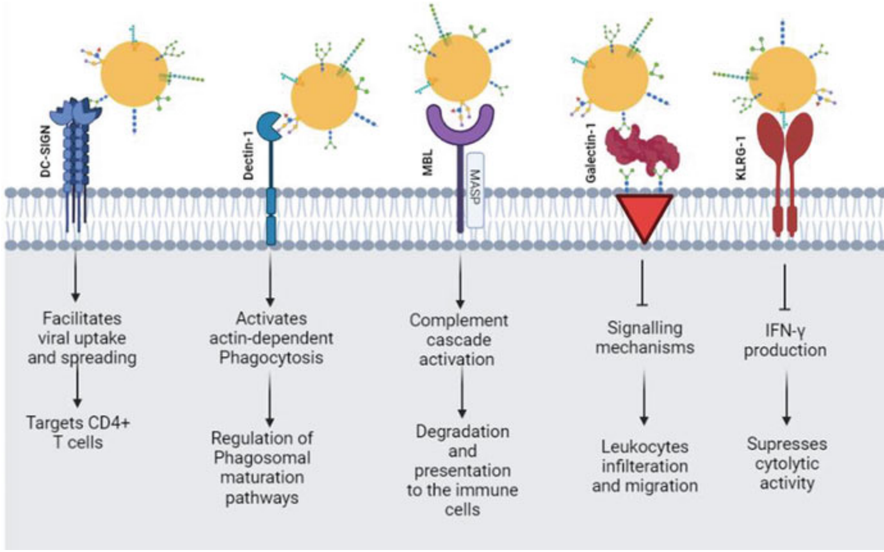

Fig. 9.1 A representative diagram show the consequences of host–lectin interaction with the glycans of the invading pathogens

Respiratory virus such as SARS-CoV-1, Influenza A virus causes infection mediated via sialic acid binding.

The viral attachment and entry into the host cell depend on the interaction of the multiple haemagglutinin (HA) with its multiple sialic acid-containing receptors. Haemagglutinin is a globular homotrimeric protein, which prefers to interact with a specific terminal of sialic acid (SA) on the host cell surface (Sriwilaijaroen and Suzuki 2012). HA is composed of two subunits, HA1 and HA2 connected by two disulphide bonds. The SA binding pocket was located in the HA1 subunit, which constituted a globular head region to interact with the host surface SA. HA1 decides the affinity and specificity of the binding of HA based on the preference of SA linkage (either α2–6 or α2–3). HA-2 is stalk-like subunits involved in the internalization of the virion into the host endosomal compartment via pH-dependent fusion (Dou et al. 2018). HA mediated human virus infection favours α2–6 linked SA in human lineage, whereas α2–3 linked SA was favoured avian lineage. The change in binding preference of viral HA from α2–3 to α2–6 is a precondition for transmission of avian virus to humans (Imai and Kawaoka 2012). However, studies related with human adenovirus type 37, human polyomavirus 1 (BK virus) indicated the human virus can bind to glycan motif of α2–3 linked SA in disialylated ganglioside GD1a, GD1b and GT1b receptors.

Viruses belong to *Parvoviridae*, *Herpesviridae*, *Hepadnaviridae*, *Rhapdoviridae*, *Coronaviridae* are well known for the recognition of host surface sialic acid with its specific viral proteins(Tong et al. 2018; Baker et al. 2020). Haemagglutinin-neuraminidase (HN) from human parainfluenza virus such as measles virus, Newcastle disease virus (NSV), mumps virus (MV), sendai virus (SV) and respiratory

syncytial virus (RSV) will interact with host surface SA (Takimoto et al. 2020). In the case of MV and NSV, the interaction of haemagglutinin with host sialic acid induces conformational changes in the fusion protein (F protein) and facilitates membrane fusion. The consequences of the viral interaction with SA differ between viruses. Coxsackievirus A24 variant and enterovirus 70 are groups of enterovirus interaction with sialylated oligosaccharides that cause acute hemorrhagic conjunctivitis (Mistry et al. 2011; Alexander and Dimock 2002). The viruses belonging to *Picornaviridae* such as hepatitis A virus and few classes of rhinovirus binding to SA resulted in upper respiratory inflammation (Greenberg 2016). BK virus causes fatal demyelinating disease and kidney graft loss (Darbinyan et al. 2016).

9.2.2 Bacterial Lectins Versus Host Glycans

Bacterial lectins are multi-subunit protein appendages known as fimbriae or pili. These protein appendages are used to adhere with the host cell surface glycans. Escherichia coli responsible for urinary tract infection, meningitis uses pili to adhere to the surface glycans of the host and tissue tropism. The variant of E. coli are known for producing lectins such as type-1 fimbriae, P fimbriae and F-17 fimbriae with specificity to mannose, galabiose and N-acetylglucosamine, respectively. Type 1 pili contain mannose binding lectin, FimH at the tip of the pili (Jones et al. 1995). Fim H present in the intestinal non-pathogenic E. coli binds to the host monomannose, whereas Fim H isolated from the uropathogenic *E. coli* (UPEC) specifically binds to the host oligomannose. The specificity of UPEC towards oligomannose was used to design of C1-modified α-D-mannoside-based Fim H antagonists. Apart from Fim H, *E. coli* has another pilus adhesion called UclD, which has specificity to host surface O-linked glycans (Poole et al. 2018).

The lectins present in the outer surface of *mycobacterium* possess an adhesion mechanism. In 1989, the first mycobacterial lectin was isolated from *Mycobacterium smegmatis*. The lectin molecular weight is 12–14 kDa. It agglutinates human A, B and O erythrocytes and the agglutination was inhibited by sugars (Kolbe et al. 2019). The lectin, mycotin, was reported from the cell surface of *Mycobacterium tuberculosis* (Mtb). The mycotin involvement in the interaction of Mtb was proved by inhibiting the adhesion of Mtb with mouse peritoneal macrophage by antimycotin antibodies (Goswami et al. 1994). Heparin binding haemagglutinin (HBHA) was reported from Mtb Rv0475 strain (Zheng et al. 2017). HBHA is a transmembrane protein consisting of canonical lysine-rich C-terminal heparin binding domains. It is involved in mycobacterial aggregation and plays a vital role spreading Mtb to extrapulmonary sites (Raze et al. 2018). HBHA-deficient Mtb showed the reduced dissemination in BALB/c mice (Esposito et al. 2010). The antibodies against HBHA inhibit the adhesion of Mtb to epithelial cells. Moreover, the humoral immune response to HBHA helps in dissemination of Mtb from lungs. The adhesion and invasion of Mtb was also achieved by two types of pili, type IV pili expressed by Mtb under broth condition and curli-like pili expressed under solid media condition

(Mann et al. 2016). Curli pili is a coiled, non branching protein consisting of β-sheet rich structure. The size of Mtb curli-like pili (MTP) expressed by Mtb Rv3312A strain is around 2–3 nm in diameter, which is similar to curli pili of *E. coli* or *Salmonella* species. Under in vitro conditions, MTP is involved in the aggregation and biofilm formation (Ramsugit et al. 2013). The MTP was expressed specifically in Mtb strains and absent in other mycobacterial species. The sera of TB patients show the IgG antibodies against the MTP. The MTP-deficient strains (mtp-null mutant) showed poorer adhesive nature than the MTP over expressing strain (Naidoo et al. 2018). The isolated MTP from Mtb has identified specificity towards laminin, a predominant extracellular matrix glycoproteins. Type IV pili (T4P) of Gram-positive and Gram-negative bacteria involves motility, adhesion, biofilm formation, DNA uptake and protein secretion. In Mtb, the expression of T4P is upregulated during interaction with A549 cells and macrophages (Kolbe et al. 2019). In silico genome analysis showed that T4P-associated genes in Rv3659 strain codes for a potential mycobacterial lectin. Currently, glycan-binding characteristics and their role in adhesion processes of Mtb T4P were not known. However, T4P from other bacteria showed lectin-like property (Kolbe et al. 2019).

Helicobacter pyroli, Gram-negative bacteria live asymptomatically in the gastric mucosa of the human population. The epithelial lining and the gastric mucosa of the human system is highly glycosylated. To survive in the gastric environment, *H. pyroli* uses lectins-like BabA and SabA adhesins. BabA is the blood group antigen-binding lectin. It recognizes Lewis B, which is a fucosylated glycoconjugate expressed in the gastric epithelial cells and mucosal mucins (Magalhães et al. 2016). The binding of BabA to glycans is pH-dependent processes, which show high affinity to the glycans of gastric mucosa at high pH. Sab A recognizes Lewis blood group antigens, sialyl Lewis X (sLeX) and sialyl lewis A13. The gastric lining of healthy individuals expresses low levels of sialic acid. But, the expression of silaic acid in the stomach lining increases after H. pyroli infection, suggesting that *H. pyroli* influences the host gastric glycome to establish the chronic infection (Magalhães et al. 2016). The evidences disclosed so far in the field suggest that glycointeractions has significant influence in bacterial pathogenesis.

9.2.3 Fungal Lectins Versus Host Glycans

Filamentous fungi and non-filamentous fungi contain adhesin, a major virulence factor that contributes to pathogenesis. Non-filamentous fungi like *Candida* uses epithelial adhesin (Epa) and agglutinin like sequence (ALS) for their adhesion during host cell invasion (Rodríguez-Cerdeira et al. 2020). The N-terminal adhesion domain of Epa proteins contains lectin activity. The haemagglutinin expressed by *Candida* species contains different classes of Epa proteins, which include Epa1, Epa6 and Epa 7. Epas recognize host oligosaccharides with terminal α-linked galactose moieties (Maestre-Reyna et al. 2012). Epa1 protein induces the adhesion of fungi to the host epithelial cell. Epa1 shows good affinity towards the

glycoproteins containing Galβ1–4 GlcNAc or Galβ1–4 Glc linkage. The adherence of Candida by Epa1 can be blocked by lactose and N-acetyl lactosamine (Ielasi et al. 2014). Likewise, Epa7 shows good binding to terminal Galβ1–3 Gal or Galβ1–4 Glc disaccharide. The ALS family consists of eight members, which are GPI-linked to the β-1,6- glucans of the cell wall (Zupancic et al. 2008). It is reported that Als2p, Als3p and Als4p are specific towards the human epithelial cells. Als1p has the ability to bind endothelial and epithelial during oropharyngeal candidiasis. Als3p helps *Candida* to damage oral epithelium through biofilm formation. Epa proteins contain PA14 ligand binding domain, which is conceivable as lectin and referred to as Pwp. The Pwp family potentially interacts with glycosaminoglycan and is involved in fungal aggregation, for instance, during host surface binding or biofilm formation.

Filamentous fungi such as *Aspergillus*, *Cephalosporium*, *Penicillium* express lectins for adhesion. *Aspergillus fumigatus*, opportunistic fungi expresses *Aspergillus fumigatus* lectin (AFL) with specificity to l-fucose and fucosylated oligosaccharides, which includes α1–6 linked core fucose (Houser et al. 2013). A. conidia express AFL as a major virulence factor for early stage infection. The lectin secreted or expressed by *Penicillium* species has specificity towards O-glycans, Glcβ1–3-GalNAc6so-3 linkages in chondroitin sulphate, galactose, xylose and N-acetyl-D-galactosamine (Singh et al. 2009). *Cephalosporium*, a filamentous opportunistic pathogen causes ophthalmic mycoses secrete a *Cephalosporium* lectin (CSL) with specificity toward fucosylated trimannosyl glycans rather than O-glycans and monosaccharides (Inamdar et al. 2016). Asialofetuin and mucin have the potential to inhibit the CSL activity, suggesting the glycointeraction is important for infection (Nagre et al. 2010). The filamentous fungi, *Fusarium solani*, cause keratitis in humans and contain lectins with specificity towards desialyted glycoproteins (Ortega-Rosales et al. 2019). Lectins isolated from other *Fusarium* species such as *F. acuminatum*, *F. chlamydosporium*, etc. shows specificity towards L-fucose, D-galactose, bovine mucin and dextran. Lectin from *Penicillum* species showed specificity towards O-glycans which can be inhibited in the presence of porcine stomach mucin. *P. griseofulvum*, *P. thomii*, *P. duclauxii* and *P. proteolyticum* secretes lectins showed specificity towards GlcAβ1–3GalNAc6SO-3 linkages present in chondroitin sulphate. The illustrated discussions suggest that lectins present on the fungi plays vital role in fungal pathogenesis.

9.3 Host–Lectin and Pathogen–Glycans Interactions in Pathogenesis

Considering the omnipresent nature of lectins, glycans of the pathogens were well recognized by host lectins to initiate host signalling mechanisms to combat the invading pathogen. The pathogens are also smarter to use the glycans to reach the host lectin to establish proliferation and spread infection to the adjacent host cells. Host lectins are classified into membrane associated lectins and soluble lectins. The

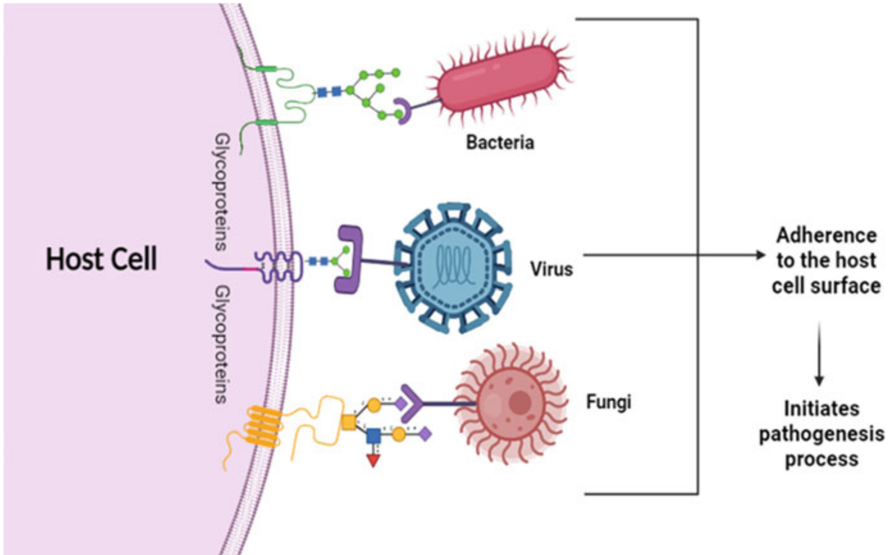

Fig. 9.2 A representative diagram illustrate the recognition of host glycan by the pathogenic lectins

importance of these lectins in the context of pathogenesis was discussed below (Fig. 9.2).

9.3.1 Membrane Associated Lectins

Membrane associated lectins serve as a pathogen recognition iota, which has the ability to bind to the pathogen and turn on signalling mechanism against the pathogen. These lectins assist in presenting antigen to the antigen presenting cells (APC) to develop adaptive immune response against pathogens (Bidula et al. 2019). The pathogen attached to surface lectins of immune cells promotes the host immune system for effective clearance and degradation of the pathogen. C-type lectin shows an immense role against the pathogen, where it can act as a pattern recognition receptors (PRRs) by which it recognizes the glycan structure on the pathogen surfaces. C-type lectins such as SP-A, SP-D, MBL, collectin liver protein 1 (CL-L1) and CL-K1 acts as an opsonins. It involves in phagocytic engulfment, complement pathway activation, modulation of inflammation and inhibition or direct killing of pathogens (Casals et al. 2019). β-glucan receptor, a membrane associated C-type lectins, activates the intracellular signalling and induces a wide range of cellular and immunological responses against the invaded pathogens (Goodridge et al. 2009). NK cells expressing C-type lectins trigger the antiviral immunity by regulating cytotoxic responses. It leads to alteration in the expression of MHC class I

or MHC class I like molecules on viral infected cells (Paul and Lal 2017). For instance, NKG2D contains MHC class I-like ligands, which is highly regulated in healthy cells through transcriptional repression of histone deacetylases (HDACs). During herpes virus infection, the expression of HDAC inhibitors by viral genes will overcome this transcriptional repression resulting in the expression of NKG2D ligands, ULBP1 (Greene et al. 2016). Viruses are evolved to overcome these defences, by inhibiting the expression of NKG2D ligands at the host surface. The expression of virus-encoded antagonists, MHC class I-like molecules, blocks the ligand binding to NKG2D and inhibits the NKG2D through the host cytokine response modulation.

C-type lectins expressed by myeloid cells recognize the viral glycoproteins to invoke antiviral responses such as degradation, clearance of virus particles and activation of adaptive immune responses. C-type lectins expressed in the Langerhans cells (CD207) recognize HIV-1 envelope protein gp120 through the high level of mannose glycans. The interaction gp120 was associated with the internalization of the virus into Birbeck granules results in virus degradation and clearance through TRIM5α- dependent autophagy pathway. C-type lectin-mediated adaptive immunity against viruses can be activated indirectly, through DCIR-mediated inhibition of damaging inflammatory responses during Chikungunya infection. The cross-presentation activities of DNGR1 mediated by C-type lectins were required to control vaccinia virus and herpes simplex virus infections (Iborra et al. 2012). Inversely, C-type lectins can have injurious effects during virus infections (Brown et al. 2018). DC-SIGN is a mannose specific C-type lectin present on the dendritic cell surface. The DC-SIGN binding to mannose of HIV-1 virion facilitates viral uptake and spreading infection in DCs and successive trans-infection of CD4+ T cells. DC-SIGN targeted by viruses affects the cellular functions, such as DC maturation, signalling induced through TLRs and activation of RIG-I-like receptors (Lester and Li 2014). Another C-type lectin known as MMR involves in HIV binding and transmission. It involves recognition of many viruses such as dengue virus, influenza virus and hepatitis B virus. Dengue, influenza and Ebola viruses are recognized by C-type lectins such as DAP12-coupled CLEC5A and CLEC4G in the liver (Huang et al. 2016). Lymph node sinusoidal endothelial C-type lectin influences the pro-inflammatory responses to support the pathogenesis of viral infections. Membrane associated C-type lectin receptors (CLRs) regulate diverse cellular functions in leukocytes and play a key role in immune response against the invaded pathogens (Bermejo-Jambrina et al. 2018). For instance, dectin 1 can bind pathogens to the leukocyte cell surface and activates the intracellular signal transduction. The activation induces actin-dependent phagocytosis against the pathogen results in regulation of phagosomal maturation pathways (Uribe-Querol and Rosales 2017). CLRs can activate the expression of cytokines, chemokines and immunomodulatory lipids such as eicosanoids. The activation of NLRP3, NLRC4 or caspase 8 inflammasomes by CLRs lead to IL-1β production through direct or indirect mechanisms (Duez and Pourcet 2021).

Galectins are a group of proteins with affinity for β-galactosides and characteristic CRD sequence motifs. Galectins play a key role in innate and adaptive immune

responses against infectious pathogens, allergic responses and even cancer. Galectins are expressed ubiquitously in mammalian tissues, includes immune cells such as dendritic cells, macrophages, mast cells, natural killer cells, gamma and delta T cells, B1 cells and activated B and T cells (Vasta et al. 2017). Mammalian galectins are classified into proto, chimera or tandem repeat types. Proto-type galectins are homodimers linked non-covalently through one CRD per subunit. Chimera-type galectins are similar to proto-type galectins, which have carboxy-terminal CRD attached to the amino terminal peptide rich in glycine, tyrosine and proline. In case of tandem repeat galectins, functional linker peptide attaches the two CRDs (Si et al. 2016). Nearly, 15 types of galectins are identified in mammals in which Galectins 1, 2, 5, 7, 10, 11, 13, 14 and 15 are belongs to proto type galectin and galectins 4, 6, 8, 9, and 12 belongs to tandem repeat type and only galectin 3 belongs to chimera type of galectin. The anti-inflammatory activity by Galectin 1 inhibits the signalling mechanism resulting in leukocyte infilteration, migration and recruitment. The viral attachment and host cell fusion was inhibited by galectin 1 on binding to virion envelope or capsid glycoproteins containing N-linked oligosaccharide (Pourrajab 2021). In Nipah viral infection, the fusion and attachment was triggered by the envelope glycoprotein to the ephrin B2 located on the endothelial cell surface. Conversely, ephrin B3 receptor inhibits the cell–cell fusion and syncytia formation. The dimeric galectin 1 oliogmerize the N-glycans of the Nipah virus envelope glycoproteins and inhibits cell–cell fusion (Levroney et al. 2005). The removal of viral N glycan regions results in higher fucogenicity and antisera neutralization with high sensitivity.

Pathogens like S. aureus expressing β,1,4-GlcNAc cause epicutaneous infection with increased skin inflammation. C-type lectin in skin recognizes the β,1,4-linked N-acetylglucosamine region on the surface of glycopolymer wall of teichoic acid of S. aureus. The glycan binding to lectin increases the level of cytokine production of Th-1 and Th-17 (Mnich et al. 2019a, b). C-type lectins are involved in antibacterial immunity related to mycobacterial infections. The glycolipids from the cell wall of the pathogens are recognized by C-type lectins. It includes the recognization of trehalose dimycolate (TDM) by minacle; phosphatidyl-inositol mannosides (MCL) by DC immunoactivating receptor 1 (DCAR 1); and mannose capped lipoarabinomannan by dectin 2 (Ishikawa et al. 2009). Even though CLRs show a strong response towards the mycobacterial ligands, the exact role of individual CLRs is not clear in antimycobacterial immunity. Bacteria such as *S. pneumoniae*, *K. pneumoniae*, *N. meningitidis* and *N. gonorrhoae* contain ligands specific to galectins (Díaz-Alvarez and Ortega 2017). The C-terminal CRD moiety of galectin 3 interacts with lactosyl moieties of lipopolysaccharides (LPS) in *K. pneumonia* (Mey et al. 1996). The N-terminal domain of Galectin interacts with the LPS lipid A moiety in Salmonella subspecies (Bertani and Ruiz 2018). During infection, the glycolipid, mycobacterial phosphatidyl mannosides engulfed in mycobacterial phagosome was recognized by galectin 3 (Weiss and Schaible 2015). These studies have established that membrane associated lectins can recognize the pathogen glycosylation during infection and assist the host to fight against infection.

9.3.2 Soluble Lectins

Soluble lectins aid in protecting the host from the infection by neutralizing and clearing the pathogen from the system. Surfactant protein A, D (SP-A, SP-D) and mannose binding lectins (MBL) belongs to soluble C-type lectin works in defence mechanism against the pathogen (Watson et al. 2019). These lectin monomers share similar structure with N-terminal cysteine rich domain, a coiled coil neck domain, collagen like domain, and C-terminal CRD (Murugaiah et al. 2020). Despite sharing similar structure, the glycan specificity and effectiveness against the infection differs significantly within the soluble lectins. For instance, SP-D and MBL binds to the Influenza A virus surface glycoproteins, haemagglutinin and neuraminidase. At the same time, SP-A recognize the glycoproteins of Influenza A virus, but the variant of Influenza A virus does not display lectin property from SP-A. Alternatively, the variants interaction depends on the SP-A CRD, which contains sialyted N-glycans with viral haemagglutinin (York et al. 2019).

The classical pathway of pathogen recognition occurs through antibodies, whereas in the lectin pathway, the glycan recognition by mannose binding lectin or ficolins is involved (Howard et al. 2018). The lectin pathway was activated through the interaction of MBL and MBL-associated serine protease (MASP). Hence, the lectin of the host cells activates either innate or adaptive immune response against a pathogen. For example, MBL recognizes surface glycans of pathogens such as viruses, bacteria, fungi, protozoan and parasites. MBL is specific to mannose, but also recognizes L-fucose, N-acetylmannosamine (ManNAc) and N-acetylglucosamine (GLcNAc) except galactose (Idowu et al. 2021). Besides sugars, MBL binds to the phospholipids, nucleic acids and non-glycosylated proteins. The broad specificity of MBL to recognize pathogens allows MBL to activate diverse biological pathways, including complement activation, lectin pathway, opsonophagocytosis and inflammation. MBL mediates the recognition by interacting with the terminal sugars of cytoskeletal proteins in apoptotic cells results in phagocytosis mediated macrophage leading to their clearance (Gregory and Devitt 2004). Ficolins are another class of pattern recognition receptors involved in activating the lectin pathway to initiate immune response against the pathogen. Ficolin-1 was found in secretory granules of monocytes, gelatinase granules of neutrophils and type II alveolar epithelial cells. Ficolins cannot be considered directly as lectins, because they bind to acetylated molecules rather than specific carbohydrates. Ficolin-1 recognizes the acetylated compounds like GlcNAc and GalNAc present in both Gram-positive and Gram-negative bacteria (Bidula et al. 2019). Among the ficolins, ficolin-1 interacts with sialic acid located on the capsular polysaccharide of pathogen, *S. agalactiae* as well as on the surface of immune cells. Ficolin-2 secreted in the liver recognizes N-acetyl-d-glucosamine (GlcNAc), N-acetylgalactosamine and N-acetyllactosamine (Kilpatrick and Chalmers 2012). It also interacts with N-acetylneuraminic acid present on encapsulated pathogens such as S. agalactiae, bacterial peptidoglycan, fungal 1,3-β-D-glucan and envelope glycoproteins of hepatitis C virus. These studies describe that the initiation of lectin pathways by pattern

recognition molecules such as MBL, CL-K1 and ficolins establish the interaction with pathogen-associated molecular patterns. The complement cascade of interaction leads to the formation of a complex called terminal complement complex or MAC. This creates pores in the cell membrane, disturb the membrane integrity and finally lead to cell death.

9.4 Reverse Lectin Approach Against Pathogen Glycans

The reverse lectin approach uses exogenous lectins as targeting moieties that target glycoproteins or glycololipids over expressed on the surface of pathogens (Fig. 9.3). Lectins shows antibacterial, antiviral and antifungal activity through the interaction with peptidoglycans, polysaccharides, lipopolysaccharides, teichoic acid and teichuronic acids present on the pathogen cell surface. The lectin isolated from the marine sponge *Chondrilla caribensis* (CCL) reduces biofilm biomass formed by *S. aureus*, *S. epidermidis* and *E. coli*. The activity of CCL was inhibited by α-lactose, suggesting that the lectin carbohydrate recognition domain (CRD) was involved in the antibiofilm activity (Marques et al. 2018). BanLec, a lectin isolated from the banana (*Musa acuminate*), has shown affinity towards mannose glycans present on viral envelops of HIV-1 and inhibits the viral entry into the cell at picomolar concentration (Swanson et al. 2010). Engineered banana lectin (H84T) is effective against influenza virus strains, including drug-resistant and currently circulating strains. The H84T recognizes viral haemagglutinin glycoportein and exerts its action

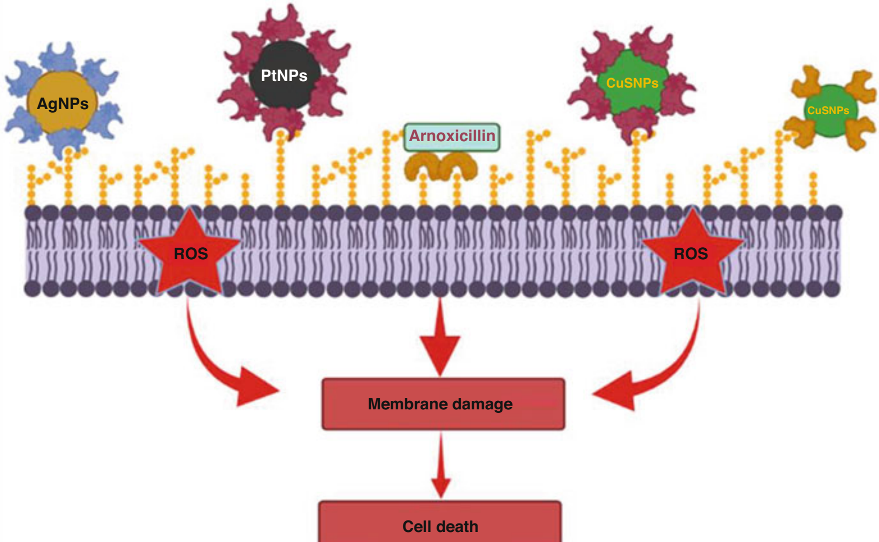

Fig. 9.3 Depicting the reverse lectin approach with nanoparticles to combat invading pathogens

through inhibiting viral membrane fusion to the host endosomal membrane (Covés-Datson et al. 2020). *Aspidistra elatior* (AEL), a rhizome lectin with affinity to mannose showed promising effect against the vesicular stomatitis virus, Coxsackie virus B and respiratory syncytial virus (Xu et al. 2015). The marine worm lectin, CvL isolated from *Chaetopterus variopedatus* shows affinity to β- galactose. CvL exhibit anti-HIV-1 activity by inhibiting the production of viral p24 antigen and the cytopathic effect induced by HIV-1, blocking the HIV-1 entry into the host cells (Wang et al. 2006).

The alage-derived lectin, Griffithsin (GRFT) was known for its high potential in inhibiting the entry of human immunodeficiency virus. The antiviral activity of GRFT is known for its ability to interact with the terminal of mannose oligosaccharide. The mannose binding crosslink the glycans present in the surface of the viral envelope glycoproteins and inhibit the HIV entry (Lusvarghi and Bewley 2016). GFRT works against genotypes I and II hepatitis C virus through binding to envelope glycoproteins E1 and E2 and blocks the viral penetration in human hepatocytes. A mannose binding lectin from red algae, *Eucheuma serra* (ESA-2) display antiviral property against HIV-1 and influenza A virus (H1N1) (Sato et al. 2011). Microvirin (MVN), mannose specific lectin isolated from *Microcystis cyanobacterium* inhibits the HIV-1 infection (Huskens et al. 2010). Sytovirin (SVN) isolated from *Scytonema varium* recognize mannose residues on the envelope glycoproteins of virus and inhibits the replication of Zaire Ebola virus (Garrison et al. 2014). Concanavalin A (ConA), a lectin extracted from the jack-bean, sugar specificity for α-MD glucopyranoside. ConA binds to *K. pneumonia* cells directly and agglutinates them without affecting their growth. However, pre-treatment with ConA protects the mice from *K. pneumoniae* infection and results in 55% survival. The consecutive ConA treatment after infection enhances the mouse survival to 83%. The plant lectin, ConBr (*Canavalia brasiliensis*) and CFL (*Cratylia argentea*) reduce the intracellular infection by Salmonella enterica serovar. ConBr and CFL treated animals showed immunomodulatory properties, which was evident by the reduced expression of IL-10 and TNF-α in the peritoneal fluid (Batista et al. 2017).

Besides acting directly on pathogens, some lectins are capable of directing drug to bugs. Wheat germ agglutinin (WGA)-conjugated liposomes possess bioadhesive properties as well as capable of encapsulating drug like amoxicillin and binds to oral epithelial cells within minutes. The amoxicillin loaded WGA-liposomes complex showed sustained drug release for effective management of oral pathogenic infection. ConA conjugated microspheres of amoxicillin trihydrate has good mucoadhesiveness and also showed controlled drug release in simulated gastric fluid. The ConA conjugates are suggested to have better therapeutic effectiveness against bacteria like *H. pylori* residing in gastric mucosa (Wijetunge et al. 2020). A lectin isolated from the seeds of *Butea monosperma* (BMSL) forms a non-covalent complex with silver nanoparticles (AgNPs) and exhibits enhanced activity against UPEC. The carbohydrate binding site plays a crucial role in targeting the AgNPs to the surface glycans of *E. coli*. The chemical modification of BMSL sugar binding site depletes the bacterial cell surface glycan activity and severely affects the antimicrobial activity BMSL-AgNPs complex (Bala Subramaniyan et al. 2020).

The AgNPs functionalized with Jacalin, a lectin from the seed of Artocarpus integrifolia kills the *S. aureus* in less than 30 min through inducing oxidative stress and membrane damage. Jacalin-AgNPs complexes also inhibit exopolysaccharide synthesis and reduce the biofilm formation ability of *S. aureus*. The antibacterial and antibiofilm activity of Jacalin-AgNPs complex was hindered by galactose, suggesting that the sugar binding site of jacalin is critical for better activity (Ahmed et al. 2018). Nanoparticles conjugated with jacalin display good therapeutic efficacy in treating bacteria-infected zebrafish (Subramaniyan et al. 2019). The platinum nanoparticles prepared with jacalin (JPtNPs) were highly effective against Gram-positive and Gram-negative bacteria. JPtNPs rescue *A. hydrophila* infected zebrafish and develop adaptive immunity against the bacterium. JPtNPs promotes the pro-inflammatory cytokines and antibody production against the bacteria which prevents the fish from re-infection (Ayaz Ahmed et al. 2018). Jacalin-copper sulphide (CuS) nanoconjugates show excellent antifungal activity against Vulvovaginal Candidiasis causing *Candida albicans* and exhibit synergistic activity with standard antifugal drugs (Senthilganesh et al. 2021). Shrimp lectin conjugated CuS nanoparticles enhance the elimination of aquatic pathogens in infected Nile Tilapia (Rubeena et al. 2020). The emerging evidences suggest the lectin could be used to extract therapeutic action from drugs at reduce concentration, thereby; the side effects associated with drugs can be negated.

9.5 Conclusion

In conclusion, the interaction between lectin and glycans plays a key role in pathogenesis process. The lectins in the pathogen surface helps in adhesion process through the interaction with the host surface glycans. The adhesion of the pathogen is facilitated by various host surface glycans varies from pathogen to pathogen. It helps the pathogen to recognize the host surface and also helps in invasion process. Upon adhesion pathogen invades to the host cell and proliferates and cause infections. The lectins in the host surface helps in the activation of immune response against the pathogen. The lectin activates the host signalling mechanism against the pathogen leads to the release of molecules which acts against the pathogen or the activation of host immune response. The exogenous lectins such as plant lectins can be used as a reverse lectin approach to target the pathogen glycans to develop the targeted drugs. Hence, understanding the interaction of lectin and glycans in pathogenesis helps us to know about the process. It also opens a wide area of research to develop a new age of lectin-mediated drug delivery.

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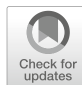
Chapter 10
Lectins in Health and Diseases: Mannan-Binding Lectin and Infectious Diseases



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Abstract Mannan-binding lectin (MBL), a pathogen recognition receptor of the innate immune system, plays a key role in all types of infections and diseases. The ability of MBL to recognize pathogens facilitates immune mechanisms to function efficiently for the clearance of disease-causing agents. MBL not only initiates activation of lectin complement pathway but also simultaneously facilitates other effector functions of the immune system, such as proinflammatory responses, generation of reactive oxygen species, and phagocytosis. Thus, appropriate levels of MBL provide protection from the majority of diseases. However, certain intracellular organisms whose phagocytosis is increased due to opsonization with MBL get benefits from higher levels of MBL and thus in such cases MBL becomes a facilitator for these organisms to cause diseases. MBL being protective or increasing the susceptibility to various bacterial, viral, parasitic, and fungal infections has been extensively studied. Few important polymorphic sites in the exon and promoter region of MBL gene have been reported and they cause extensive variations in serum MBL concentration. Association of occurrence of diseases with the genetic variants has also been scientifically proven. Even though the immune system of the host is

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well equipped, pathogens have evolved to employ various survival strategies and evade immune response to cause disease.

Keywords Mannan-Binding Lectin · MBL-associated serine protease MASP · Disease association

Abbreviations

AIDS	Acquired Immunodeficiency Syndrome
BBB	Blood–Brain Barrier
C1–C9	Complement Proteins C1–C9
CMV	Cytomegalovirus
COPD	Chronic Obstructive Pulmonary Disease
CRD	Carbohydrate Recognition Domain
DV	Dengue virus
HA	hemagglutinin
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV-1	Human Immunodeficiency Virus 1
IAV	Influenza A Virus
MAC	Membrane attack complex
MASPs	MBL-associated Serine Proteases
MBL	Mannan-binding Lectin
NA	Neuraminidase
PAMPs	Pathogen Associated Molecular Patterns
PID	Pelvic Inflammatory Diseases
PRR	Pattern Recognition Receptor
RTI	Respiratory Tract Infections
SARS-CoV	Severe Acute Respiratory Syndrome Coronavirus
UTI	Urinary Tract Infection
WNV	West Nile Virus

10.1 Introduction

Infectious diseases are the disorders caused by the invasion and multiplication of pathogens into the host. These include bacteria, viruses, parasites, and fungi. Pathogens invariably induce the immune response when they cross the primary barriers of the host system. The most important act of the immune system is to recognize these infectious agents for their proper clearance from the host's body. The pathogens' presence itself initiates the process of their recognition by the host's immune system. Hence, the initial defense is the recognition, which is done by a set of innate

proteins. A few of these proteins act as pattern recognition receptors (PRR, also referred to as pattern recognition molecules or PRMs), which help in recognizing patterns of glycoproteins and glycolipids, almost exclusively present on pathogens and known as Pathogen Associated Molecular Patterns (PAMPs). Recognition of PAMPs on pathogens by PRRs is the key to initiate the process of killing any disease-causing agent. This process is facilitated by innate immune cells and supported by inflammatory responses, thus the elimination of non-self, i.e. disease causing agent is achieved by host's immune system.

Mannan-Binding Lectin (MBL) has been identified as one of the important key proteins of the innate system, which plays a key role in recognition of the pathogens followed by a series of processes which enable the host immune system in its removal without causing disease. It is only when the host defense dips in favor of the intrinsic virulence of the infectious agent, the disease occurs. This may also happen when the key protein MBL has either its inappropriate levels or the altered structure. This chapter describes various mechanisms initiated, activated, and operated by MBL, its polymorphic genetic variants and its significance in various infectious diseases.

10.2 MBL-Mediated Immune Response

MBL is an acute phase protein produced mainly by the liver and some other organs such as the small intestine. It is a C-type (Ca^{2+} dependent) lectin belonging to the Collectins superfamily (Murugaiah et al. 2020). It also acts as a pattern recognition receptor (PRR) that is able to recognize PAMPs present on a wide variety of infectious agents. It is able to differentiate the invading non-self, microorganisms from self and initiate pathways to kill and remove them by various processes. Figure 10.1 depicts the strategies adopted by MBL to counter various infections. The PPRs mediated recognition of PARMs on pathogen triggers the receptor mediated effector function of MBL which leads to initiation of activation of lectin pathway (LP) of complement activation, which involves sequential cascade of proteolytic reactions of complement proteins leading to the formation of terminal membrane attack complex and eventually killing of the pathogen. Ability of MBL to activate complement is due to its association with three serine proteases known as MASPs (MBL-Associated Serine Proteases) which gets activated to initiate the complement cascade only when MBL binds to its targets. Three serine proteases which remain associated with MBL are MASP-1, MASP-2, and MASP-3. Two non-enzymatic fragments of MASPs, i.e. MAp44 and Map19, are also found to be associated with the recognition complex.

According to Héja et al. (2012) MASP-1 controls the full activation process. It begins with the autoactivation of MASP-1, which activates zymogen form of MASP-2, which then cleaves C4 into C4a and C4b. The surface of the target pathogen being bound with MBL gets deposited with C4b fragments on it. This follows binding of C2, which gets cleaved mainly by MASP-1 (60%) and by MASP-

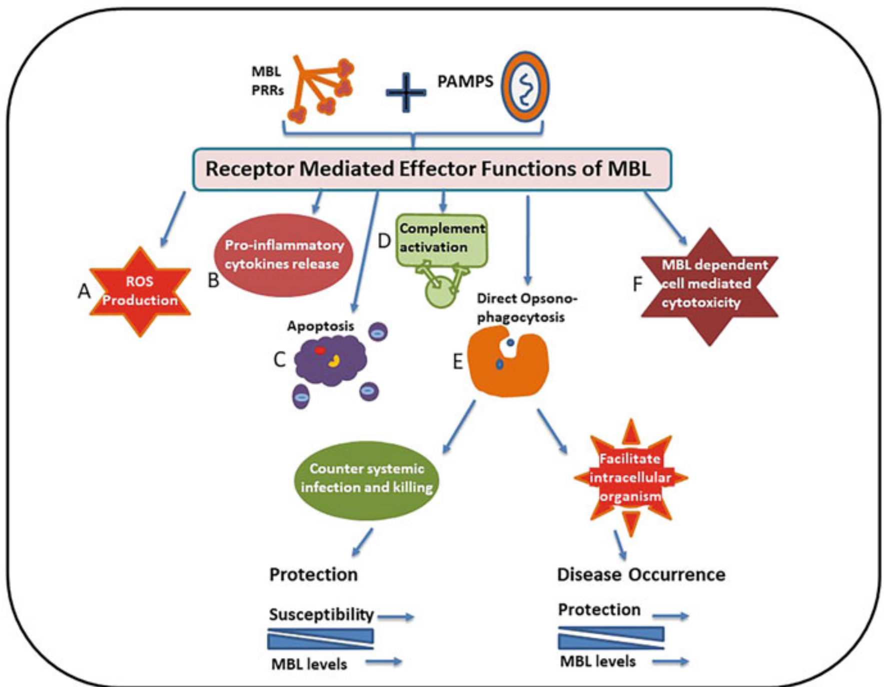

Fig. 10.1 MBL-mediated Immune responses (Kawasaki et al. 1983): (**a**) increased production of reactive oxygen species, (Hartshorn et al. 1993), (**b**) enhanced release of pro-inflammatory cytokines (Zhou et al. 2019), (**c**) enhanced apoptosis, (Nauta et al. 2003) (**d**) activation of complement pathway (Matsushita and Fujita 1992) by PAMPs by MBL, (**e**) activated direct opsono-phagocytosis (Kuhlman et al. 1989) which helps to counter systemic infections (Protection), however, it may also facilitate intracellular organism leading to disease occurrence (susceptibility), (**f**) activated MBL dependent cell mediated cytotoxicity (Ma et al. 1999)

2 (40%), resulting in the formation of the C3 convertase (C4b2a). Complement C3 being substrate of C3 convertase gets cleaved into C3a and C3b which are anaphylatoxin and opsonin, respectively. Furthermore, C3b gets deposited on the surface of the pathogen and C5 convertase activity is generated which cleaves C5 into C5a, another anaphylatoxin and C5b. This follows deposition of C5b and activation of complement components C6, C7, and C8. This eventually leads to the formation of terminal membrane attack complex (MAC) by C9 resulting in lysis of target pathogen. This pathway of complement is able to kill pathogens in an antibody independent manner providing immediate response to invasion (Fig. 10.2).

In parallel, the process of target killing is further facilitated by anaphylatoxins, i.e. C3a and C5a which attract neutrophils at the site of infection. The C3b opsonized target easily gets phagocytized by these neutrophils which have receptors for C3b. Thus, the process of opso-phagocytosis of the target is also facilitated by MBL. This further includes production of Reactive Oxygen Species, proinflammatory cytokine

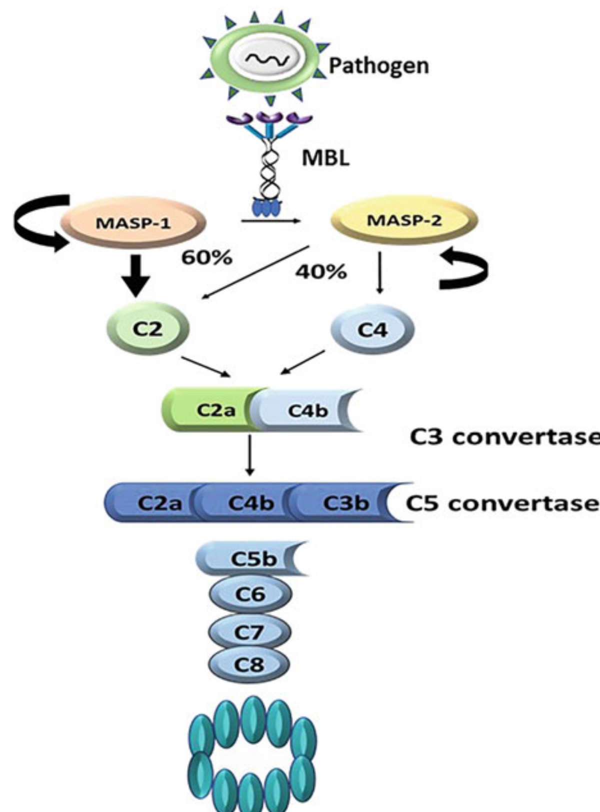

Fig. 10.2 Lectin pathway (LP) of complement: Activation of LP of complement by recognition of PAMPs by MBL. This binding activates MBL-associated serine protease (MASPs), which cleave C2 and C4 to generate C4b2a, a C3 Convertase. C3 convertase cleaves C3 into C3b, and C3a. C3b fragments opsonize the pathogen and generate C5 Convertase activity (C4b2a3b) which cleaves C5 into C5b and C5a. C5b fragments attach on the surface of the pathogen to which subsequent additions of complements 6, 7, 8, and 9 generate a terminal complement complex, a membrane attack complex (C5b-9). Fig modified from Héja et al. (2012)

release, and MBL dependent cell mediated cytotoxicity. Hence, MBL's role in innate immune response greatly impacts the target killing.

10.3 MBL Structure

The human MBL gene is known as *MBL2*. In addition to this a pseudogene of MBL that is *MBL1P1* is also present in humans and they are closely situated at chromosome number 10 (10q11.2-q21) (Sastry et al. 1989; Guo et al. 1998). Although MBL1 and MBL2 have been detected in Rhesus monkeys, (Mogues et al. 1996) but only MBL2 represents MBL in humans and chimpanzees.

The protein-encoding region of the MBL2 gene is covered by *four exons* separated by three introns (Taylor et al. 1989). The first exon encodes a signal peptide, a cysteine-rich domain, and seven copies of Gly-Xaa-Yaa (glycine motif) repeats

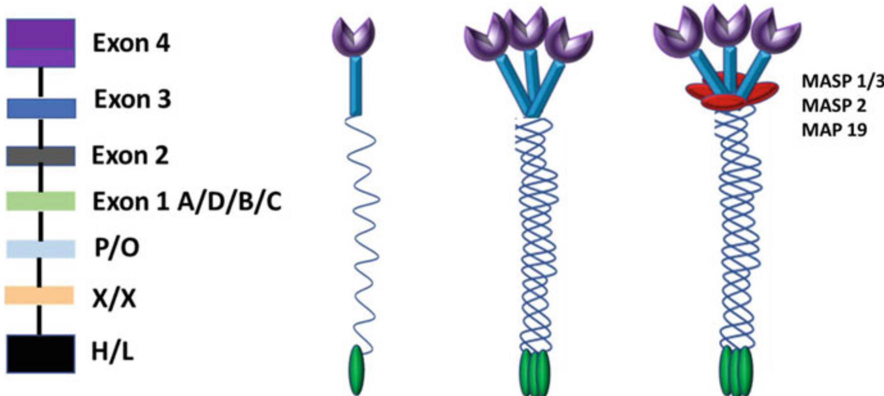

Fig. 10.3 Structure of MBL protein and its gene: Different exons on MBL gene encode different regions of the protein. Three identical 32 kDa polypeptide form a triple helix. Higher oligomers of different sizes are formed, tetramer being the most common one. Fig modified from Dommett et al. 2006

followed by exon second which encodes 12 repeats of Gly-Xaa-Yaa. The neck region and carbohydrate recognition domain (CRD) are encoded by exon third and fourth, respectively. Figure 10.3 schematically depicts the structure of MBL.

The secreted MBL is a 228 amino acids long polypeptide chain found as an oligomer of homotrimeric helical structured form. The single polypeptide has 20 cysteine-rich residues on the N-terminal side, followed by 19 Gly-Xaa-Yaa (where X and Y are any amino acids) collagenous repeats, a neck region, and a CRD on the C-terminal side. The CRD recognizes carbohydrates on the pathogen or self-altered structure in Ca^{2+} dependent manner (Fig. 10.3). Metal binding site of CRD is of approximately 1°A diameter. Metal ions greater than this are not able to enter the site (Patel et al. 2015).

10.4 MBL Polymorphic Variants

Different allelic variants of MBL have been reported due to polymorphism found in the promoter and structural region of MBL2 gene. These variants of MBL play a pivotal role in various disease susceptibilities. The stability and the concentration of MBL protein are influenced by variations in the alleles. Three allelic variants referred to as B, C, and D have been reported in MBL2, while the wild type is A. These variants are due to three frequent polymorphisms in exon1 of the MBL2 gene, as revealed by MBL2 gene sequencing. Point mutations at codon 52, 54, and 57 change the amino acids. Arginine gets replaced by cysteine at codon 52 (CGT to TGT), (Lipscombe et al. 1993; Madsen et al. 1994), glycine by aspartic acid at codon 54 (GGC to GAC) (Sumiya et al. 1991), and glycine by glutamic acid at codon 57 (GGA to GAA) (Table 10.1). All three alleles have a strong dominant effect

Table 10.1 MBL2 Structural Variations due to SNPs in exon1

Codon	Reference SNP (rs) cluster ID	Existing codon and its encoded amino acid	Replaced codon and its encoded amino acid	Allele Name	Level of functionally active MBL oligomers in circulation
Codon 52	rs5030737	CGT- arginine	TGT- cysteine	D	Decrease
Codon 54	rs1800450	GGC-glycine	GAC-aspartic acid	B	Decrease
Codon 57	rs1800451	GGA- glycine	GAA- glutamic acid	C	Decrease

Table 10.2 Promoter polymorphism of MBL2

S. No.	Position in promoter	Reference SNP(rs) cluster ID	Nucleotide substitution	Variants
1.	−550	rs11003125	G to C	H/L
2.	−221	rs7096206	C to G	X/Y
3.	+4	rs7095891	C to T	P/Q

(heterozygotes) on MBL levels in serum, lowering functional MBL levels by roughly 90%. These MBL variants are very unstable, easily degradable, which reduces their oligomeric formation, and also have a shorter half-life in blood circulation. This could lead to a decrease in the absolute concentration of variant MBL polypeptide chains in the bloodstream, as well as a loss of function. According to epidemiological studies, these three MBL variations are distributed in varying frequencies in different populations around the world (Garred et al. 2006). MBL deficiency due to allelic variations has been reported to be associated with increased susceptibility to infectious diseases (Garred et al. 2006).

In addition to these structural variants, sequencing of promoter regions revealed three additional variants, which have a direct impact on MBL concentration in the circulation (Madsen et al. 1994; Madsen et al. 1998). In these promoter polymorphisms, G to C nucleotide substitution (H/L variants) at position −550, C to G nucleotide substitution (X/Y variants) at position −221, and C to T nucleotide substitution (P/Q variants) were observed at position +4 of the 5′ untranslated region of exon 1 (Table 10.2).

Such promoter and exonic polymorphisms result in various MBL haplotypes. Seven common MBL secretor haplotypes have been identified: LYPA, HYPA, LXPA, LYQA, LYPB, LYQC, and HYPD (Madsen et al. 1998). HYPA have been linked to high levels of MBL in circulation, LYQA, and LYPA are linked to intermediate levels, whereas LXPA, LYPB, LYQC, and HYPD have been linked to low levels of the same (Madsen et al. 1998). However, in addition to the seven common MBL haplotypes, many population-specific investigations have discovered additional MBL haplotypes also.

MBL deficiency is very common with frequency of 10–20% depending on cut-off. Levels in serum below 500 ng/ml are considered as deficient as this much is essential for opsono-phagocytosis (Keizer et al. 2014). As the serum level of MBL is genetically determined, genotyping of promoter including exon should essentially be used to define deficient individuals and not the serum levels of the same.

10.5 MBL in Bacterial Infections

Binding of MBL to a wide range of bacteria by recognition of sets of specific carbohydrates on their surfaces has been studied extensively and reported (Table 10.1). The MBL being an innate protein provides the first line of defense, counters the infection by its ability to activate the complement pathway leading to lysis of bacteria, and also promotes bacterial phagocytosis (Turner 2003; Kilpatrick 2002). Thus, appropriately high levels of MBL in serum (sMBL) provide protection, whereas insufficient levels increase the susceptibility to the diseases (Ambrosio and De Messias-Reason 2005; De Miranda Santos et al. 2001). Many studies have reported MBL deficiency associated with susceptibility to various diseases (Areeshi et al. 2016; Svejgaakd 1994).

The fact that low sMBL levels may lead to inappropriate complement activation and ineffective opsono-phagocytosis of the pathogen, the deficiency of MBL may be one of the predisposing factors to many infections specifically caused by the extracellular pathogens.

In bacterial meningitis including invasive meningococcal disease, the protective role of MBL has been reported (Lewis and Ram 2014). The central nervous system with efficient blood–brain barrier (BBB) remains protected from most extracellular pathogens such as *Escherichia coli K1* and *Streptococcus agalactiae* (Group B Streptococcus) in the new borns. In the same way the children and adults get protection from *Neisseria meningitidis, Haemophilus influenzae type b,* and *Streptococcus pneumoniae* (Pong and Bradley 1999; Huang et al. 2000; Schut et al. 2008), however, these pathogens do cross the BBB using their virulence factors (Nassif et al. 2002; Coureuil et al. 2012). Since the children get more affected by meningococcal disease, the role of MBL, being a protein of innate immunity, becomes even more important in the early life where adaptive immunity is still to be developed and this could be the reason that the childhood meningococcal disease has been found to be associated with MBL exon1 structural variant in an age dependent manner (Faber et al. 2007). Other studies, in an ex vivo model, have reported that MBL binding to *Neisseria meningitidis* increases the process of phagocytosis mediated by human neutrophils, monocytes, and monocyte derived macrophages, Also, MBL has been reported to increase the infection risks with *N. meningitidis* and reinforce the significance of epistatic genetic interactions in disease susceptibility (Bathum et al. 2006).

Low sMBL levels, leading to low complement activation and little opsono-phagocytosis of the pathogen, may offer advantage in protecting the host from

many intracellular bacterial infections where the parasitic bacteria employ phagocytosis to enter into the cells and cause disease (Kalia et al. 2021). Leprosy and tuberculosis are examples of such diseases, where MBL-initiated complement activation, for early defense against bacterial infections, facilitates parasitization of bacteria to host cells. Garred et al. (1994) have reported significantly higher levels of serum MBL in Ethiopian patients with lepromatous/or borderline lepromatous leprosy (Svejgaakd 1994) and also, reduction in complement activation to reduce chances of parasitization was proposed to minimize the infection (Green et al. 1994). Low serum MBL levels and polymorphism in structural or promoter region are protective in clinical progression to severe forms of leprosy (do Carmo et al. 2021). In contrast low MASP-2 level was found to be associated with susceptibility to leprosy (Boldt et al. 2013). Polymorphism of MASP-2 and ficolins increases susceptibility of HBV infection among leprosy patients (Boldt et al. 2021). Susceptibility to leprosy has also been linked with the polymorphism regulating MASP-3/MAp44 availability in serum. This has also highlighted the importance of lectin pathway modulation in the fight against infections that use phagocytosis to infiltrate host macrophages (Mendes et al. 2020). Similarly, *Mycobacterium tuberculosis* also, through MBL binding (Bartlomiejczyk et al. 2014), gets entry into the macrophages through phagocytosis and strategically hides itself in the immune cells to evade the host immune protection mechanism (Søborg et al. 2003). High MBL levels in such cases may be detrimental and increase the susceptibility of hosts to these diseases. The presence of low MBL will reduce the infection because of reduction in MBL-mediated phagocytosis. High frequency of variant alleles indicate the advantageous nature of functional MBL deficiency (Table 10.3).

10.6 MBL in Parasitic Infections

MBL has an equally important role in parasitic infections too. Though all pathogens including parasites have emerged with mechanisms to evade the host immune system, these infections do induce the host's immune system. As a part of innate immune response, MBL through its ability to recognize PAMPs on parasites, binds and follows various strategies to eliminate it. The role of MBL activated complement system (LP) in eliminating invading parasites which includes the single celled protozoan parasites and the multicellular parasites such as helminths and ectoparasites has been studied extensively and reported (Shao et al. 2019). The complement system serves as the host's first line of defense against parasitic invasion. An efficiently robust proteolytic cascade, mediated by MBL, which serves as PRRs in the process, eventually results in the opsonization and lysis of the same. Also, the complement regulatory mechanisms maintain a tight balance between the efficient clearance of pathogens and a well-regulated complement activation in the host tissue (Schmidt et al. 2016).

Hence, MBL levels are critical in offering protection and or susceptibility to various parasitic infections. Since the MBL allelic variants show different levels of

Table 10.3 Binding of MBL to various bacteria

S No	Bacteria	Site of presentation of bacteria	Infections/ Diseases	MBL BINDING	References
1	*Actinomyces israelii*	Intracellular	Chronic suppurative infection, actinomycosis	Normal MBL binding	(Townsend et al. 2001)
2	*Bifidobacterium bifidum*	Extracellular	Blood infection with bifidobacteria in critically ill infants	Normal MBL binding	(Townsend et al. 2001)
3	*Burkholderia cepacia*	Intracellular	Cystic fibrosis, often late in the course of the disease	Normal MBL binding	(Davies et al. 2000)
4	*Chlamydia pneumonia*	Intracellular	Pneumonia	Normal MBL binding	(Swanson et al. 1998)
5	*Chlamydia trachomatis*	Intracellular	Trachoma, lymphogranuloma venereum, nongonococcal urethritis, cervicitis, salpingitis, PID	Normal MBL binding	(Swanson et al. 1998)
6	*Enterococcus spp.*	Extracellular	UTI, endocarditis, bacteremia, and nosocomial infections	No MBL binding	(Neth et al. 2000)
7	*Escherichia coli*	Extracellular	Diarrhea, UTI, RTI, and pneumonia	Normal MBL binding	(Emmerik et al. 1994)
8	*Fusobacterium*	Intracellular	Periodontal disease, tonsillitis, peritonsillar abscess, and thrombophlebitis of the jugular vein (Lemierre syndrome)	Normal MBL binding	(Townsend et al. 2001)
9	*H. Pylori*	Extracellular	Gastritis, peptic ulcer, and stomach cancer	High MBL binding	(Neth et al. 2000)
10	*Haemophilus influenzae*	Intracellular	Pneumonia, meningitis, and bacteremia	Weak MBL binding	(Neth et al. 2000; Emmerik et al. 1994)
11	*Hafnia alvei*	Intracellular	Diarrhea, BSI, meningitis, urinary tract infection (UTI), wound infection, intra-abdominal abscess	High MBL binding	(Man-kupisinska et al. 2018)
12	*Klebsiella aerogenes*	Intracellular	Infections, such as urinary tract infection (UTI), pneumonia, intra-abdominal infection, bloodstream infection (BSI), meningitis, and pyogenic liver abscess	Weak MBL binding	(Neth et al. 2000)
13	*Klebsiella spp.*	Intracellular	Pneumonia, UTI, sepsis, meningitis, and bacteremia	Normal MBL binding	(Man-kupisinska et al. 2018)
14		Intracellular		High MBL binding	

	Leishmania braziliensis		A deforming monocutaneous disease deforming the nose and palate		(De Miranda Santos et al. 2001)
15	*Leishmania Chagasi*	Intracellular	Infections with Leishmania infantum (chagasi)	MBL variant susceptible for disease	(Asgharzadeh et al. 2007)
16	*Leptotrichia buccalis*	Intracellular	Systemic infections in immunocompromised hosts, including fusobacterium species, peptococcus species, and leptotrichia buccalis	Normal MBL binding	(Townsend et al. 2001)
17	*Listeria monocytogenes Non-encapsulated*	Intracellular	Febrile gastroenteritis or Listeriosis-meningitis, encephalitis, intrauterine infection, etc.	High MBL binding	(Emmerik et al. 1994)
18	*Mycobacterium avium*	Intracellular	Chronic lung diseases, pulmonary disease (COPD), lymphadenitis, etc.	Normal MBL binding	(Polotsky et al. 1997)
19	*Mycobacterium leprae*	Intracellular	Leprosy	MBL variant protective for disease	((do Carmo et al. 2021)
20	*Mycobacterium tuberculosis*	Intracellular	Tuberculosis	MBL variant protective for disease	(Areeshi et al. 2016)
21	*Mycoplasma pneumonia*	Intracellular	Mild infections of the respiratory system (the parts of the body involved in breathing)	Normal MBL binding	(Hamvas et al. 2005)
22	*Neisseria cinerea*	Intracellular	Infections including acute meningitis	Normal MBL binding	(Emmerik et al. 1994)
23	*Neisseria gonorrhoeae*	Intracellular	Genitourinary infections, PID ophthalmia neonatorum	Normal MBL binding	(Devyatyarova-Johnson et al. 2000)
24	*Neisseria meningitidis Non encapsulated*	Extracellular	Meningitis and septicaemia	High MBL binding	(Emmerik et al. 1994)
25	*Neisseria subflava*	Intracellular	Meningitis, endocarditis, or bacteremia	High MBL binding	(Emmerik et al. 1994)
26	*Propionibacterium acnes*	Intracellular	Skin diseases—Acne vulgaris, sarcoidosis, and prostate cancer	Normal MBL binding	(Townsend et al. 2001)
27	*Pseudomonas aeruginosa*	Extracellular	Nosocomial infections, UTI, RTI, soft tissue infections	Weak MBL binding	(Davies et al. 2000)

(continued)

Table 10.3 (continued)

S No	Bacteria	Site of presentation of bacteria	Infections/ Diseases	MBL BINDING	References
28	*Salmonella enterica*	Intracellular	Gastroenteritis, bacteremia, enteric fever, and an asymptomatic carrier state (7)	Normal MBL binding	(Devyatyarova-Johnson et al. 2000)
29	*Salmonella Montevideo*	Intracellular	Enterocolitis—Diarrhea, abdominal pain, and fever	Normal MBL binding	(Emmerik et al. 1994)
30	*Salmonella typhimurium*	Intracellular	Gastroenteritis	Normal MBL binding	(Chong et al. 2014)
31	*Staphylococcus aureus*	Intracellular	Skin and soft tissue infections such as abscess and cellulitis	Normal MBL binding	(Chong et al. 2014)
32	*Streptococcus pneumoniae*	Intracellular	Community-acquired pneumonia (CAP), bacterial meningitis, otitis media, as well as sinusitis, septic arthritis, osteomyelitis, peritonitis, and endocarditis	MBL variant susceptible for disease	(García-Laorden et al. 2013)
33	*Veillonella dispar*	Intracellular	Serious infections such as meningitis, endocarditis, and osteomyelitis	Normal MBL binding	(Townsend et al. 2001)

this protein, protection/ susceptibility to disease is also manifested differently in polymorphic variants (Frank 1997).

Even though one study found no direct genetic link between malarial parasitic infection and MBL levels, but MBL levels do have an impact on parasite load (Jha et al. 2014). Lower plasma MBL levels associated with MBL-2 polymorphisms (codon 54 and Y-221X) have been reported to be linked with increased susceptibility to multi-organ dysfunctions in *Plasmodium falciparum* malaria infections (Garred et al. 2003). Similar associations of low MBL levels and genetic polymorphisms in the MBL gene have been reported in severe cases of malaria, particularly in children (Luty et al. 1998; Holmberg et al. 2008).

MBL recognizes the surface glycoproteins of *Schistosoma mansoni cercariae* adult worms and activates the lectin pathway (Klabunde et al. 2002), however, not so different sMBL levels were observed in patients infected with Schistosoma and in healthy controls (Klabunde et al. 2002). Furthermore, a study has reported that high sMBL levels were protective in schistosomiasis (Antony et al. 2013), whereas lower MBL levels were protective in infection against *S. haematobium* and *S. mansoni* and MBL2 promoter and variants LY and LL increased susceptibility to *S. haematobium* infection (Rutendo et al. 2015) .

Certain MBL2 genotypes were found to be predictive of risk for developing visceral leishmaniasis (VL) and clinical complications in *Leishmania chagasi* infections (Alonso et al. 2007; Silva et al. 2019). Similarly, there was a strong correlation found between sMBL levels and the probability of developing VL (Santos et al. 2001). There is also an association of allelic variants of MBL2 with cutaneous leishmaniasis (CL) caused by *Leishmania guyanensis*.

It is noteworthy that the uniformity in association of MBL2 polymorphisms in leishmaniasis is lacking worldwide, thus, suggesting that the influence of genetic variations can differ in different population and with differences in parasite/vector relationship (Silva et al. 2019).

In a study conducted in Tanzania for a short term (1 year only) individuals expressing low MBL were three times more likely to get infected with filarial infection as compared with high MBL2 gene expressing individuals. In a long term study (three decades) it was found that the low expressing genotypes were almost ten times more likely to be positive for infection than those with high MBL genotype expressing individuals (Meyrowitsch et al. 2010). In a separate study, results showed no significant association between MBL2 polymorphism at codon 54 in *filarial chyluria* (FC) but Polymorphism at the 221 promoter region is linked with FC (Pant et al. 2019). In another study, MBL genotype (XX) of the promoter region was found to be associated with susceptibility to filariasis (Choi et al. 2001) (Table 10.4).

Table 10.4 MBL variants and association with parasitic infection

Parasite	Association type	Reference
Schistosoma haematobium	High serum MBL have protective role in infections	(Antony et al. 2013)
Trypanosoma cruzi	Lower serum MBL have protective role in in chronic CD cardiomyopathy	(Luz et al. 2016)
Wuchereria bancrofti	Lower MBL level associated with higher filarid infections	(Meyrowitsch et al. 2010), (Choi et al. 2001)
Plasmodium falciparum	Lower plasma level associated with increased multi-organ dysfunction susceptibility	(Garred et al. 2003) (Boldt et al. 2009)
Cryptosporidium sporozoites	Lower serum MBL associated with infection	Carmolli et al. 2009)
Leishmania infantum	High MBL serum level are associated with visceral Leishmaniasis	(Asgharzadeh et al. 2007), (De Miranda Santos et al. 2001)
	Lower MBL level have protective role in visceral Leishmaniasis	(Asgharzadeh et al. 2007), (De Miranda Santos et al. 2001)
Leishmania guyanensis	Lower serum level associated higher risk of cutaneous leishmaniasis	(De Araujo et al. 2015)
Leishmania braziliensis	Higher MBL associated with infections visceral Leishmaniasis	(De Miranda Santos et al. 2001)
Leishmania chagasi	Higher MBL associated with infections	(Asgharzadeh et al. 2007)
Leishmania donovani	Higher MBL associated with infections	(Mishra et al. 2015)

10.7 MBL in Viral Infections

Role of MBL in providing protection against viral infections has been considerably investigated. Recognition of various viruses by MBL has been listed in Table 10.5. The possible mechanisms adopted by MBL to limit viral spread are (a) inhibition of viral binding to host cell; (b) indirectly neutralizing virus by opsonization; (c) phagocytosis of virus; (d) pattern recognition mediated complement activation; and (e) viral agglutination (Fig. 10.4) MBL recognition of viral glycoproteins mediated processes, which may or may not involve complement activation, facilitates neutralization of various types of viruses which includes influenza A virus (IAV) (Chang et al. 2010; Kase et al. 1999), hepatitis C virus (HCV) (Brown et al. 2010), severe acute respiratory syndrome coronavirus (SARS-CoV) (Zhou et al. 2019; Ng et al. 2005), Dengue virus (DV), West Nile virus (WNV) (Avirutnan et al. 2011), and hepatitis C virus (HCV) (Brown et al. 2010). The mechanism through which MBL neutralises diverse viruses may not always involve lectin complement activation (Jack et al. 2005). This has been documented in HIV-1 infection where MBL interacts with the surface protein gp120 of HIV-1 to arrest its entry to the host

Table 10.5 Action of MBL on different viruses

S. No.	Virus	Mode of action of MBL	MBL Role	References
1	Influenza A virus (IAV)	Binds to hemagglutinin (HA) and neuraminidase (NA)	High serum MBL level is protective	(Chang et al. 2010), (Kase et al. 1999), (Al-Ahdal et al. 2018)
2	Human immuno-deficiency Virus-1 (HIV-1)	Interacts via N-linked glycosylated envelope glyco-protein, gp120	Lower MBL levels associ-ated with susceptibility	(Garred et al. 1997), (Saifuddin et al. 2000), (Wei et al. 2003)
3	Severe acute respi-ratory syndrome coronavirus (SARS-CoV)	MBL interferes with the entry of SARC-CoV and binding of S protein to host receptors and trigger downstream antiviral Innate immune response	Lower MBL levels associ-ated with susceptibility	(Zhou et al. 2010), (Hoffmann et al. 2020), (Letko et al. 2020)
4	Hepatitis C virus (HCV)	MBL directly bind E1 and E2 glycoproteins of HCV and trigger complement activation via MASP-2, causing neu-tralization of HCV particles	High serum MBL level is protective	(Brown et al. 2010)
5	Hepatitis B virus (HBV)	MBL bind to hepatitis B surface antigen and N-linked glycosylated forms [210], MBL-HBsAg interac-tion leads to complement activation and enhance C4 deposition.	Lower MBL levels associ-ated with susceptibility	(Chong et al. 2005), (Xu et al. 2013)
6	Dengue virus (DV)	MBL binds to flaviviruses Such as dengue and neutral-izes infection through complement-dependent and independent mechanisms	High serum MBL level is protective	(Avirutnan et al. 2011)
7	Herpes simplex virus	MBL bind to glycoprotein structures on the surface of HSV-2 virus	Lower MBL levels associ-ated with susceptibility	(Seppänen et al. 2009)
8	Cytomegalovirus (CMV)	MBL interacts with virally infected cells, leading to the activation of MBL-associated serine proteases (MASP) 1 and 2. The MASPs activate C4	Lower MBL levels associ-ated with susceptibility	(Kwakkel-van Erp et al. 2011), (Manuel et al. 2007)
9	Ebola virus	MBL bind to Ebola virus via glycoprotein envelope (high mannose glycan sites), and inhibit the binding of Ebola Viruses blocking attachment to host cells and neutralize The virus through comple-ment activation	Lower MBL levels associ-ated with progression	(Brudner et al. 2013), (Ji et al. 2005)

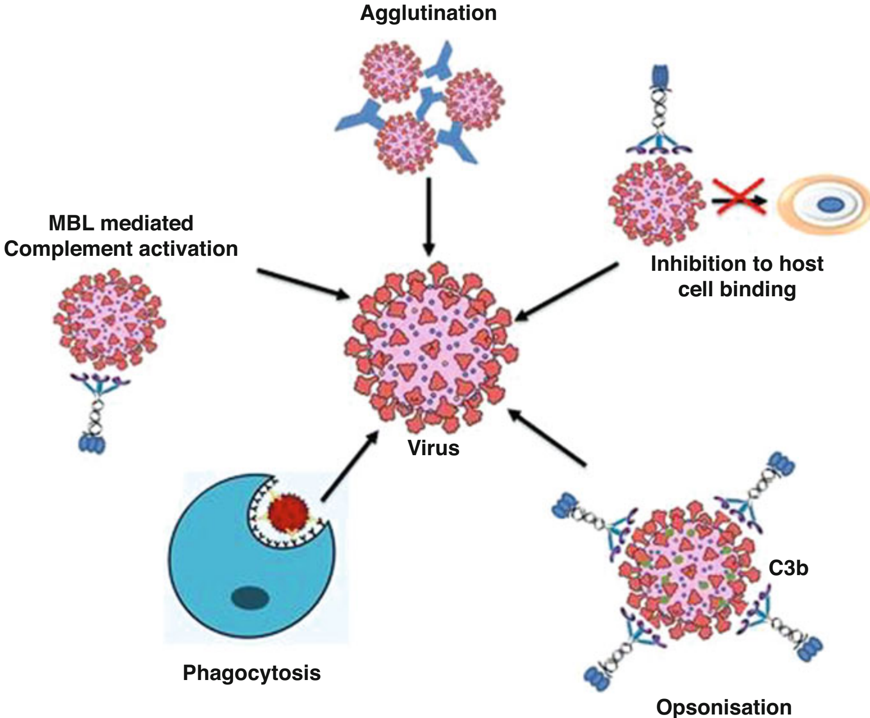

Fig. 10.4 MBL-mediated antiviral Strategies (**a**) Activation of MBL-mediated complement system on virus, (**b**) MBL blocks the viral surface molecule meant for host cell binding (**c**) promotes virus agglutination, (**d**) promotes phagocytosis, (**e**) enhance opsonization (Mason and Tarr 2015)

cell (Ying et al. 2004; Spear et al. 2003), thereby, limiting HIV-1 transfection by direct spatial blocking (Ying et al. 2004). This also suggests the critical role of MBL beyond complement activation in neutralizing viruses. Furthermore, lower MBL levels have been associated with an increased transmission risk of HIV-1 or progression to Acquired Immunodeficiency Syndrome (AIDS) (Ballegaard et al. 2014; Takahashi and Ezekowitz 2005; Garred et al. 1997). However, positive correlation and no correlation between the MBL plasma levels and rate of AIDS progression have also been reported (Mangano et al. 2008; Nielsen et al. 1995; Mcbride et al. 1998). Nevertheless, homozygous and heterozygous allelic variants have been found to increase the risk of HIV-1 infection (Garred et al. 1997; Prohászka et al. 1997) and disease progression owing to poor MBL-mediated viral elimination (Catano et al. 2008; Vallinoto et al. 2006). The MBL variants have been found to influence dengue virus infection and disease progression (Avirutnan et al. 2011). Its deficiency has also been linked to cytomegalovirus (CMV) reactivation after transplantation of lung or liver (Kwakkel-van Erp et al. 2011; Manuel et al. 2007) and susceptibility to SARS-CoV (Ng et al. 2005), yet displayed no correlation with IAV H1N1 infection (Eisen et al. 2011). Certain strains of IAV are resistant to the functions of MBL due

to the degree of glycosylation on the viral HA globular domain (Reading et al. 1997; Job et al. 2010; Tokunaga et al. 2011). Table 10.2 comprehensively lists MBL binding to viruses.

10.7.1 MBL in SARS-CoV-2 Infection

Scientific studies have demonstrated that MBL is an important primary defense molecule against SARS-CoV infection (Ip et al. 2005). Earlier in vitro study has also shown that MBL binds SARS-CoV in a dose dependent manner that could be inhibited by mannan and also enhanced the deposition of C4 on the surface of the virus. Furthermore, structurally altered polymorphic MBL (codon 54 variant) was found to be significantly associated with susceptibility to SARS-CoV infection (Zhang et al. 2005) and this finding further confirmed MBL's relevance in viral infections especially SARS-CoV.

It is reported that binding of MBL, FCN-2, and CL-11, to SARS-CoV-2 S- and N-proteins, activate LP which mediates C3b and C4b deposition (Ali et al. 2021). It was also found that the N-protein of SARS-CoV-2 binds directly to the MASP-2, the LP- effector enzyme, leading to unusual complement activation and aggravated inflammatory lung injury. Inhibition of the LP of complement by an inhibitory monoclonal antibody against MASP-2 effectively blocks its activation. (Ali et al. 2021).

Furthermore, significantly low levels of MBL have been found in SARS-CoV-2 patients compared to controls (Ip et al. 2005). MBL genetic variants, revealed that the B variants of MBL2 gene (codon 54), associated with lower MBL2 levels, were related to higher risk for severe clinical course of COVID-19 infections in parts. Also, the individuals with MBL deficiency may be at greater risk of re-infection than the general population.

In context with SARS-CoV-2, it is speculated that the interaction of SARS-CoV-2 with MBL in blood may lead to activation of inflammatory and coagulation cascades in lungs and blood (Polycarpou et al. 2020). The autopsy of lung tissues of COVID-19 patients with acute respiratory distress syndrome showed deposition of MBL, MASP-2, C4, C3, and C5b-9. This clearly indicates the significantly important role of the lectin pathway in epithelial cells and the overwhelmed response of complement activation which led to cell injury. Hence, Eculizumab, a humanized antibody to C5 is being used as a complement inhibitor in clinical practice and in clinical trials in serious COVID-19 patients (Annanea et al. 2020).

It is possible that viral particles in circulation may trigger LP of complement activation causing microvascular injury (Polycarpou et al. 2020) and also activating the procoagulant effect of MASP-1 and MASP-2 leading to microvascular thrombosis. Our earlier work has shown MASP-1 to have thrombin-like activity, is capable of cleaving fibrinogen (Hajela et al. 2002) and thrombin substrates (Presanis et al. 2004) and MASP-2 can convert prothrombin to thrombin (Gulla et al. 2010; Krarup et al. 2008) thus causing coagulopathy subsequent to activation.

10.8 MBL in Fungal Infections

Several primary and opportunistic fungal pathogens have been shown to interact with MBL.

Infections caused by fungi are more common in people with low MBL levels (Mullighan et al. 2008; Granell et al. 2006). MBL is capable to bind with *Candida neoformans, Candida albicans, Candida parapsilosis, Aspergillus fumigatus, Blastomyces dermatitidis* (Neth et al. 2000; Koneti et al. 2008; van Asbeck et al. 2008; Ip and Lau 2004). MBL binds to *Candida albicans* and *Candida neoformans* via mannan and manno-protein, while *Blastomyces dermatitidis* and *Aspergillus fumigatus* use 1,3-glucan and mannose, respectively (Neth et al. 2000, Koneti et al. 2008, Ip and Lau 2004) .

When *Aspergillus fumigatus* binds to MBL, it causes aggregation, phagocytosis, and complement deposition (Kaur et al. 2007). However, *Aspergillus fumigatus* was not always killed by phagocytes on binding with MBL (Kaur et al. 2007, Madan et al. 2005). A further consideration is that MBL being primarily a serum protein may not be present in significant amounts in the lungs. Despite this, polymorphisms in the MBL gene have been linked to severe aspergillosis (Crosdale et al. 2001; Vaid et al. 2007). Likewise immunocompromised patients, transplant recipients, and cancer patients with MBL deficiency are at risk for aspergillosis. It is possible that in Aspergillus fumigatus infection, MBL role is dependent on both routes of infection as well as host immunosuppression level (Kaur et al. 2007). MBL binds to yeast and pseudohyphae of *Candida albicans*, as well as to yeast cells of Candida parapsilosis (van Asbeck et al. 2008, Brummer et al. 2007). The aggregate of MBL with Candida albicans results in deposition of complement (C4b and C3b) and inhibition of growth through MASPs (Van Asbeck et al., 2008, Ip and Lau 2004). Candida parapsilosis also showed similar levels of complement deposition mediated by MBL (van Asbeck et al. 2008; Ip and Lau 2004; Zimmerman et al. 1992; Van De Wetering et al. 2004). It appears that Candida albicans-induced macrophage responses in THP-1 cells are inhibited by MBL via TLR-2 and TLR-4. TLR signalling pathways of macrophage are altered by Candida albicans (Wang et al. 2013). MBL, on the other hand, increases the phagocytosis of both Candida albicans and Candida parapsilosis yeast cells in neutrophils (van Asbeck et al. 2008). When Candida albicans is captured by neutrophils via the CR1 receptor, MBL greatly increases the production of reactive oxygen species by Dectin-1, that identifies the phagocytosed −1,3 glucan of the fungal pathogen(Li et al. 2012). TNF-α production by monocytes in vitro and in vivo is also increased by MBL binding to *Candida albicans* (Ghezzi et al. 1998; Lillegard et al. 2006).

Vulvovaginal candidiasis affects millions of women worldwide. In women vaginal candidiasis is a significant mycosis. Vaginal secretions contain the MBL protein (Pellis et al. 2005). There is a higher risk of vulvovaginal candidiasis in women with MBL variant allele. The MBL2 codon 54 mutation has been associated with decreased vaginal concentration of MBL and increased incidence of recurrent vulvovaginal candidiasis (Nedovic et al. 2014). This suggests that lectins play a

protective role in the female genital tract infections by aiding in the development of innate resistance to Candida (Murugaiah et al. 2020, Donders et al. 2008, Milanese et al. 2008). Yet, the exact role of MBL in candidiasis has not been completely explored. MBL does not cause Candida neoformans yeast cells to aggregate when bound to their capsular yeast cells (Eisen et al. 2011; Ip and Lau 2004).

On the contrary, MBL binding to *Candida neoformans* results in increased complement deposition and neutrophil phagocytosis (van Asbeck et al. 2008). Additionally, *Candida neoformans* manno-protein stimulates the production of TNF-α in peripheral blood mononuclear cells, and this was augmented by MBL. The binding of MBL with *Pneumocystis carinii* or *Histoplasma capsulatum* is still unclear. Since the cell wall of *H. capsulatum* comprises 1,3-glucan, MBL is implausible to bind to it (Rappleye et al. 2007).

Nevertheless, MBL may be bound to the cell surface of *Pneumocystis carinii,* as it contains −1,3-glucan (Williams 1997). Whether MBL interacts with *Coccidioides* species is also unclear. However, patients with active coccidioidomycosis had lower serum MBL levels than healthy individuals who had previously been infected with Coccidioides (Pellis et al. 2005).

10.9 Pathogens' Survival Strategies

All non-self-disease-causing agents confront the host's immune system for their survival. Pathogens and even self-cells transsformed into cancer cells try to evade the immune response in different ways for their existence in the host. Various strategies adopted by various pathogens to deceive the host's immune system are depicted in Fig. 10.5. Pathogens employ sequential mechanisms to mislead the immune mechanism. The recognition mediated by MBL is bypassed by masking their PAMPs (Sahly et al. 2009). The LP activation may also be inhibited by certain decoy proteins secreted by pathogens (Hair et al. 2010).

Certain proteases of pathogens hydrolyze complement proteins to smaller non-functional fragments disrupting LP operation (Jusko et al. 2012; Jusko et al. 2015). Some microorganisms use host cells mechanisms to prevent excessive complement activation by using natural human complement inhibitors and thus survive in the system. The regulators of complement pathways C1-inhibitor (C1-INH) (Davis et al. 1986), C4b-binding protein (C4BP), MBL/ficolin/CL-associated protein-1 (MAP-1), and small MBL-associated protein (sMAP) (Schmidt et al. 2016) are utilized by various pathogens for their survival. *Escherichia coli* and *Bordetella pertussis* use C1-INH (Lathem et al. 2004; Marr et al. 2007), many pathogens exploit C4BP as part of their survival strategy (Blom and Ram 2008, Hovingh et al. 2016). As coagulation inhibitors can affect LP, such as anti-thrombin inactivation of MASPs (Presanis et al. 2004), it may also be part of the survival strategy of various pathogens. Furthermore, certain parasites such as Schistosoma and Trypanosoma prevent formation of C3 convertase. They express a surface protein known as Complement Receptor Inhibitor Trispanning (CRIT) which

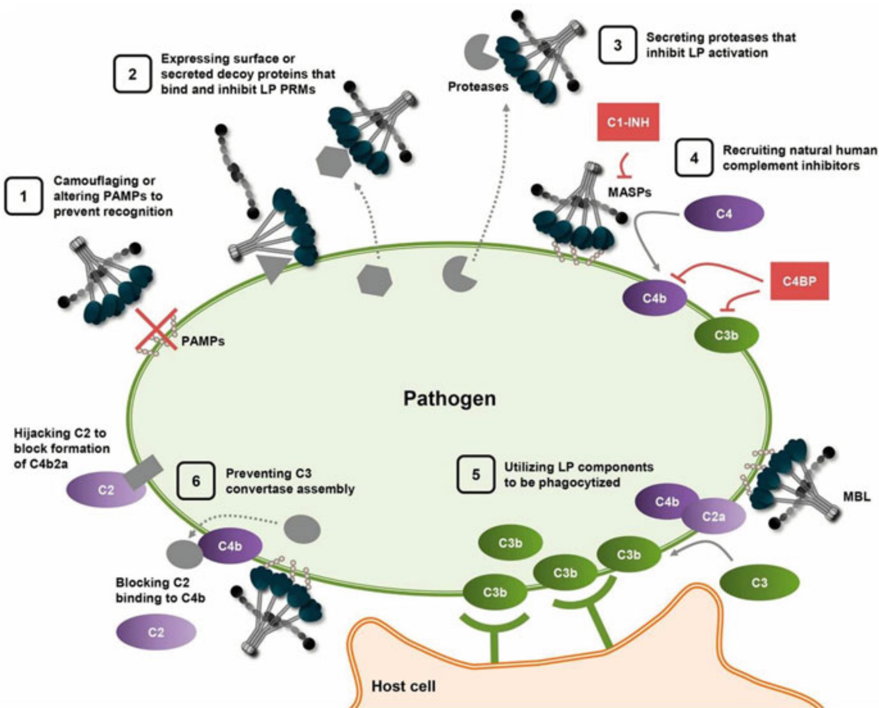

Fig. 10.5 Mechanisms employed by microorganisms to evade complement actions and interfere with LP activation: (1) masking of the PAMPs and thus avoiding recognition by PRMs/PRR, (2) surface expression or secretion of inhibitor proteins that bind PRMs on MBL and impair PRM-PAMPs ligand-binding, (3) secretion of proteases that cleave and destruct LP components, (4) recruitment of the host's complement inhibitory proteins; C1-INH that inhibits the MASP activity and C4BP that inactivates C4b, (5) utilization of LP components (opsonins) for opsonization by intracellular pathogens, (6) prevention of C3 convertase assembly by hijacking C2 via a surface expressed protein or by blocking the C2 binding-site on C4b via a secreted protein. Reprinted from Rosbjerg et al. 2017

binds to C2 and interfere in binding to C4b, hence inhibiting the formation of C3 convertase (C4b2b) (Inal and Sim 2000,Cestari et al. 2008). Similarly, the bacteria *Staphylococcus aureus* reduce the complement activity by the use of extracellular adherence protein (Eap) which binds C4b and blocks assembly of the C3 convertase C4b2a (Logan and Gordon 2014) (Fig. 10.5). Last but not the least, all intracellular pathogens get their access into the host cells by the normal process of opsono-phagocytosis. Thus, their survival is facilitated by the opsonins of LP (Fig. 10.1).

10.10 Perspectives

MBL replacement therapy or MBL supplementation is an area which still remains hitherto unexplored. Although MBL has passed phase-I clinical trials for its use in severe recurrent infections due to opsonic defects, cystic fibrosis and lung infections (Keizer et al. 2014), further research in this domain is warranted to explore therapeutic use of MBL. There has been a lack of well-designed trials with large number of patients on adoption of MBL supplementation which restricted widespread clinical use of MBL. Thus, further exploratory research on a large number of patients is essential to strengthen the MBL supplementation as effective treatment in different diseases.

10.11 Conclusion

MBL in fact is a double edged sword where low serum levels may be responsible for susceptibility to certain pathogens on one hand and high serum levels may be responsible for susceptibility to another. The intermediary levels of MBL may most likely be responsible for protection against a broad range of pathogens.

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Conflict of Interest The authors have no conflicts of interest to declare.

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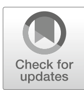
Chapter 11
Lectins in Health and Diseases: Galectins and Cancer



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Abstract Galectins, the glycan-binding family of lectins, have been known to mediate a multitude of biological functions with the help of their unique 'sugar-sensing' carbohydrate recognition domain (CRD). They read and decipher glycan coded information systematically patterned over the cellular surface and regulate growth, development, cell–cell and cell–matrix interactions, vascularization, apoptosis, and many other physiologically relevant processes. The 'hallmarks of cancer' include ten, biologically distinctive, yet complementary, characteristics manifested within tumour cells in a multistep cascade process during carcinogenesis. They armour the tumour cells with ability to evade immunogenic response along with growth suppressors, resist apoptosis, promote angiogenesis, trigger genomic instability and inflammation and reprogram cellular metabolomics, thereby conferring invasiveness and replicative immortality, eventually leading to the metastatic spread of the tumour. Aberrant glycosylation in transformed cells plays a defining role in

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dictating the biological and clinical outcome of each of these hallmarks. By taking control of an array of physiological and immunological cellular processes during different stages of cancer, Galectins meticulously mould the prognostic behaviour of the transformed cells, and hence are identified as effective biomarkers for disease diagnosis and potential targets for therapeutic intervention in cancer. Targeting Galectins or their cognate glycoconjugate partners with specific inhibitors, alone or in combination with existing methods of treating cancer, provides a promising vision in designing effectual next-generation therapeutics for cancer treatment in the near future.

Keywords Lectin · Galectin · Cancer · Tumour microenvironment · Inhibitor · Galectin-targeted therapy

Abbreviations

CAF	Cancer associated fibroblast
CLRs	C-type lectin receptors
CRC	Colorectal cancer
CRD	Carbohydrate recognition domain
CSC	Cancer stem cells
CTD	C-terminal domain
DCs	Dendritic cells
EC	Endothelial cells
ECM	Extracellular matrix
EMT	epithelial-to-mesenchymal transition
HNSCC	Head and neck squamous cell carcinoma
LacNAc	*N*-acetyllactoseamine
MCAM	Melanoma cell adhesion molecule
MCP	Modified citrus pectin
MDSC	Myeloid derived suppressor cells
MMP	Matrix metalloproteinase protein
NLS	Nuclear localization signal
OSCC	Oral squamous cell carcinoma
RCC	Renal cell carcinoma
TAM	Tumour-associated macrophage
TDG	Thiodigalactoside
TF	Thomson–Freidenreich
TIL	Tumour infiltrating lymphocyte
TME	Tumour microenvironment
TN	Triple negative
VEGF	Vascular endothelial growth factor
VEGFR	Vascular endothelial growth factor receptor

11.1 Introduction

Recent trends have witnessed a major surge in the studies of glycosylation changes occurring during cancer progression. These aberrant glycosylation patterns occur at various levels of cellular synthetic events and include modification in terminal sialic acid or fucose, truncation in *O*-glycan and branching in *N*- and *O*-glycosylation patterns (Pinho and Reis 2015; Cagnoni et al. 2016). These alterations are responsible for changes in cell surface associated glycoconjugates present in the form of glycoproteins, glycolipids and/or glycosaminoglycans (Peracaula et al. 2008). Therefore, these aberrant glycosylation signatures vary from one cancer type to another and thus provide a unique opportunity not only for their use as diagnostic markers but also for designing new therapeutic strategies. The information encoded in the form of these glycans is decoded by glycan-binding proteins commonly known as lectins which include sialic acid binding Ig-like lectins (Siglecs), c-type lectin receptors (CLRs) and Galectins. Lectins which are specific in their carbohydrate recognition properties are widely used in recognition of these glycosylation signatures (Rabinovich and Toscano 2009; Gupta et al. 2020). Among these lectins, Galectins have taken a great lead in the recent times, due to their multifaceted role in cancer, prognostic value and as therapeutic targets. Indeed, overexpressions of Galectins have been correlated with aggressiveness of the tumour and their conversion into metastatic form whereas reduced expression of Galectins results in decreased tumour survival (Liu and Rabinovich 2005; Girotti et al. 2020). Therefore, the ability of Galectins to modulate various events in cancer makes them attractive targets for studying the role of various Galectins in cancers, their utility as prognostic biomarkers as well as in the development of therapeutics. In this focused chapter of Galectins and cancer, we have covered (a) the basic details of Galectins including their structure, function and binding activity; (b) the subcellular localization and unconventional secretion of Galectins; (c) a brief discussion on different 'hallmarks of cancer' and role of Galectins in regulating them; (d) Galectins in tumour microenvironment; and last but not the least, (e) Galectins as diagnostic markers and therapeutic targets in cancer and their inhibitors.

11.2 What Are Galectins?

Lectins are multivalent carbohydrate binding proteins which are widely available in nature and are classified based on their carbohydrate binding specificity (Lis and Sharon 1998; Kilpatrick 2002). Galectins were identified based on the hypothesis that cell surface glycans take part in cell adhesion. They were later on tested on tissues for their ability to agglutinate erythrocytes or to be purified on β-galactosides-coupled affinity columns (common cell surface carbohydrates that were available at

that time in reasonable quantity for biochemical experiments, for example in lactose, and asialofetuin) (Teichberg et al. 1975; Nowak et al. 1976). Bound proteins were eluted with lactose, analysed and these lactose binding lectins were given names depending on their source and the discoverer (e.g., electrolectin, CLL-I and -II, L-14, L-29, L-31, CBP-35, and galaptin) (Hirabayashi and Kasai 1993; Leffler et al. 2002). Galectins are found in tissues of almost all animals ranging from lower invertebrates like sponges and nematodes to mammals including humans. In humans, a total of 15 Galectins have been discovered till now. Previously, Galectins were named as S-type lectins because of their dependence on sulphydryl (S) or thiols group, which is required to preserve their activity under reducing condition. However, this idea has been dropped after studies showing that this property is restricted to only two members of the Galectin family, namely Galectin-1 and Galectin-2 (Drickamer 1988; Dodd and Drickamer 2001). The term Galectin was introduced in 1994 and these are classified as a group of proteins (14–35 kDa) belonging to the lectin superfamily and having β-galactoside binding activity (Barondes et al. 1994).

11.3 Structure, Function and Binding Activity of Galectins

Membership in the Galectin family requires two major key criteria to be fulfilled: Presence of a highly conserved carbohydrate recognition domain(s) (CRDs) of approximately 130 amino acids along with an affinity for β-galactoside binding activity) (Barondes et al. 1994). The CRD of Galectins consists of 2 sheets (F and S) bent with 5 and 6 strands on the convex and concave side, respectively forming a β-sandwich (approximately 135 amino acids). The carbohydrate binding site is

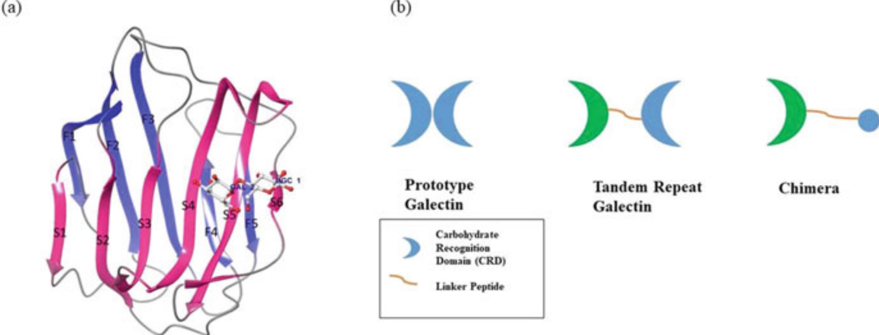

Fig. 11.1 (**a**) X-Ray crystal structure of human Galectin-1 (PDB ID: 1GZW) Carbohydrate bound CRD BGC 1 (β-D-glucopyranose), GAL 2 (β-D-galactopyranose) showing β strands in six anti parallel beta sheets (S1–S6) and five strands in antiparallel β sheets (F1–F5) (López-Lucendo et al. 2004). (**b**) Three types of Galectins based on the number of CRDs present; Prototype Galectins (Galectin-1, -2, -5, -7, -11, -13, -14, and -15), Tandem Repeat Galectins (Galectin-4, -6, -8, -9, and -12), and Chimeric Galectin (Galectin-3)

present on the antiparallel β-strands S4–S6 on the concave face of one β-sheet (Fig 11.1a) (López-Lucendo et al. 2004). Based on the number and characteristic of the CRDs present, the Galectins are classified into three different types: (a) 'prototype' Galectins (Galectin-1, -2, -5, -7, -11, -13, -14, and -15) which have a single CRD and can dimerize; (b) 'tandem-repeat' type Galectins (Galectin-4, -6, -8, -9, and -12) that are comprised of two non-identical CRDs which are joined by a linker peptide; and (c) 'chimera type' Galectin-3 which is the only member of this type that possesses two distinct functional domains consisting of one lectin domain, CRD and a non-lectin N-terminal region of about 120 amino acids connected to CRD (Fig. 11.1b) (Cooper and Barondes 1999; Leffler et al. 2002; Rapoport et al. 2008).

Galectins bind to the carbohydrate moiety attached to the lipid or protein components adorning on the cellular surface. They are highly renowned for their binding activity with β-galactosides containing glycans, found linked to the asparagine-(N) and serine/threonine-(O) residues of membrane proteins, membrane lipids and of the ECM glycoconjugates. A series of events like sulfation, sialylation, fucosylation, and the presence of repeating *N*-acetyllactosamine units, and β1,6-GlcNAc branching on the glycan portion of the glycoconjugate maintain the diversity in their glycan-binding specificity of Galectins (Dimitroff 2015; Méndez-Huergo et al. 2017). For example, using glycan microarray it was shown that fucosylated human blood group A and B determinants show higher affinity towards Galectin-2 and -3 than Galectin-1. The differential glycan binding of Galectins has also been assessed using sialylated glycans, where Galectin-2 exhibits a diminished binding activity, slightly contrasting with Galectin-1 and Galectin-3, which shows binding preferences depending on the branching on the glycan core (Stowell et al. 2008). Additionally, using neoglycoproteins with diverse sugar determinants, Galectin-1 has been shown to be interacting with terminal β4Gal residues and the affinity is further augmented by the repetitive presence of this determinant on long-chain polysaccharides (André et al. 2004; Stowell et al. 2004). Individual Galectins usually choose a particular set of glycosylated receptors, which emphasize the significance of protein–protein interactions and glycan density in establishing Galectin-receptor preferences. Another distinctive feature of lectin activity is the aggregation of cells by their ability to cross-link surface glycans (Fred Brewer 2002; Brewer et al. 2002; Rabinovich and Croci 2012). This confers thorough effects on various biophysical characteristics of the plasma membrane, for instance on its fluidity or osmotic property (Gupta et al. 2006). They interact with distinct surface glycans enabling migration/ invasion, regulating cell adhesion as well as modulating cellular growth. A growing body of evidence indicates that Galectins' multivalent properties are important for their biological functions (Thiemann and Baum 2016). Depending on specific conditions, Galectins with a single CRD can exist as a bivalent molecule forming monomers, dimers or sometimes even oligomers whereas Galectin-3 exists as a pentamer owing to its unique N-terminal region (Lin et al. 2017). This enables them to form lattices with an array of multivalent glycoconjugates (Nabi et al. 2015). Galectin oligomers bridging glycoprotein conjugates maintain a harmonious discord by limiting and

facilitating the protein-protein interaction, both at the same time (Ahmad et al. 2004a, b). This is of much importance as a lot of Galectins and their ligands have been discovered to play a major role in cancer progression. This interaction forms the basis of tumour angiogenesis, immunoregulation, homotypic aggregation or heterotypic adhesion with the purpose of facilitating cancer progression (Cedeno-Laurent and Dimitroff 2012; Thijssen et al. 2013; Häuselmann and Borsig 2014). Galectin-1 and -3 are the most notorious of Galectins when it comes to modifying cancer cell behaviour. As per most studies, both Galectin-1 and -3 bolster pro-tumorigenic activity upon interaction with cancer cell ligands (Yang et al. 2008; Häuselmann and Borsig 2014). Few notable ones expressed on the cancer cell surface would be membrane proteins, Lysosomal associated membrane protein-1/2 (LAMP-1/2), 90k/MAC-2BP, Carcinoembryonic antigen (CEA), and melanoma cell adhesion molecule (MCAM) among many others (Ohannesian et al. 1994; Inohara et al. 1996; Tinari et al. 2001). Recognition of these ligands by Galectins is ensured by the adequate presentation of β-galactoside determinants on the poly-*N*-acetyllactosamines of the *N*- and *O*-glycans, the key drivers of metastatic behaviour. Depending on the cancer type, *O*-glycans being capped or undergoing a chain extension, can contribute considerably to Galectin binding. In addition to these, recent studies have elucidated the role of ST6GalNAcs with respect to Galectin-1 and -3 interactions with *O*-glycans in impacting cancer growth and metastasis. Blocking or hindering these interactions of Galectins with cell surface ligands of cancer cells serves as an appealing target in the development of cancer therapeutics thereby focusing more studies on the binding activities of Galectins in cancer has become imperative (Dimitroff 2015; Nabi et al. 2015; Wdowiak et al. 2018; Laaf et al. 2019).

11.4 Subcellular Localization and Unconventional Secretion of Galectins

Galectins are found to be present intracellularly, both within the nucleus and the cytoplasm, as well as in extracellular compartment as free secretory extracellular matrix protein or bound to glycoproteins adorning the cell surface. Galectins, despite lacking a conventional signal sequence, are secreted outside the cell via unconventional secretory pathways (Rabouille 2017). However, the mechanism has been very elusive so far. The varied role of Galectins is sustained by its multi-localization capability even within different subcellular territories. This becomes much more important in the light of their involvement with Survival of the Motor Neurons (SMN) multiprotein complex assembly within the cytoplasm which congregates snRNPs and aid pre-mRNA splicing within the nucleus. The relatedness of the functions both in cytoplasm and nucleus is highly indicative of shuttling machinery that facilitates Galectins' journey in and out of the nucleus. This was demonstrated using human–mouse heterodikaryons where Galectin-3 can efficiently depart from one nucleus, travel across the cytoplasm and eventually recruited in the other

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                        210       220       230       240       250       260       270
                    ....|....|....|....|....|....|....|....|....|....|....|....|....|..
P09382|Galectin-1  | VAEVCITFDQANLTVKLPDGYEFKFPNRLN-LEAINYMAADGDFKIKCVAFD---------------
P05162|Galectin-2  | EVKFTVTFESDKFKVKLPDGHELTFPNRLG-HSHLSYLSVRGGFNMSSFKLKE--------------
P17931|Galectin-3  | PFKIQVLVEPDHFKVAVNDAHLLQYNHRVKKLNEISKLGISGDIDLTSASYTMI-------------
P56470|Galectin-4  | AFELVFIVLAEHYKVVVNGNPFYEYGHRLP-LQMVTHLQVDGDLQLQSINFIGGQPLRPQGPPMMPPY
P47929|Galectin-7  | PFEVLIIASDDGFKAVVGDAQYHHFRHRLP-LARVRLVEVGGDVQLDSVRIF---------------
O00214|Galectin-8  | SFEIVIMVLKDKFQVAVNGKHTLLYGHRIG-PEKIDTLGIYGKVNIHSIGFSFSSDLQS--------
O00182|Galectin-9  | PFDLCFLVQSSDFKVMVNGILFVQYFHRVP-FHRVDTISVNGSVQLSYISFQNPRTVPVQPAFSTVPF
Q3B8N2|Galectin-9B | PFDLCFLVQSSDFKVMVNGSLFVQYFHRVP-FHRVDTISVNGSVQLSYISFQNPRTVPVQPAFSTVPF
Q6DKI2|Galectin-9C | PFDLCFLVQSSDFKVMVNGSLFVQYFHRVP-FHRVDTISVNGSVQLSYISFQNPRAVPVQPAFSTVPF
Q05315|Galectin-10 | EFELSISVLPDKYQVMVNGQSSYTFDHRIK-PEAVKMVQVWRDISLTKFNVSYLKR-----------
Q96DT0|Galectin-12 | SFLILFLFGNEEVKVSVNGQHFLHFRYRLP-LSHVDTLGIFGDILVEAVGFLNINPFVEGSREYPAGH
Q9UHV8|Galectin-13 | QFELCIYVHYNEYEIKVNGIRIYGFVHRIP-PSFVKMVQVSRDISLTSVCVCN--------------
Q8TCE9|Galectin-14 | PFELCIYVRHKEYKVMVNGQRIYNFAHRFP-PASVKMLQVFRDISLTRVLISD--------------
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Fig. 11.2 Multiple sequence alignment of different human Galectins using ClustalW (Thompson et al. 1994) for nuclear localization sequences (NLS) and nuclear export sequence (NES)

nucleus, perfectly at par with the definition of nucleocytoplasmic shuttling (Park et al. 2001). Interaction of Galectin-1 and -3 with Gemin4, a component of SMN complex, has been implicated in the biogenesis of snRNP and its subsequent delivery into the nucleus along with the bound Galectin (Park et al. 2001). Further, an array of nuclear localization signal like sequences (NLS like NHRVKKLNE) and nuclear export sequences (NES, LNEISKLGI) was found to be present within the CRD of Galectin-3. This was also implied in an experiment by Kim and Chun (2020) where Galectin-3 showed nuclear transport independent of CRD recognition and triggered oncogenic development (Kim and Chun 2020) However, no such sequences have yet been reported in the case of other members of the family (Fig. 11.2). Based on their localization Galectins have been found to be involved in several processes and their alteration in spatial localization is associated with several pathologies such as cancer progression from adenoma to carcinoma (Park et al. 2001). On the other hand, Galectins in the extracellular space may form components of extracellular matrix (ECM) or remain bound to ECM and membrane glycoproteins involved in various physiological events including cellular adhesion and signalling (He and Baum 2006) There are different ways through which Galectins can exit the cellular periphery and enter ECM; two of which deal with direct transportation of Galectins across the lipid bilayer, either in an energy independent fashion (Type I pathway) or through ABC transporters aiding translocation of lipid modified proteins (Type II pathway) (Rabouille et al. 2012). These two pathways conduct the non-vesicular export of Galectins while the other two pathways of Unconventional protein secretion (UPS) consider dispatch of leaderless cytoplasmic cargo into the extracellular space in double-membrane-bound vesicles. These vesicular pathways are classified into Type III pathway; involving membrane-bound endosomes or autophagosomes that become 'secretory' as a response to stress and Type IV Golgi bypass pathway. Proteins to be secreted by Type IV pathway harbour signal peptide and/or transmembrane domains and is manufactured within the ER itself. When stress pathways are activated by the accumulation of unfolded or partially folded proteins, they exit the ER and directly reach plasma membrane by tactfully steering away from the Golgi, with the purpose of being secreted out (Popa

et al. 2018). The multifaceted role of Galectins is well synchronized with their ability to reside in different cellular/extracellular locations and decipher an assorted sets of sweet glycan residues. Based on their localization, Galectins have been found to be involved in several pathophysiological processes which may sometimes have opposing roles. For example, Galectin-1 and Galectin-3 stimulating apoptosis in extracellular space, is potentiated by their interaction with surface glycans (Sotomayor and Rabinovich 2000; Stillman et al. 2006). whereas, Galectin-3 inhibits the same intracellularly, by means of protein–protein interactions (Sano et al. 2003). Further, the role of Galectins in cancer progression is highly dependent upon their place of localization. Extracellular secretion of both Galectin-1 and Galectin-3, or Galectins in general, is extremely crucial for preserving its 'cluster glycoside effect' but how it is maintained in cancer cells is still a matter of speculation.

11.5 Cancer and Its Hallmarks

Scientific research conducted on Galectins over the years has revealed the importance of Galectins in several physiological processes including inflammatory and immune responses, atherosclerosis, diabetes, wound healing, developmental processes, apoptosis, tumour growth, and metastasis. Cancer is the second most leading cause of death globally. As per the statistical data of National Cancer Institute, in 2018, 18.1 million new cases along with 9.5 million deaths were recorded worldwide (Bray et al. 2018). The conversion of a normal healthy cell into a highly malignant form is a multistep process termed tumorigenesis. It involves genetic alterations within the normal cell and the disruption of regulatory mechanisms of the cell that ensure normal cell proliferation and homeostatic maintenance. With this knowledge come several sets of questions that must be answered for a better understanding of what exactly goes wrong within a healthy cell in order to make it cancerous: (a) How many regulatory circuits within a healthy cell must be dismantled before it transforms into a cancer cells? (b) Do different cancer cell types face anomaly in the same set of cellular regulatory circuits? or (c) What role could the enormously diversified collection of cancer associated genes play in the functioning of the regulatory circuits? In the year 2000, Hanahan and Weinberg, in their article, 'The Hallmarks of Cancer' (Hanahan and Weinberg 2000), beautifully showcased all that goes haywire within a normal cell, transforming it into a cancerous one. Normal cell acquires a distinguished set of characteristics known as the hallmarks of cancer; in succession, which push them towards a neoplastic state. Hanahan mentioned six such hallmarks that are manifested in tumour cells during their oncogenic development. These are: (1) Self-sufficiency in growth signals; (2) Insensitivity to growth inhibitory signals; (3) Uncontrolled replicative potential; (4). Sustained angiogenesis; (5) Resisting cell death; and (6) Invasion and Metastasis (Hanahan and Weinberg 2000). 'The Hallmarks of Cancer' can be thought of as a collected set of acquired

functional capabilities of tumour cells which allow them to proliferate, spread, and sustain themselves within a TME. These act as an organizing principle justifying the intricacies of human tumours and determine the degree of tumour complexity. Such functional acquisition might occur at different times in different tumours during the course of tumorigenesis involving distinct sets of mechanisms. In 2011, four additions were made to the existing list of six hallmarks by Hanahan and Weinberg (2011). 'Genetic instability' and 'Tumour promoting inflammation' were categorized under 'Enabling Characters' named so, as they enable the acquisition of the existing hallmarks of cancer by a normal cell. The disruption of the genetic stability of a normal cell allows the accumulation of random mutations within the cell thereby leading the cell to acquire one or more hallmark features sequentially. The inflammatory states of the premalignant and malignant lesions facilitate the evasion of the immune response by the cells, yet again enabling hallmark characteristics to creep in. The other two were characterized as 'Emerging Hallmarks' including 'Deregulating cellular energetics' and 'Avoiding immune destruction' (Hanahan and Weinberg 2011). Based on these principles, a new concept has been formulated, which became to be known as the 'Hallmarks of Metastasis' (Perrotta et al. 2021) For the normal cell to progress towards a metastatic state, it must acquire four hallmarks of metastasis which appear in a sequence of intertwined events known as the metastatic cascade. The four hallmarks of metastasis proposed are: (1) motility and invasion that helps the tumour cells to disseminate to the pre-metastatic niche; (2) modulation of the microenvironment enables the interaction of the tumour cells with the stroma; (3) Plasticity ensuring survival in the hostile new environment; and lastly (4) colonization, to take control of the pre-metastatic niche and make it suitable for seeding and growth. Recent developments in the field of glycobiology and oncology have declared Galectins as the master regulators of these metastatic cascades facilitating cancer progression. Apart from having a role in tumour cell migration, their significant contribution in invasion and intravasation processes is also undeniable (Girotti et al. 2020; Perrotta et al. 2021) The binding of Galectins to glycan receptors on cancer cells induces homotypic or heterotypic aggregation in order to increase their chances of survival within circulation. Moreover, adherence of tumour cells to endothelium too depends upon Galectin-glycan interactions, facilitating extravasation and colonization events during metastasis (Dange et al. 2014; Arnal-Estapé and Nguyen 2015). Galectins also play an important role in the disruption of the basement membrane by the tumour cells enabling them to invade the stromal habitat (van Seijen et al. 2019). Galectin-1, -3 and -9 have direct impact on motility, migration and invasion properties and they influence the plasticity of tumour cells (Perrotta et al. 2021) Tables 11.1 and 11.2 summarize the effects of various Galectins on cancer hallmarks.

Table 11.1 Effect of Galectins on cancer hallmarks

Cancer hallmarks	Galectins	Effects
1. Evasion of growth suppressors	Galectin-3	Promotes as well as inhibits growth suppressors (Cecchinelli et al. 2006; Lavra et al. 2009; Raimond et al. 1995; Wang et al. 2009)
	Galectin-7	Promotes growth suppressors by showing pro-apoptotic activity (Ueda et al. 2004)
2. Sustaining persistent proliferation	Galectin-1	Promotes proliferation (Paz et al. 2001; Chung et al. 2012)
	Galectin-3	Promotes proliferation (Elad-Sfadia et al. 2004; Wang et al. 2009, 2013)
	Galectin-7	Suppress tumour proliferation (Kopitz et al. 2003; Ueda et al. 2004; Luo et al. 2018)
3. Resisting cell death	Galectin-1, -3, and -7	Promotes evasion of cell death (Kuwabara et al. 2002; Ueda et al. 2004; Oka et al. 2005; Mathieu et al. 2007; Barkan et al. 2013; Su et al. 2016)
4. Avoiding immune destruction	Galectin-1, -3, and -9	Prevents immune destruction (Wang et al. 2014; Silva et al. 2017; Nambiar et al. 2019)
5. Enabling replicative immortality	Galectin-1, and -3	Positive effect on induction of replicative immortality (Venuta et al. 2016)
6. Invasion and metastasis	Galectin-1, and -3	Promotes invasion and metastasis (Takenaka et al. 2002; Wu et al. 2009; Fortuna-Costa et al. 2014)
7. Angiogenesis	Galectin-1, -3, and -9	Induce angiogenesis in tumours (Nangia-Makker et al. 2000; Thijssen et al. 2006; Heusschen et al. 2014; Aanhane et al. 2018)
8. Genome instability	Galectin-3	Have both pro and inhibitory effects (Gebert et al. 2012; Carvalho et al. 2014)
9. Tumour promoting inflammation	Galectin-1	Both promoting and inhibitory effect (Rubinstein et al. 2004).
	Galectin-3, and -9	Promote inflammation, pro-tumour effect (Chen et al. 2013; Enninga et al. 2018)
	Galectin-4	Inhibits tumour promoting inflammation (Hokama et al. 2004).
10. Deregulating cellular energetics	Galectin-3, and -9	Inhibitory effect (Partridge et al. 2004; Lee et al. 2013; Silva et al. 2017)

11.6 The Tumour Microenvironment (TME)

Cancer cells are not solitary entities; they require other accessory cells and a nurturing niche for their growth and nourishment. Such a niche is termed as the tumour microenvironment (TME) which is different in different cancers. The TME can be thought of as a complex tissue comprising a diverse set of cell types that indulge in heterotypic interactions with one another. Such heterotypic interactions impact the development as well as expression of certain cancer hallmarks (Hanahan and Weinberg 2011). Summarizing just the traits of the cancer cells would not suffice in the understanding of how the normal cells are pushed towards a neoplastic state, hence the contributions of the TME must also be taken into consideration.

Table 11.2 Effect of galectins on hallmarks of metastasis

Metastasis hallmarks	Galectins	Effects
Motility and invasion	Galectin-1	Promotes invasion by facilitating mesenchymal transformation and enhancing the number and length of filopodia on tumorigenic cells via the Rho-dependent signalling pathway (Wu et al. 2009) Promotes invasion in transformed mammary cells (Bhat et al. 2016)
	Galectin-3	Active member of the human trophoblast invasion machinery (Bojić-Trbojević et al. 2019)
	Galectin-7	Regulates keratinocyte migration in mouse corneas and epidermis (Cao et al. 2002)
Modulation of TME	Galectin-1	Promotes expression of matrix metalloproteinases (MMP-2 and MMP-9) and induces rearrangement of actin cytoskeleton Promotes activation of CAF, thereby facilitating TME remodelling (Guo and Li 2017) Prevents T-cell migration into tumour bed (Nambiar et al. 2019)
	Galectin-3	Also promotes the expression of MMPs via PI3K/AKT and β-catenin signalling pathways (Zhang et al. 2012) Encourages immunosuppressive behaviours to tumours by modulating T-cell signalling and anergy (Zhang et al. 2013)
	Galectin-7	Promotes invasion via ERK and JNK signalling with the facilitative expression of MMPs (Guo and Li 2017)
Plasticity	Galectin-1	Induces EMT in cancer cells via activation of the JNK/p38 signalling pathway (Zhu et al. 2019)
	Galectin-3	Promotes pro-angiogenic activity facilitating metastatic spread of tumour cells by TGF-β1 and VEGF crosstalk (Machado et al. 2014) Acts as an endothelium activator impacting the initial events of disseminating tumour cells (DTC) intravasation process (Zhao et al. 2009)
Colonization	Galectin-1	Induces both homotypic and heterotypic aggregation of tumour cells by cross-linking surface glycans (Tinari et al. 2001) Interacts with oncogenic RAS proteins to facilitate cancer cell proliferation (Paz et al. 2001)
	Galectin-3	Promotes and induces heterotypic and homotypic aggregation by interacting with core 1 antigen (TF; Galβ1,3GalNAcα-1-0-Ser/Thr) (Zhao et al. 2010) Interacts with oncogenic RAS proteins to facilitate cancer cell proliferation (Paz et al. 2001)

Genetic and epigenetic instability of the cancer cells allow random mutations to accumulate that further activate signalling networks within the cells, which, otherwise remain switched off. These signalling networks promote the formation of the TME by communicating with the neighbouring cells and the extracellular matrix (ECM) proteins. The main components here are ECM proteins along with several stromal cells like endothelial cells, fibroblasts, immune cells, adipocytes, inflammatory cells, all contributing significantly to tumour growth and development. Tumorigenesis begins as a result of the crosstalk between these cells controlling inflammation, angiogenesis and immune evasion (Birbrair 2020). The endothelial cells offer protection to the cancer cells from the immune system and provide them

with nutritional support for growth and development. The fibroblasts enable migration of the cancer cell from their primary location into circulation facilitating systemic metastasis (Truffi et al. 2020). The fibroblasts and the myofibroblasts, together known as the Cancer Associated Fibroblasts (CAFs), facilitate the production of ECM and its components like cytokines, proteinases, growth factors etc. (Elola et al. 2018). Besides, they drive the epithelial-to-mesenchymal transition (EMT) by changing the morphology or behaviour of the epithelial cells to resemble that of the fibroblast mesenchymal cells, augmenting invasiveness and motility of the tumour cells (Albini et al. 2015). CAFs directly impact the intratumoural immunity and tumour cell metastasis by inducing intratumoural fibrosis, as a result of cross-linked collagen matrix deposition within the tumour (Yamauchi et al. 2018). Immune cells like lymphocytes, granulocytes and especially macrophages are a major part of the TME that elicits inflammatory responses resulting in a pro-tumorigenic atmosphere. Altered phenotypes are often observed in the immune cells recruited to the TME as compared to those in normal tissues. Tumour-Associated Macrophages (TAMs) are by far the most notable cells for their ability to promote the escape of the immune cells into the bloodstream, leading to extravasation and colonization. They enable immune evasion by the tumour cells and majorly contribute to pro-tumour angiogenic signalling (Anderson and Celeste Simon 2020). The ECM serves as more than just a physical scaffold within TME. It is the home to a well-knit network of macromolecules including different enzymes, array of glycoproteins and collagen that impact the events of cell adhesion, cell–cell and cell–matrix interactions, and tumour cell proliferation. It directly regulates migration of the tumour cells by modifying their composition, topology, and other physical properties. Cytokines, growth factors and chemokines act as soluble mediators synchronizing the interaction between a diverse set of co-dependent cells (fibroblasts, endothelial cells, immune cells) and sustain tumour growth (Fig. 11.3) (Balkwill et al. 2012; Arneth 2020; Anderson and Celeste Simon 2020)**.** Additionally, Cancer Stem Cells (CSCs) are also one of the emerging key elements of TME providing dynamic support in order to foster a pro-tumour environment and facilitate metastatic burgeoning (Albini et al. 2015). Thus, targeting different pharmacologically relevant components of TME has given a new edge to the therapeutic development in treating cancer. However, there cannot be a universal strategy here since the TME is different in different cancers. This is where the Galectins come in like a knight in shining armour. Being ubiquitously present and more or less similar in function, Galectins make for a suitable target to elucidate the events going on and modulate the various pro-survival cells present within the TME.

11.6.1 Galectin-1 Within the TME

Galectin-1 is significantly expressed by the tumour cells, the stromal components as well as its deposition in the ECM (Martínez-Bosch and Navarro 2020). It has been linked to the induction of angiogenesis showing distinct role in fibroblast activation

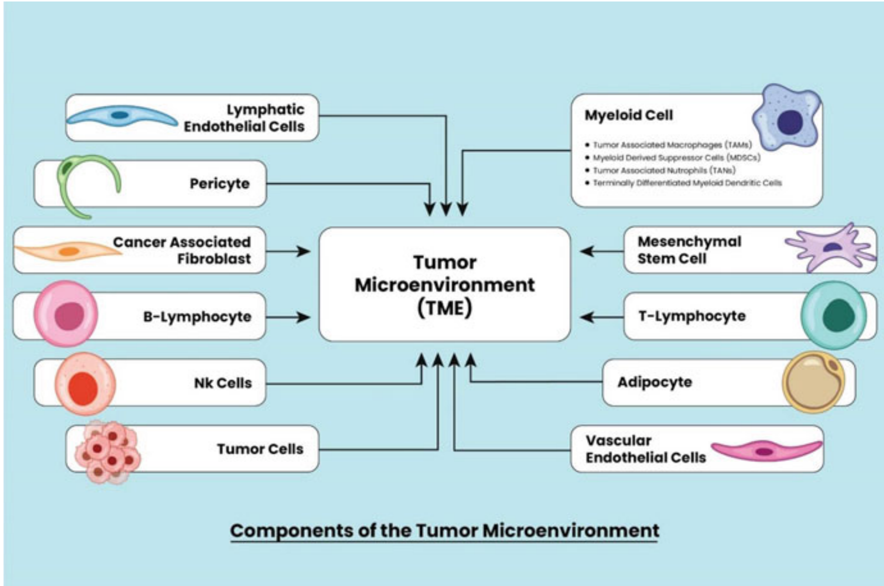

Fig. 11.3 Components of the Tumour Microenvironment (TME). TME is a complex tissue comprising a family of diverse groups of co-dependent cells that work in synchrony to sustain the tumour cell growth and progression. Other than the malignant cells, TME is home to the cells of the immune system, fibroblasts, pericytes, adipocytes and endothelial cells as shown in the figure. Tumour cells being the principal component of TME, control the actions of the other components which maintain a dynamic crosstalk among themselves. The endothelial cells (ECs) form a major bulk of TME offering protection and nutritional support to the tumour cells. Vascular endothelial cells are required to bolster cancer growth whereas lymphatic endothelial cells form lymphatic vessels in the TME crucial for dissemination of the cancer cells. The pericytes offer structural support to the blood vessels. Cancer associated fibroblasts (CAFs) can also be abundantly found in TME facilitating the production of ECM components like cytokines and growth factors, driving epithelial-to-mesenchymal transition (EMT) in malignant cells and are also responsible for immune escape and migration of tumour cells. Adipocytes are the source of fatty acids that serve as a fuel for growth and sustenance of cancer cells as well as assist in malignant cell recruitment in several cancers. The members of the immune system—the lymphocytes, natural killer (NK) cells, myeloid derived suppressor cells (MDSC), tumour-associated macrophages (TAMs), etc. are also abundant in TME. The lymphocytes can be found in the invasive margins of the tumour and in the draining lymph nodes and lymphoid structures surrounding the TME. TAMs mediate immune cell escape, migration, colonization and provide a pro-tumour environment for tumour growth. Experiments conducted in the past decade have revealed the 'mode of conduction' by Galectins within TME in regulating cancer cell behaviour like enhanced tumorigenesis and angiogenesis, regulating growth signals or evading immune system and cell death in some cases and eventually the metastatic spread of tumour cells. Most of the work done so far focused mainly on Galectin-1 and Galectin-3 and testified against their infamous pro-tumour code of behaviour

and modulation of the endothelial cells (ECs) inducing migration, proliferation, and tubulogenesis (Hsieh et al. 2008; Thijssen et al. 2010). Galectin-1 knockdown eventually led to inhibition of CAF induced tumour growth and intravasation as it normalized CAFs upon activation (Elola et al. 2018). In comparison to normal

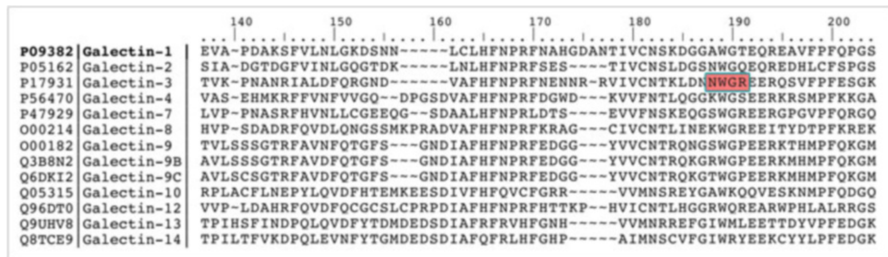

Fig. 11.4 Multiple sequence alignment of different human Galectins for NWGR, a conserved motif in BH1 domain using ClustalW (Thompson et al. 1994)

pancreatic stroma, significantly high amount of CAFs was found in the tumour ECM upon immunostaining of pancreatic carcinoma. Galectin-1 is thus, one of the prime modulators of pancreatic TME (Berberat et al. 2016), and thereby, it is partially responsible for shaping the TME by modulating fibroblast signalling programs. Galectin-1 secreted by activated ECs directly impacts angiogenesis. The uptake of tumour cell secreted Galectin-1 by the ECs can induce EC migration and proliferation via H-Ras and downstream Raf/MAPK/ERK pathway (Thijssen et al. 2010). Blocking Galectin-1 resulted in vessel normalization, reduction of tumour hypoxia and increased number of immune cell migration into the tumour parenchyma (Croci et al. 2014). In addition, it also contributes to the suppression of immune surveillance and immune evasion by tumour cells within the TME (Elola et al. 2018). Impairing T-cell function by inducing apoptosis in the T cells and changing the T helper cytokine balance is one of the tactics employed by Galectin-1 here, within the TME. Galectin-1 blockade enhanced Th1 response simultaneously enhancing tumour rejection (Rubinstein et al. 2004). Tumour-derived Galectin-1 diminished the rate of T-cell recruitment to the tumour parenchyma, IFN-γ secretion and promoted T-cell apoptosis, finally impairing T effector activities (Soldati et al. 2012). It has notable contributions in Th2 cytokine shift, selective deletion of Th1 and Th17 lymphocytes via their differential glycosylation (Toscano et al. 2007), which is crucial for generating an immunosuppressive microenvironment. Galectin-1, like several other soluble factors, secreted by the tumour cells instructs the conversion of dendritic cells (DCs) to tolerogenic DCs, thereby suppressing T-cell responses. Normal functioning of NK cells is restricted by Galectin-1 in the TME, thereby directly hindering their cytotoxic activity against transformed cells or secretion of pro-inflammatory cytokines (Baker et al. 2014). Apart from the DCs and NK cells, it also targets macrophages and myeloid derived suppressor cells (MDSC) (Elola et al. 2018). Additionally, MDSCs can trigger events leading to anticancer immunity by altering the other set of immune cells like macrophages, NK cells, and T effector cells (Fig. 11.4) (Schupp et al. 2019).

11.6.2 Galectin-3 Within the TME

As already discussed, the alteration of the immune cells within the TME leading to tumour progression, metastasis and immune evasion, could be attributed to Galectins, especially Galectin-3. It negatively regulates the immune cells in any abnormal pathological scenario (Farhad et al. 2018). Moreover, it is also responsible for the immune evasion by tumour cells, supporting cancer cell survival through an array of interconnected intracellular and extracellular mechanisms. The Galectin-3 inactivates the NK cells by interacting with Nkp30 receptor allowing the tumour cells of the TME to evade NK cell recognition and subsequent attacking (Wang et al. 2014). Additionally, interaction between Major histocompatibility complex class I-related chain A (MICA) with NKG2D, an NK cell activating receptor, is hindered by Galectin-3, thereby further facilitating immune evasion by cancer cells (Tsuboi et al. 2011). It has been established for being invaluable in promoting T-cell apoptosis and regulating T-cell receptor (TCR) signalling, thereby contributing to immunomodulation of the TME (Fukumori et al. 2003; Hsu et al. 2009). Thus, it has been rightfully called ‘the guardian of the TME (Ruvolo 2016), as it suppresses immune surveillance by destroying T cells and it interferes with the NK cells thereby protecting the tumour niche. Further, the role of Galectin-3 in fibroblast proliferation was also established by gene silencing (Tadokoro et al. 2009). It is also involved in senescence regulation, as was evident from the fact that reduction of Galectin-3 in human skin NFs (normal fibroblasts) resulted in diminished cell proliferation and escalated premature senescence (Kim et al. 2014). A precisely synchronized network of Galectins and their ligands helps in regulating the fibroblast signalling within the TME. Galectin-3 is one of the most prominent Galectins, apart from Galectin-1, to be found in the ECs. They help in capillary tube formation, and migration of the ECs, facilitating angiogenesis in vivo (Fig. 11.4) (Nangia-Makker et al. 2000).

Because of these functions of Galectins displayed within a TME, they make themselves a desirable candidate as diagnostic biomarkers in cancer. Out of the 15 Galectins discovered in humans, Galectin-1, -3, and -9 have been extensively studied in the context of cancer which is elucidated thoroughly in the following section (Califice et al. 2004a; Heusschen et al. 2013; Astorgues-Xerri et al. 2014; Compagno et al. 2020).

11.7 Role of Different Galectins in Cancer

‘The hallmarks of cancer’ as described by Hanahan and Weinberg are distinctive yet complementary, with acquired biophysiological traits that are manifested within cancer cells in order to foster their oncogenic development. Each of these traits contributes to the metastatic behaviour of cancer cells. The invasive property allows them to bid farewell to their original tissue and migrate towards the pre-metastatic niche. They interact with stromal cells to encourage pro-metastatic behaviour in the

contemporary tissue and impact the subsequent TME. Another significant feature of Galectins impacting metastatic cascade is their plasticity and ability to colonize. It helps them to cope up with the aggressive environment of pre-metastatic niche and establish a supportive neighbourhood with facilitative carcinogenic outgrowth. Understanding these unique abilities of cancer cells becomes even more important in order to understand the metastatic progression of cancer and further, the role of interacting and counter-interacting molecules at play. One such important family of molecules in the context of cancer is Galectins. A growing number of evidences strongly point towards aberrant Galectin expression in tumours and tumour-associated stroma as a mediating factor behind metastatic progression of tumour cells (Méndez-Huergo et al. 2017). We will discuss in details the impact of Galectins in governing these so-called 'Hallmarks of cancer' and will also focus on their subsequent interactions at the molecular level to reveal potential guiding candidates for therapeutic intervention with the aim of treating cancer.

11.7.1 Galectin-1 and Cancer

11.7.1.1 Galectin-1 Structure and Its Binding Activity

Galectin-1, the first discovered member of the Galectin subfamily, is a prototype Galectin with a single CRD (Barondes et al. 1994) which is mainly coded by *LGALS1* gene, located on the chromosome 22q12. Usually, it exists in dual forms, as a 14 kDa monomer as well as a noncovalent homodimer having one CRD in each subunit. The monomeric forms anchor with one another through hydrophobic interactions, forming dimers where the CRDs are terminally placed facing in the opposite direction at a distance of 5 nm from one another (López-Lucendo et al. 2004; Belardi et al. 2012). This ability of Galectin-1 to form multivalent homodimers facilitates cell signalling, cell–cell and cell–matrix interactions, and thus making homotypic and heterotypic aggregation possible (Leffler et al. 2002; Camby et al. 2006). The preferred binding partners of Galectin-1 are glycoconjugates, especially those containing *N*-acetyllactoseamine. Though, it binds to individual lactosamine, the binding affinity increases significantly when lactosamine disaccharides form a chain-like arrangement (Ahmad et al. 2004a, b; Schwarz et al. 1998). Multivalency of Galectin-1 allows it to cross-link glycoproteins within the TME. Besides, multivalent carbohydrate dependent as well as carbohydrate independent interactions are also responsible for Galectin-1 mediated biophysiological activities like cell mobility, invasion, apoptosis, and tumour-induced angiogenesis within the TME (Rabinovich 2005; Cousin and Cloninger 2016).

11.7.1.2 Intracellular and Extracellular Activities of Galectin-1

Although being a free cytoplasmic protein, traces of Galectin-1 could also be found within the ECM components. They are secreted non-classically out in the extracellular space without the mandate of containing an N-terminal signal sequence (Astorgues-Xerri et al. 2014). It has also been observed to be secreted by stromal components including fibroblasts, DCs, ECs, neutrophils and macrophages (Birbrair 2020). In the extracellular compartments, Galectin-1 indulges in carbohydrate dependent interactions whereas intracellularly it binds to proteins through domains other than the CRDs. (Camby et al. 2006). A vast array of cell surface glycoproteins and glycolipids along with components of the ECM like laminin, vitronectin, thrombospondin, osteospondin, and fibronectin comprise the binding partners for Galectin-1 (Moiseeva et al. 1999, 2000; Satelli et al. 2008). By enabling the cross-linking between the ECM proteins and the cell surface receptors, it either promotes or inhibits their interactions at a molecular level and contributes to its pro and anti-adhesive functions (Van den Brûle et al. 1995; Martinez et al. 2004). Within the cell, it also binds to several cytoplasmic or nuclear proteins involved in different biological functions. Usually, these intracellular and extracellular activities of Galectin-1 have immense implications in the development of cancer. It is known to be expressed in many types of tumours including melanoma, prostate, colon, ovarian and bladder carcinomas (Park and Kim 2016; Balakrishnan et al. 2018; Huang et al. 2019; Zhu et al. 2019; Goud and Bhattacharya 2021). Galectin-1 mediates homotypic and heterotypic aggregation of melanoma cells, in a carbohydrate dependent manner, by interacting with glycoprotein 90K/Mac-2BP (Tinari et al. 2001). Galectin-1 was found to interact with the markers of breast cancer and colon cancer stem cells, CD44 and CD326 that might help in metastatic development by promoting cancer cell attachment to the endothelium. Further, extracellular Galectin-1 binds to glycoproteins on cell surface like integrins, ganglioside GM1, neuropilin-1 (NPR1) and CD146 (Gu et al. 1994; Kopitz et al. 1998; Moiseeva et al. 2003; Hsieh et al. 2008; Jouve et al. 2013), thereby transmitting signals to the target cells and regulating events like tumour cell growth; tumour proliferation, migration and cell adhesion. In colon cancer cells, interaction between Galectin-1 and the intracellular domain of protocadherin-24 regulates cytoplasmic Galectin-1 levels by anchoring it to the cell membrane and thereby preventing subsequent PI3K activation. This leads to the inhibition of the β-catenin signalling (Ose et al. 2012). Gemin4 is an intracellular binding partner of Galectin-1 modulating pre-mRNA splicing (Park et al. 2001).

11.7.1.3 Galectin-1 in Cellular Transformation

Tumour transformation and its salient features like cell growth, adhesion, apoptosis, migration and metastasis can be attributed to the oncogenic activities of H-Ras protein. Ras oncogene mutation is a common feature shared among several cancers.

The activities of the H-Ras depend majorly on its association with the cell membrane. Such anchorage of the H-Ras to the cell membrane mediates Galectin-1 recruitment from the cytoplasm to the cell membrane, which in turn stabilizes the interaction between activated H-Ras-GTP complex and the cell membrane. However, Galectin-1 shows no effect on membrane-bound inactivated H-Ras. Evidently, Ras binding to GTP and its subsequent activation is required for Galectin-1 interaction with H-Ras. (Paz et al. 2001). Overexpression of Galectin-1 increases membrane-associated H-Ras-GTP, thereby procuring more Raf-1 binding sites, which further sets off sustained activation of MEK-ERK pathway, mediating cellular transformation (Elad-Sfadia et al. 2002; Rotblat et al. 2004). It was observed that transformation of cells was prevented in presence of a dominant negative mutant of H-Ras and Galectin-1 antisense RNA. This interaction between activated H-Ras and Galectin-1 mostly involves intracellular Galectin-1, which is completely independent of its extracellular activities (Prior et al. 2003). In a therapeutic context, such interactions between Galectin-1 and H-Ras can be hijacked to target oncogenic Ras protein subsequently blocking cell transformation (Paz et al. 2001).

11.7.1.4 Galectin-1 in Mediating Angiogenesis, Cancer Cell Adhesion, and Metastasis

Vascular endothelial growth factors (VEGF) secreted by the tumour cells induce the formation of new capillaries from pre-existing vessels thereby remodelling the vascular structures (Abedi and Zachary 1997). Extracellular Galectin-1 has been shown to induce angiogenic signalling in endothelial cells of VEGF refractory tumours by binding to vascular endothelial Growth Factor Receptor (VEGFR) in absence of putative VEGF-A (Croci et al. 2014). Though, it is scarcely expressed in the endothelial cells of the normal tissues, elevated levels of Galectin-1 are seen in the ECs of the TME (Thijssen et al 2006, 2010). High expression of endothelial cell marker CD34 was shown by Galectin-1 obtained from the tumour cells of the advanced stages of prostate cancer (Laderach et al. 2013). Studies conducted in xenograft models showed knocking down Galectin-1 in the cancer cells impeded endothelial proliferation or migration and eventually led to decreased tumour angiogenesis (Mercier et al. 2008; Thijssen et al 2006). In Galectin-1 null mice tumour growth and angiogenesis were seemingly disrupted. Furthermore, Galectin-1 uptake by endothelial cells stimulates H-Ras/MEK/ERK signalling pathway, consequently inducing endothelial cell proliferation and migration (Thijssen et al. 2010). Galectin-1 has been associated with tumour invasion in hepatocellular, lung, pancreatic, and ovarian tumours (Spano et al. 2010; Xue et al. 2011; Chung et al. 2012; Kim et al. 2012). It is known that the detachment of tumour cells from primary tumour site and their subsequent attachment to distant ECs occurs during tumour metastasis (Al-Mehdi et al. 2000). This attachment of tumour cells to the ECM and ECs is facilitated by Galectin-1 in TME through two functional CRDs of its dimers. Galectin-1 induces tumour invasion by eliciting the enzymatic actions of matrix metalloproteinase proteins (MMP) 2 and 9, in oral squamous cell carcinoma model

(Wu et al. 2009). Thus, tumour originating Galectin-1 shows pro-angiogenic features and a significant role in the development of tumour vasculature (Fig. 11.5).

11.7.1.5 Immune Regulation of TME via Galectin-1

Tumour cells express immunomodulatory Galectin-1 in order to impair the T-cell effector function drawing a correlation between Galectin-1 expression and aggravated behaviour in cancer. Galectin-1 helps in the evasion of host immune response by cancer cells as suggested by experiments conducted on melanoma tissue by blocking the biological activities of Galectin-1 that resulted in reduced tumour mass and generation of T-cell response against the tumour in question (Rubinstein et al. 2004). As already mentioned, Galectin-1 has a huge impact on a wide variety of immune cells. One of the first functions of Galectin-1 to be identified was the regulation of the immune system homeostasis (Perillo et al. 1995). It can trigger polarization, migration, activation, differentiation and apoptosis of a variety of immune cells by interacting with their glycosylated receptors (Rabinovich and Conejo-García 2016). Galectin-1 directly targets different immune cells thereby having a crucial immunosuppressive role to play in immune-privileged sites like testes, placenta, etc. Because of its immunosuppressive roles, it is one of the key players in the sustenance of tumour. The importance of the immunosuppressive properties of Galectin-1 was assessed by regulating its expression in cell lines and injecting it into wild type or Galectin-1 knockout mice models. Interestingly, it has been found in transgenic model of neuroblastoma, that host derived Galectin-1 regulated intratumoural infiltrates without affecting the tumour growth as opposed to tumour-derived counterparts (Büchel et al. 2016). It was also responsible for tumour immune evasion in melanoma as the blockade of Galectin-1 generated T-cell mediated responses causing tumour regression (Rubinstein et al. 2004). Further, Galectin-1 is known to regulate both innate and adaptive immune responses and hence could induce tumour immune suppression in different types of tumours. Tumour immune evasion thus seems to be one of the major roles of Galectin-1 in cancers as downregulation of Galectin-1 resulted in reduced tumour growth and increased survival in immunocompromised mouse model (Banh et al. 2011). Galectin-1 being an important part of the TME emerges as a vital target for anticancer drug development. Additionally, Galectin-1 inhibitors are also being vividly studied for the development of cancer immunotherapy since Galectin-1 plays a crucial role in immune evasion (Cedeno-Laurent and Dimitroff 2012) (Table 11.3).

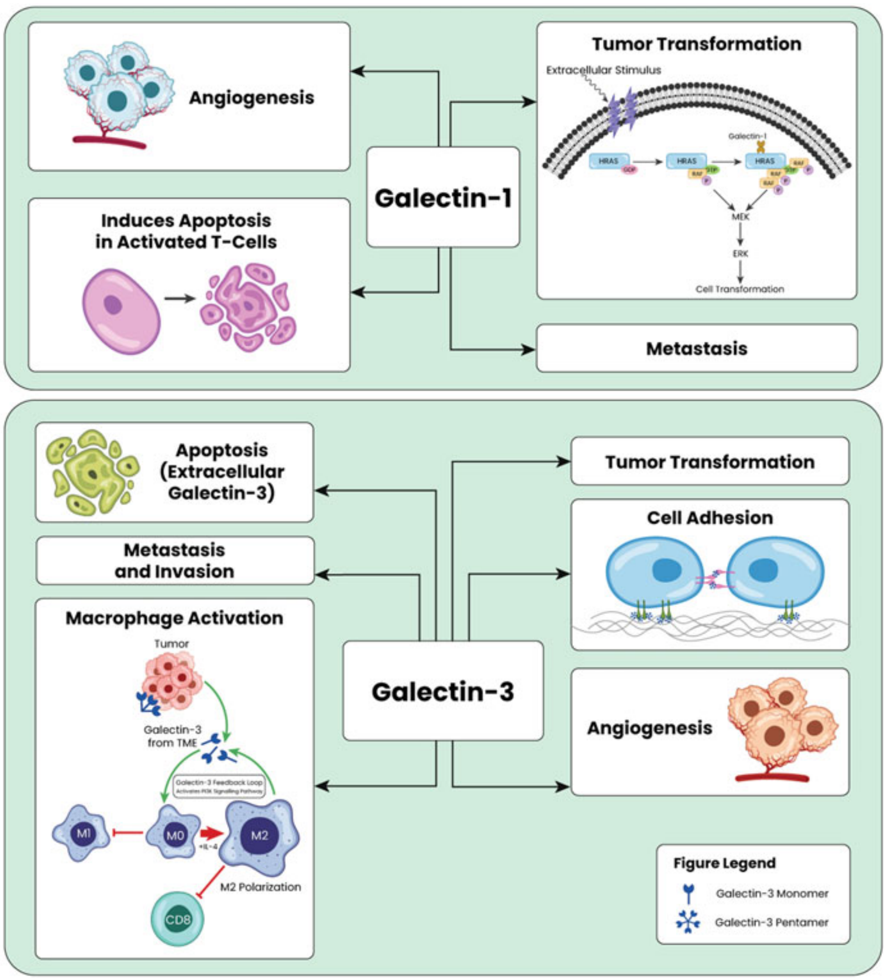

Fig. 11.5 Role of Galectin-1 and Galectin-3 in Cancer: Galectin-1 and Galectin-3 have been established notoriously for being the poor prognostic markers in many types of cancer, having a direct or indirect impact on the early tumorigenic transformation or late metastatic events. Galectin-1 facilitates tumour transformation by regulating tumour growth, adhesion, mortality, migration, and moreover the metastasis spread of cells in an H-Ras dependent manner. It has also been identified as one of the key regulators of VEGF signalling pathway and plays a crucial role in sustaining angiogenesis and proliferation in tumour cells. On the other hand, Galectin-3 promotes neoangiogenesis in a VEGF-dependent manner and ensures the oncogenic viability within the tumour microenvironment. It also facilitates cellular invasiveness by disrupting cell–matrix adhesion and allows tumour cells to leave its primary tumour site and migrate. Additionally, Galectin-3 has also been instrumental in the activation of pro-tumorigenic M2 macrophages. It engages in a feedback loop upon IL-4 stimulation, by binding to CD98 or Beta integrin complex, subsequently activating a sustained PI3 K signalling. This leads to the alternative activation of M2 macrophage and the simultaneous down regulation of CD8 function. M2 macrophage further secretes TGF-β that finally contributes to immunosuppression within TME

11.7.2 Galectin-3 in Cancer

11.7.2.1 Galectin-3 Structure and Binding Activities

The sole member of the chimeric group, Galectin-3 is one of the most talked about in the world of Galectins. Because of its diverse roles, both intracellularly and extracellularly, Galectin-3 has been studied the most among the other Galectins. It has a single CRD with a C-terminal domain (CTD) of 130 amino acids linked to an N-terminal domain (NTD) via a flexible linker region (110–130 aa) (Birdsall et al. 2001; Dumic et al. 2006). The CTD is formed by a stretch of 130 aa, a globular structure forming a β-sandwich (Seetharaman et al. 1998). The NTD resembles the structure of alpha 1(II) chain, having 7–14 repeats of a 9 aa sequence (Raz et al. 1989). The CTD of the Galectin-3 is acknowledged in tumour invasiveness and metastasis (Gong et al. 1999). By contrast, NTD plays an important role in Galectin-3 secretion and nuclear localization along with Galectin-3 multimerization (Gong et al. 1999; Menon and Colin Hughes 1999; Massa et al. 2002). Multimerization of Galectin-3 allows lattice formation via cross-linking with the cell surface ligands (Fred Brewer 2002; Brewer et al. 2002; Ahmad et al. 2004a, b). This lattice formation helps to initiate cell signalling, cell–cell and cell–matrix interaction in healthy tissue as well as cancer cell adhesion, angiogenesis and metastasis in cancerous tissues. Formation of pentamers with glycoproteins helps them to organize cell surface molecules on the plasma membrane (Yang et al. 1998; Ahmad et al. 2004a, b). Interaction with extracellular proteins like fibronectin and integrins as well as certain intracellular proteins like caveolin 1 allows Galectin-3 to carry out such organization on the plasma membrane. Cleavage of NTD within the TME directly affected tumour angiogenesis and progression by hindering Galectin multimerization (Nangia-Makker et al. 2010). NTD region also shows involvement in anti-apoptotic activity via highly conserved Ser6 residue (Yoshii et al. 2002) and the Tyr102 residue is implicated in carbohydrate binding activity (Seetharaman et al. 1998; Barboni et al. 2000). The Galectin-3 CRD, crucial for its β-galactoside binding activity, contains a NWGR motif which is also found in the members of the BCl2 family involved in apoptosis regulation (Akahani et al. 1997; He et al. 2012). Thus, it is not very hard to speculate that NWGR motif might be the one that is responsible for the anti-apoptotic activity of Galectin-3 (Yang et al. 1996). On the other hand, the CRD region of Galectin-3 is a highly conserved structure within the animal kingdom. Galectin-3 binds more strongly to galactose-terminated glycans like lactose (Lac) and *N*-acetyllactosamine (LacNAc) than to galactose. A Five times higher affinity for Galectin-3 is shown by LacNAc when compared with that of Lactose (Sato and Hughes 1992; Agrwal et al. 1993).

Table 11.3 Galectin-1 expressions in different types of cancer

Type of cancer	Role of Galectin-1 in cancer	Reference
Prostate cancer	Key player behind the recurrence of cancer	Laderach et al. (2013)
Cervical cancer	Increased expression in stroma results in increased pathogenesis	Kohrenhagen et al. (2006)
Epithelial ovarian cancer	Peritumoural Galectin-1 intensity positively correlates poor survival rate	Van den Brûle et al. (2003), Kim et al. (2012)
Breast cancer	Increased galectin-1 level correlates with increased rate of metastasis	Jung et al. (2007)
Colon cancer	Increased level of galectin-1 with tumour progression	Sanjuan et al. (1997)
Laryngeal carcinoma	Positive correlation with EC, negative correlation with CD45. Increased expression in tumour stroma as compared to normal stroma	Sven Saussez et al. (2007)
Myeloma	Galectin-1 expression in extracellular space	Abroun et al. (2008)
Non-Hodgkin lymphoma	Galectin-1 in blood vessels	D'Haene et al. (2005)

11.7.2.2 Intracellular and Extracellular Activities

Galectin-3 is omnipresent within the nucleus, cytoplasm, on the surface of the cells and also out in the extracellular matrix. Like all other Galectins, it is secreted non-classically outside the cell, by skirting around the Golgi ER pathway and into the ECM. Surprisingly, it can even traverse into circulation. After being synthesized in the cytoplasm, translocation of the Galectin-3 from the cytoplasm to the nucleus is majorly attributed to the NTD of the CRD (Gong et al. 1999). The transport of Galectin-3 from the nucleus to the cytoplasm is due to the involvement of a nuclear export sequence within its CRD (Gaudin et al. 2000). Studies have shown that the operations carried out by Galectin-3 are relative to its spatial location within the TME. For instance, Galectin-3 is produced by the macrophages, neutrophils, mast cells, immature DCs as well as the Mesenchymal Stromal Cell (MSC) (Dumic et al. 2006). The set of roles executed by Galectin-3 intracellularly or extracellularly is completely contrasting. Intracellularly, Galectin-3 can be either cytoplasmic or nuclear. Whereas the cytoplasmic Galectin-3 shows anti-apoptotic activity (Barboni et al. 2000), within the nucleus, it helps in pre-mRNA splicing and forms a complex with nuclear protein Gemin 4 to initiate spliceosome assembly (Dumic et al. 2006; Yang et al. 2008). It also shows interaction with activated GTP bound K-Ras and also impacts Ras mediated Akt signalling within the cytoplasm (Lee et al. 2003; Elad-Sfadia et al. 2004; Oka et al. 2005; Shalom-Feuerstein et al. 2005). The nuclear

Galectin-3 on the other hand, enhances the transcription factor association with Sp1 and CRE elements present within the gene regulatory regions thereby regulating gene transcription (Dumic et al. 2006). Extracellular secretion of Galectin-3 can be observed in the cell matrix, serum and on endothelia with high implications in tumour occurrence, growth, and metastasis. Galectin-3 helps in anchorage-independent growth by indulging in cell–extracellular matrix interaction (Inohara and Raz 1994). Matrix metalloproteases (MMPs) are crucial for matrix degradation and thus invasion. Extracellular Galectin-3 via its interaction with membrane glycoproteins has been shown to induce MMP-9 secretion (Dange et al. 2015). Strong evidences show that intracellular and extracellular activities of Galectin-3 are involved in transformation, apoptosis, angiogenesis, immunosuppression, tumour development and progression. Expression of cytoplasmic Galectin-3 positively impacts the tumour size in non-melanoma skin cancer. The expression within the cytoplasm increases drastically as compared to nuclear Galectin-3 in melanoma (Radosavljevic et al. 2011). It promotes tumour progression in prostate cancer (Barboni et al. 2000). In contrast, decreased expression of nuclear Galectin-3 regulates the progression of non-melanoma skin cancer. It is also linked to tumour progression in skin cancer (Prieto et al. 2006; Radosavljevic et al. 2011). Nuclear Galectin-3 shows antitumour activity in prostate cancer (Califice et al. 2004a). Extracellularly, increased levels of Galectin-3 in the serum are correlated with the occurrence and metastasis of NSCLC (Liang et al. 2009). Metastatic melanoma has been shown to be contributed to a certain extent by serum Galectin-3 (Vereecken et al. 2006). Serum Galectin-3 expression was shown to be upregulated in hepatocellular carcinoma as compared to chronic liver disease (Matsuda et al. 2008). As already discussed, cytoplasmic Galectin-3 shows anti-apoptotic role in the TME. It inhibits apoptosis by communicating with a calcium-dependent, phospholipid binding protein, Synexin (Yu et al. 2002). Synexin facilitates the translocation of cytoplasmic Galectin-3 into the mitochondria where Galectin-3 further interacts with BCl2, thereby stopping the mitochondrial membrane potential from changing and eventually impeding the release of cytochrome *c* (Matarrese et al. 2000; Yu et al. 2002). Nuclear Galectin-3 is responsible for induction of apoptosis in human prostate cancer cells through mechanisms yet to be deduced. So far it is only known that interaction with Nucling, an apoptosis associated protein, could be responsible for such pro-apoptotic function of nuclear Galectin-3 (Califice et al. 2004b; Liu et al. 2004).

11.7.2.3 Galectin-3 in Cellular Transformation

Galectin-3 overexpression has as intriguing contribution in the transformation of the cells, by acting either as a tumour promoter or as a tumour suppressor. Galectin-3, upon suppression reversed the altered morphology of tumour cells, led to the loss of anchorage and serum independent growth in highly malignant breast cancer, thus causing tumour growth inhibition in immunologically suppressed mice (Honjo et al.

2001). Additionally, Galectin-3 maintains the transformed phenotypes in malignant breast cancer and thyroid cancer. These transformations are linked to the interaction of Galectin-3 with K-Ras. It increases the Ras anchorage activity to the plasma membrane by consequently activating Ras dependent phosphotidylinositol 3-kinase (PI3-K) and Raf-1 (Elad-Sfadia et al. 2004) It also contributes to tumorigenic potential by interacting with β-Catenin and other transcription factors enhancing the expression of cyclin D as well as c-Myc and thereby promoting cell cycle progression (Shimura et al. 2004). The overexpression of Galectin-3 is observed in diffuse B-cell lymphoma, in clear cell renal carcinoma (CC-RCC) and in Gastric cancer tissue (Cheng et al. 2004; Merseburger et al. 2008; Sakaki et al. 2010; Ito et al. 2012; D'Haene et al. 2016). This seemingly resulted in increased cell motility, cell adhesion to ECM compartments and cancer invasion in lung cancer as reported by O'Driscoll et al. (2002). Overexpression of Galectin-3 is also correlated to metastasis in melanoma and pancreatic cancer proliferation and invasion. As opposed to these, it also acts as a tumour suppressor in certain cancers. In benign, adjacent benign as well as prostate cancers, decreased Galectin-3 expressions were seen. Reduction in tumour cell migration, proliferation, invasion, tumour growth, and anchorage-independent colony formation consequently followed the Galectin-3 knockdown by siRNA in nude mice with prostate tumours (Ellerhorst et al. 2002; Merseburger et al. 2008; Wang et al. 2009; Glen et al. 2010; Knapp et al. 2012). Downregulation of Galectin-3 resulted in an increased aggressiveness of the tumour in breast cancer and endometrial cancers further resulting in an increased cancer cell motility and reduced matrix binding ability in the former (Potemski et al. 2007). Galectin-3 expression was found to be significantly less in the primary prostate tissues as compared to normal tissues.

11.7.2.4 Galectin-3 Mediating Angiogenesis, Cancer Cell Adhesion, and Metastasis

Angiogenesis is an important step for the sustenance of the tumour cells, tumour invasion and metastasis. It is crucial for supplying nutrients and oxygen for persistent tumour growth. In the early steps of angiogenesis, Galectin-3 interacts with aminopeptidase N/CD13, an enzyme secreted on the endothelial cell surface and controls neovascularization (Yang et al. 2007) Endothelial cell motility was increased by the action of exogenous Galectin-3 in vitro and enhanced capillary formation was observed in mice in vivo (Nangia-Makker et al. 2000). Galectin-3 promotes angiogenesis by communicating with integrins or cell surface glycans. The clusters of Galectin-3 and its ligands further activate focal adhesion kinase that mediates angiogenesis via the modulation of fibroblast growth factor (FGF2) and VEGF (Fukushi et al. 2004; Markowska et al. 2010). In order to metastasize successfully, the tumour cells must travel in circulation via the blood or lymph and upon invasion, the tumour cells must adhere to the ECM. Overexpression of Galectin-3 causes subsequent increased tumour cell–matrix adhesion and a fastened escape of tumour cells from the primary tumour site (Ochieng et al. 2002). It has

been observed that high levels of Galectin-3 corresponded to the increased invasiveness of the tumour, like in ovarian, thyroid, melanoma, and colorectal cancer cells (Dumic et al. 2006; Barrow et al. 2011). ECM glycoproteins namely, fibronectin, elastin, collagen IV and laminin indulge in cell–matrix interactions with Galectin-3. For example, Mgat5-modified *N*-glycans (Lagana et al. 2006), on lysosome associated membrane glycoproteins, laminin (Dumic et al. 2006; Lagana et al. 2006) are suggested to be involved in Galectin-3 mediated cell adhesion. Galectin-3 circulating in the blood or lymph is crucial for cancer metastasis. In cancer patients, especially those showing metastasis, significantly high levels of Galectin-3 in the bloodstream are observed as compared to that of healthy people (Takenaka et al. 2002; Song et al. 2014).

11.7.2.5 Immune Regulation of TME via Galectin-3

Galectin-3 is popular for its immunosuppressive activities in the TME. It has a great influence over the majority of the immune cells especially, lymphocytes and macrophages. It negatively regulates these immune cells within the TME thereby assisting the tumour cells to escape immune destruction. The normal cells evade autoimmune reactions by the host immune system through highly precise regulatory mechanisms. Such in-built mechanism of the immune systems, to check autoimmunity, is exploited by the cancer cells in order to defend themselves from detection and attack by the immune cells (Farhad et al. 2018). A lot of such components negatively regulating the immune system, like chemokines and suppressive cytokines are secreted in the TME by Cancer Associated Fibroblasts (CAFs), Tumour-Associated Microphages (TAMs) and the tumour cells. Galectin-3 in TME modulates the secretion, expression and functioning of these immune cells. Tumour-driven immune suppression is promoted by Galectin-3 within the TME by altering the resident immune cells (Ruvolo 2016). It inhibits T-cell activation and mediates the transformation of the normal fibroblasts into CAFs (Erez et al. 2010) and activates pro-tumour macrophages. It also acts as a suppressor of tumour reactive T cells. Studies showed amplification of tumour reactive T cells on blocking tumour-expressed Galectin-3, in a culture of lymphocytes comprising peripheral blood derived T cells mixed with autologous tumour cells (Melief et al. 2017). Galectin-3 not only impedes T-cell receptor assembly, but also sets off apoptosis in $CD8^+$ T cells and initiates M1 macrophage polarization to pro-tumour M2 macrophage, thereby facilitating immune escape by the tumour cells (Demotte et al. 2008).

Effect on Lymphocytes

The role of Galectin-3 in suppression of lymphocytes has been studied in vitro. The secretion of extracellular Galectin-3 in tumour tissues or even normal tissues have shown to impact lymphocyte activities like TCR signalling, IL-5 production, migration, apoptosis and cell adhesion. Extracellular Galectin-3 binds to glycoprotein

receptors; CD45 and CD71, thus inducing apoptosis in human thymocytes. On the other hand, overexpression of Galectin-3 inside the intracellular compartment of Jurkat T cells, quite contrastingly, inhibited apoptosis upon induction by anti-Fas antibody and Staurospine (Yang et al. 1996). Intracellular Galectin-3, however, elicits TCR signalling and cell growth (Yang et al. 1996; Chen et al. 2009). $CD4^+$ T cells showed increased TCR expression and IFN-gamma secretion to wild type $CD4^+$ T cells, upon Galectin-3 knockout (Chen et al. 2009). The effect of Galectin-3 on $CD8^+$ T-cell activity is of great significance as these cells play a crucial role in the context of immunotherapy. However, a very little information exists on the effects of intracellular or extracellular Galectin-3 on these $CD8^+$ T cells (Demotte et al. 2008; Melief et al. 2017).

Effect on Macrophages

Macrophages play one of the most important roles in host defense and maintenance of tissue homeostasis. Macrophages can be polarized into two functional groups M1 and M2 based on the type of stimuli received from the microenvironment, where the M1 class is classically activated macrophages stimulated by IFN-γ and Lipopolysaccharides (LPS). The M2 class of macrophages is alternatively activated, has immunosuppressive activities and is further sub categorized as M2a and M2c types (Mantovani et al. 2007). Galectin-3 is highly expressed by macrophages suggesting their prodigious importance in moderating the natural physiology of these cells (Liu et al. 1995; Sano et al. 2000; Norling et al. 2009). Classically activated M1 macrophage prevents Galectin-3 synthesis and secretion as opposed to the alternatively activated macrophages that bolster the biosynthesis and release of Galectin-3 (MacKinnon et al. 2008; Novak et al. 2012). Thus, Galectin-3 upregulation can serve as a highly specific marker for M2 type macrophage (Shanshiashvili et al. 2017). Since IL-4 initiates M2 macrophage activation, it simultaneously upregulates Galectin-3 expression, which in turn participates in a feedback loop by binding to CD98 or Beta integrin complex and subsequently activating PI3 K leading to M2 macrophage activation (Novak et al. 2012). It can also alter the classically activated M1 macrophage to its alternate phenotype, M2 macrophages, that add more Galectin-3 to the TME.

Expression of Galectin-3 along with its localization within the cell, in the extracellular matrix or on the cell surface, has different implications for different types of cancers. The pleiotropic role of Galectin-3 in regulating cancer cell activities is greatly attributed to its interaction with its intracellular and extracellular binding partners. Be it angiogenesis, tumour invasion, tumour transformation, immune evasion or metastasis, Galectin-3 has been shown to vastly impact all. With such diverse actions in different cancers, it is only fair to target Galectin-3 as a biomarker for different steps of cancer development as well as utilize its potential to develop effective anticancer therapeutics. An array of inhibitory compounds, discussed later in the chapter, has been designed to block Galectin-3 activities that have wide implications in cancer therapy (Fig. 11.5, Table 11.4).

Table 11.4 Role of Galectin-3 in different cancers

Type of cancer	Role of Galectin-3	Reference
Pancreatic cancer	(a) Tumour progression by binding to Ras and activating Ras signalling pathway. (b) Regulating mucin1/epidermal growth factor receptor (MUC1/EGFR).	Xie et al. (2012)
Breast cancer	(a) Inducing Bcl-2-like anti-apoptotic activity enhances the metastatic potential of breast carcinoma. (b) By binding to and activating wt K-Ras increases the oncogenic potential of the breast cancer cells.	Nangia-Makker et al. (2007), Balan et al. (2008)
Colorectal cancer	(a) Membrane localization of β-catenin in CRC mediated by Galectin-3 via protocadherin-24 dependant pathway results in the suppression of tumour growth and inhibition of cell proliferation in colon cancer. (b) Negative regulation of Galectin-3 by Galectin-3 binding proteins resulting in an increased motility of the cancer cells. (c) Enhancement of cell migration via K-Ras-Raf ERK1/2 pathway. (d) Galectin-3 suppression along with reduced hnRNP Q expression results in slower proliferation of cancer cells and increased susceptibility to 5 uracil treatment.	Yoo et al. (2009), Kim et al. (2011), Wu et al. (2013)
Prostate cancer	(a) Inhibition of Galectin-3 by its inhibitors like GCS100 modified pectin results in the increased efficacy of cisplatin induced apoptosis. (b) Low molecular weight compounds interrupting β-galactoside mediated interactions could prevent metastasis. (c) Galectin-3 inhibiting anticancer drug induces apoptosis by suppressing mitochondrial apoptosis.	Wang et al. (2009), Glinskii et al. (2012), Guha et al. (2013), Knapp et al. (2012)
Leukaemia	Galectin-3 expression is directly proportional to the increased leukaemia cell survival and stabilization. It could act as a therapeutic target to overcome drug resistance.	Cheng et al. (2011)
Lung cancer	Galectin-3 acts in lung cancer through two different pathways: (a) by interacting with cell surface proteins and inducing the expression of *N*-acetyllactoseamine (b) by disruption of Galectin-3 that may diminish lung carcinogenesis as it regulates B-cell receptor, ERK/MAPK signalling pathways	Buttery et al. (2004), Abdel-Aziz et al. (2008)

11.7.3 Galectin-9 in Cancer

11.7.3.1 Galectin-9 Structure and Binding Activities

Galectin-9, a protein of approximately 39.5 kDa, is a tandem repeat Galectin encoded by *LGALS9* gene on chromosome 17q11.2. It possesses two CRDs joined by a linker region which gives rotational freedom to both the CRDs and it is also responsible for Galectin-9 multivalency. It has been observed that Galectin-9 protein function greatly depends on its posttranscriptional splicing. The elimination of exons 5, 6 and 10 from the full-length mRNA transcript, determines the major difference between the splice variants formed. This splicing event of Galectin-9 can likewise affect its specificity or affinity for glycoconjugates as well as its cellular localization (Heusschen et al. 2013). Commonly, it is one of the key players in several biological processes such as cell adhesion, chemotaxis, immunomodulation, angiogenesis, and apoptosis (Heusschen et al. 2013). Like other Galectins it can be found both in intracellular and extracellular spaces (Thijssen et al. 2008). Extracellular ligands of Galectin-9 are T-cell immunoglobulin mucin-3 (Tim-3), CD44 and Glucose Transporter-2 (Glut-2) whereas intracellular ligands include Nuclear Factor for Interleukin-6 (NF-IL6) (Katoh et al. 2007; Matsuura et al. 2009). Interestingly, the distinctive isoforms generated as a result of posttranscriptional splicing event, have shown distinctive roles in terms of vascular outgrowth or angiogenesis depending on their concentration and subsequent environmental stimuli (Aanhane et al. 2018). For instance, the Gal9Δ5 isoform elicits in vitro sprouting and invasiveness in human umbilical vein endothelial cells (HUVEC), while paradoxically having antagonistic effect on vascularisation in vivo (Heusschen et al. 2014). Additionally, Matrigel plug angiogenesis assays using human dermal microvascular endothelial cells (HMVEC) revealed the role of Galectin-9 in promoting angiogenesis and subsequent monocyte migration in order to generate a pro-inflammatory response in arthritis (O'Brien et al. 2018). Galectin-9 also impacts cellular plasticity by facilitating epithelial-to-mesenchymal transition (EMT) and stemness (Wnt/β-catenin), having regulatory effect on cell motility, migration and ultimately metastasis (Perrotta et al. 2021). In accordance with this, triple-negative (TN) and $HER2^+$ breast cancer patients display a remarkable stromal prevalence of Galectin-9 along with Galectin-1 and -3, indicative of the poor prognosis of the disease (Grosset et al. 2016). It is also known to express on the surface of thymocytes and hence play a key role in the development of acquired immune response. During thymic negative selection of T cells, it induces apoptosis in the T lymphocytes, and exogenous administration of Galectin-9 results in the apoptotic cell death of the cancerous cells as well as cells of the immune system responsible for anticancer action both in vivo and in vitro. Functional studies suggest that it can induce cell death in T_H1 cells but not in the T_H2 cells and this induction of death of the T_H1 cells relies on Tim-3. Thus, the evolution of Tim-3-Galectin-9 pathway could impede the action of effector T_H1 (Zhu et al. 2005).

11.7.3.2 Galectin-9 in Tumour Regulation

In gall bladder carcinoma, Galectin-9 suppresses the growth of tumour cells by altering miRNA gene expression and by promoting apoptosis (Tadokoro et al. 2016). Silencing of Galectin-9 resulted in an increased progression and migration of cancer in a study performed in hepatocellular carcinoma (HCC) cell models (Zhang et al. 2012). Mostly it is seen that the level of Galectin-9 in healthy tissues is more than in tumour cells like breast, lung, liver, prostate, melanoma and kidney (Lahm et al. 2001; Kageshita et al. 2002; Irie et al. 2005; Zhang et al. 2012; Laderach et al. 2013). Galectin-9 modulates cell survival in tumour cells just like Galectin-1 and -3. Galectin-9 induces apoptosis in tumour cells like human melanoma cell lines, in leukaemia cell lines having a pro-apoptotic effect. For instance, lack of Galectin-9 in melanoma cells shows no colony formation as opposed to cells with Galectin-9 expressed in them (Kageshita et al. 2002).

11.7.3.3 Immune Regulation via Galectin-9

Galectin-9 has been extensively studied for its role in immune surveillance and inflammation. Latest scientific studies were able to bring into light the immunosuppressive effects of Galectin-9 in human malignancies. It induces M2 polarization of macrophages in skin cancer and enhances the expansion of myeloid derived suppressor cells (MDSC) by upregulating the expression of several pro-inflammatory cytokines (Wiersma et al. 2013). Thus, Galectin-9 has been reported to induce myeloid-lineage mediated immunosuppression in the TME. It plays a role similar to that of cytokines controlling the activity of most of the immune cells. However, the role of Galectin-9 in tumour cell immune escape remains mostly elusive but certain observations do suggest that they have similar functions like Galectin-1 in this regard (Croci et al. 2012). It is yet to be determined whether loss of Galectin-9 results in the escape of tumour from antitumour effects of the immune cells like eosinophil. The pleiotropic role of Galectin-9 in tumour biology is apparent, however many questions are yet to be addressed for which a better understanding of the molecular mechanisms underlying the various effects displayed by Galectin-9 is required (Heusschen et al. 2013) (Table 11.5, Fig. 11.6).

11.7.4 Other Galectins in Cancer

Galectin-1, -3, and -9 are the most extensively studied Galectins when it comes to cancer immunotherapy or combination therapy. However, other Galectins are also studied for their roles in tumour biology. The roles of Galectin-4, -7, and -8 in cancer have been discussed below.

Table 11.5 Summary of the role of Galectin-9 in cancer

Type of cancer	Role of Galectin-9	Reference
Breast cancer	Increased Galectin-9 expression in triple-negative breast cancer cells. Antitumour immunity provided by Galectin-9 expression and Tim 3 pathway.	Bazhin et al. (2019)
Colorectal cancer	Inhibition of cell proliferation in colon cancer by Galectin-9 through induction of apoptosis.	Morishita et al. (2021)
Chronic lymphatic leukaemia cancer	Increased expression of Galectin-9, Tim 3 and PD-L1 corresponds to T-cell exhaustion in $CD8^{+}$ cells and results in evasion of antitumour immune response.	Taghiloo et al. (2017)
Lung cancer	Galectin-9 is highly expressed in non-small cell lung cancer (NSCLC) with increased expressions of TIM, PD-L1 and might be involved in T-cell exhaustion.	He et al. (2019)
Renal cell carcinoma	Galectin-9 predicts the possibility of tumour recurrence and survival chances of the patients.	Fu et al. (2015)
Oral squamous cell carcinoma (OSCC)	Galectin-9 promotes adhesion of OSCC cell lines.	Kasamatsu et al. (2005)

11.7.4.1 Galectin-4 in Cancer

Galectin-4 is a tandem repeat Galectin, first extracted from the small intestine of rat (Leffler et al. 2002). It has a molecular weight (MW) of 36 kDa with 2 distinct yet homologous CRDs of 130 aa, joined by a linker region of 30 amino acid residues (Oda et al. 1993; Jiang et al. 1999). It serves as a natural cross-linker that connects two distinct sets of ligands with its two distinct CRDs that have different carbohydrate binding specificity (Fred Brewer 2002). Galectin-4 has been shown to have implications in physiological processes of apical protein trafficking, lipid raft stabilization, wound healing, inflammatory diseases and in cancer (Cao and Guo 2016). Studies have shown that Galectin-4 expression in many cancers, namely, colorectal cancer (CRC), hepatocellular carcinoma, breast carcinoma, pancreatic carcinoma and lung cancer, had contradictory roles (Rechreche et al. 1997; Belo et al. 2013; Hayashi et al. 2013; Cai et al. 2014). Unfortunately, only limited information is available on the role of Galectin-4 in cancer. Role of Galectin-4 in colorectal cancer has been explicitly studied. Galectin-4 inhibited tumorigenesis of the CRC cells via Wnt/ β-catenin and IL-6/NF-κB/STAT3 signalling pathway (Satelli et al. 2011; Kim et al. 2013). A similar course is taken by Galectin-4 within the pancreatic cancer cells as well. Traces of Galectin-4 can be found freely circulating in the serum of cancer patients like that in colorectal cancer, breast cancer, hepatocellular cancer, and metastatic cancer (Barrow et al. 2011, 2012; Kim et al. 2013; Cai et al. 2014). High levels of expression of Galectin-4 are seen in metastatic colorectal cancer. On the other hand, low levels of Galectin-4 were associated with the more advanced form of colorectal cancer. The difference in the degree of expressions of Galectin-4 in healthy tissue with that of cancerous tissues is

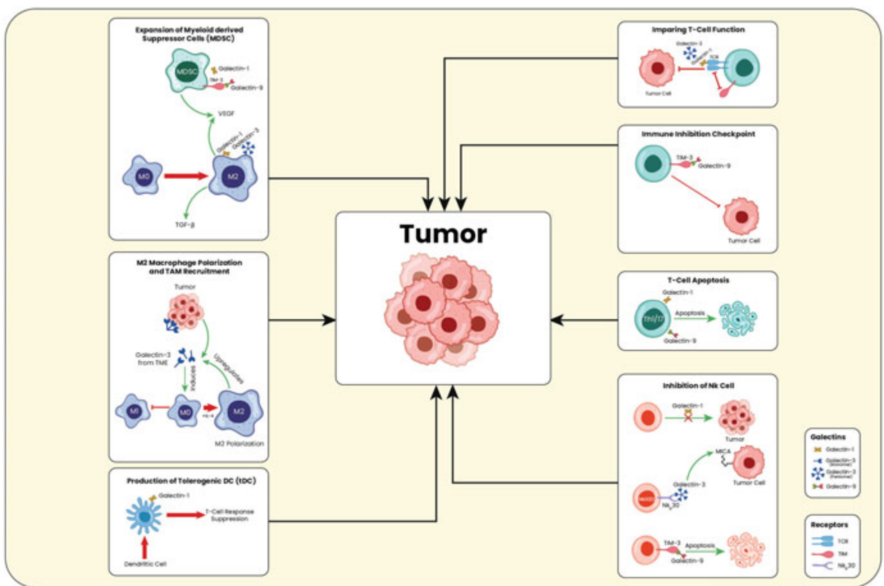

Fig. 11.6 Immune regulation of TME via Galectins (focus on Galectin-1, -3, and -9). Galectin-1, -3 and -9 play an immunomodulatory role in TME helping the tumour cells to escape immune destruction. These immunomodulatory roles involve—(1) Impairment of the T-cell function; Galectin-1 and Galectin-3 block TCR-mediated signalling and their simultaneous activation. (2) Modulation of Immune Inhibitory Checkpoint Activities; Galectin-9 engages with immune inhibitory checkpoint TIM-3 thereby impeding tumour cell death. (3) T-cell apoptosis; selective elimination of Th1 and Th17 cells via Galectin-1 and Galectin-9 induces apoptotic pathway within T-cells. (4) Inhibition of NK cells via Galectin-1, -3 and -9; blockade of NK cell recruitment to TME by Galectin-1 renders it dysfunctional; Galectin-9 disables NK cell cytotoxicity and cytokine production; Galectin-3 on the other hand inhibits NKp30 signalling and modulates MICA affinity for NkG2D. (5) Expansion of myeloid derived suppressor cells (MDSC) by the action of Galectin-1 and Galectin-9-TIM-3 interaction that leads to the production of pro-angiogenic growth factor VEGF. (6) M2 Macrophage Polarization induced by Galectin-3 which further enables the recruitment of Tumour-Associated Macrophages (TAMs) secreting immunosuppressive growth factor TGF-β. (7) Conversion of normal dendritic cells to tolerogenic DCs (tDCs) by Galectin-1 ultimately leads to T-cell suppression

the sole way to decipher the importance of Galectin-4 in cancer. Galectin-4 when present in the lymph node tissues in lung cancer patients is considered as one of the risk factors (Wdowiak et al. 2018).

11.7.5 Galectin-7 in Cancer

Galectin-7 is a prototype Galectin, encoded by the *LGALS7* gene on the chromosome 19q13.2. Galectin-7 with its single CRD, shows binding activity with the majority of

the Galectin receptors (Kaur et al. 2016). It is majorly expressed by the keratinocytes (Magnaldo et al. 1995) with restricted expression in the stratified epithelial cells of the tongue, lip, oesophagus, cornea, urinary system and Hassall's corpuscles of the thymus, also by the stratified squamous epithelial cells of the stomach and myoepithelial cells of the mammary gland (Magnaldo et al. 1998; Saussez and Kiss 2006). Galectin-7 transfection in human colon carcinoma cell lines has been shown to reduce tumour growth (Ueda et al. 2004). However, its expression is shown to promote the proliferation of activated T cells which is associated with pro-inflammatory environment (Luo et al. 2018). Numerous neoplasms like throat, thyroid, breast cancers and squamous cell cancer show elevated levels of Galectin-7 (Demers et al. 2010; Kim et al. 2013; Zhu et al. 2013). Galectin-7 increases the metastatic potential and resistance to apoptosis in breast cancer, showed curative role in cervical cancer and suppressing roles in gastric cancer (Demers et al. 2010; Kim et al. 2013; Zhu et al. 2013).

11.7.6 Galectin-8 in Cancer

Galectin-8 is another type of tandem-repeat Galectin with two identical yet unique CRDs connected by a polypeptide linker region. Galectin-8 is the most unique member of the Galectin family with six existing isoforms possessing either one or two CRDs encoded by a single gene. These isoforms are formed as a result of posttranscriptional alternative splicing, sometimes in combination with multiple polyadenylation signals, generating a much complex variant of mRNAs that will code for the different splice variants of Galectin-8 (Bidon et al. 2001; Bidon-Wagner and Le Pennec 2002). Galectin-8 knockout is associated with elevated chances of urinary bladder cancer (Bidon-Wagner and Le Pennec 2002). Galectin-8 might act as a biomarker for the detection of papillary thyroid cancer, early prediction of renal cancer recurrence. It induces metastasis in prostate cancer, has enhanced expression in breast and colorectal cancer and it acts as a modulator of angiogenesis (Savin et al. 2009; Kramer et al. 2011; Gentilini et al. 2017). However, the exact role of Galectin-8 in tumour biology remains heavily understudied and elusive.

11.8 Galectin-Targeted Therapeutic Strategies in Cancer

With the increasing scientific evidence that establishes the modulatory roles of Galectins in tumour growth, sustenance and metastasis, Galectins have become one of the most obvious targets for the development of cancer therapy. Designing and synthesis of anti-Galectin compounds have been pursued by scientists for over a decade now to tackle the influence of Galectins in several important areas of Biology, especially for diseases like cancer and asthma (Sörme et al. 2003; Zuberi et al. 2004). Given the significance of Galectins in many cellular processes and

diseases, Galectin inhibitors have been quite in demand to unravel the importance of Galectins in those areas. The success of such inhibitory compounds in inhibiting Galectins came with the idea of using these compounds as a potential therapeutic target for treating these diseases involving Galectins as a major player and cancer being the most obvious target (Ingrassia et al. 2006; Astorgues-Xerri et al. 2014; Blanchard et al. 2014). Cancer being the second most leading cause of death globally, exploiting the potential of Galectin inhibitors in generating anticancer therapeutics seemed like a silver lining on a cloudy day. Galectin inhibitory compounds have been designed keeping in mind the natural ligands of Galectins i.e., carbohydrates, especially galactosides. However, peptide ligands have also been synthesized as anti-Galectin compounds. The elucidation of the structural interaction between glycan and Galectins has enabled the designing of highly effective Galectin antagonists in the form of small-molecule glycomimetics and glycoconjugates (Laaf et al. 2019; Bertuzzi et al. 2020). Inhibitory compounds against Galectin-1 and -3 have been most extensively researched. Therefore, inhibitors of Galectins have been broadly categorized which includes carbohydrate-based small-molecule inhibitors, natural polysaccharide-based inhibitors, peptide-based inhibitors, and blocking anti-Galectin antibodies.

11.8.1 Carbohydrate-Based Small-Molecule Inhibitors

These inhibitors are based on the chemically modified natural Galectin ligands, such as the common disaccharide inhibitors like lactose (Lac) or *N*-acetyllactosamine (LacNAc) which are commonly used for targeting the CRD of the Galectins. These small-molecule inhibitors are based on the understanding of the Galectin–ligand interactions in general, however, more selective inhibitors targeting individual Galectins (more specifically Galectin-1, -3, and -7) with high affinity and selectivity are required. The major disadvantages associated with such type of inhibitors under clinical settings are low in vivo bioavailability, susceptibility to hydrolysis by glycosidases, and fast clearance from circulation (Cagnoni et al. 2016; Dings et al. 2018).

Galectins showed binding to β-galactosides such as lactose, lactulose and LacNAc with low inhibitory potency and K_d values of ~1.0 to 2.0 mM, whereas thiodigalactoside (TDG) which is a readily available disaccharide, has a higher affinity with a K_d of ~78 μM for Galectin-1, and is a simple synthetic non-metabolizable disaccharide. It is an alternative disaccharide to overcome the poor bioavailability of lactose derivatives (Cumpstey et al. 2005; Rabinovich 2005). In murine models of breast and colon tumours, TDG administration decreased the tumour progression and metastasis. TDG induced effect was due to inhibition of binding of Galectin-1 to CD44 and CD326 receptors on cancer stem cells (CSC) (Ito et al. 2011; Ito and Ralph 2012). Furthermore, symmetrical modifications of TDG at O3 and O3' positions increase its binding toward Galectin-3 (Cumpstey et al. 2005).

Modified derivatives include C2-symmetric TD139 which is available from Galecto Biotech and its asymmetrical derivatives TAZTDG (Hsieh et al. 2016)

11.8.2 Natural Polysaccharide-Based Inhibitors

These natural compounds like pectins are usually derived from the plant. Pectins have emerged as high-affinity inhibitors of the Galectins and are found in large amount in cell wall of plants (Cagnoni et al. 2016). These natural polysaccharides bind to the Galectin CRD and inhibit them naturally. Modified citrus pectin (MCP), prepared by subjecting natural pectins to adverse temperature and pH conditions, is a soluble, orally ingested dietary carbohydrate fibre (Pieters 2006). MCP is mostly used in inhibition of Galectin-3 and suppressed tumour growth in vivo (Platt and Raz 1992; Pienta et al. 1995). Various forms of pectins are used as Galectin inhibitors and have been tested in human clinical trials, namely DAVANAT (GM-CT-01), PectaSol-C, GCS100, GBC-590, GR-MD-02 (Streetly et al. 2010; Yan and Katz, 2010; Klyosov et al. 2012; Davis et al. 2014; Ruvolo 2016). GM-CT-01 (DAVANAT) is isolated from the seeds of *Cyamopsis tetragonoloba* (Guar gum) and subjected to controlled partial chemical degradation. GM-CT-01 is a 1,4-β-D-Galactomannan-based compound and the backbone of galactomannan is composed of (1→4)-β-D-mannopyranosyl units, to which single α-D-galactopyranosyl is attached by [1→6]-linkage (Klyosov et al. 2012). Another variant of GM-CT-01 (DAVANAT) known as GR-MD-02 (Belapectin) is agalactoarabino-rhamnogalacturonan polysaccharide (Traber and Zomer 2013). Both GM-CT-01 and GR-MD-02, alone or in combination are under clinical trials. Extracellular Galectin-3 was found to be involved in impairment of the tumour infiltrating lymphocytes (TILs) functions. In an experiment, treatment of TILs with GM-CT-01 (an agonist of Galectin) improved the cytotoxic potential of $CD8^+$ TILs and their IFN-γ secretion in a dose-dependent manner (Demotte et al. 2014). This observation indicates the potential benefits of the Galectin inhibitor in patients and paves the way to use these inhibitors in cancer alone or in combination with cancer vaccination. An ongoing trial using GM-CT-01 vaccine in patients undergoing diffuse melanoma is also in progress. Tumor-bearing mice (with MCA-205 sarcoma, TRAMP-C1 prostate adenocarcinoma and 4T1 mammary carcinoma) treated with GR-MD-02 (Galectin-3 inhibitor), anti-OX40 antibody or in combination therapy show reduced monocytic-MDSC (M-MDSC) mediated immunosuppression and thereby $CD8^+$ T-cell recruitment in tumour which further leads to increased tumour regression and survival (Sturgill et al. 2021). Furthermore, in a phase I clinical study of Galectin-3 blockade with belapectin (GR-MD-02) plus anti-PD-1 antibody (pembrolizumab) in patients with advanced metastatic melanoma (MM) and head and neck squamous cell carcinoma (HNSCC) showed significant increased effector memory T-cell activation and reduced M-MDSCs in responders compared with non-responders which was correlated with clinical response (Curti et al. 2021). Both the inhibitors have shown comparable inhibition of Galectin-1 and -3,

however, more work is needed to establish the link of the therapeutic activity of these inhibitors with the mechanistic determinants of Galectin binding and its subsequent function.

11.8.3 Peptide-Based Inhibitors of Galectins

Peptide-based inhibition of Galectins employs use of peptides or peptidomimetics to target Galectins at their CRD or at distant sites. G3-C12 oligopeptide (ANTPCGPYTHDCPVKR) has been obtained by combinatorial bacteriophage display technique. This peptide binds to Galectin-3 CRD with $K_d = 72$ nM (Zou et al. 2005). In an experiment, adhesion of multiple breast carcinoma cell lines to purified Galectin-3 and Thomson–Freidenreich (TF)-mimic in presence/absence of G3-C12 result in average reduction of cellular adhesion. Furthermore, G3-C12 also significantly reduced the metastasis of breast cancer cells to the lung in mouse model (Newton-Northup et al. 2012). Anginex is a unique artificial cytokine-like β-sheet-forming peptide, which is designed using basic folding principle. Anginex contains short sequences from antiangiogenic agents such as platelet factor-4, interleukin-8, and bactericidal-permeability increasing protein-1 and is a potent antiangiogenic agent. Galectin-1 is identified as molecular target of anginex (Wang et al. 2012). Further, its binding to Galectin-1 on activated endothelial cells (ECs) inhibited the adhesion and migration of tumour ECs and induced apoptosis which results in inhibition of tumour angiogenesis (Thijssen et al. 2006). Anginex also blocks Galectin-1 uptake by ECs which prevents the translocation of H-Ras-GTP and impairs phosphorylation of Raf/Mek/Erk after treatment with anginex (Thijssen et al. 2010). Anginex was also found interacting with other Galectins with lower affinities such as Galectin-2, -7, and N-terminal of Galectin-8 and -9 (Salomonsson et al. 2011). Another nonpeptidic inhibitor, OTX008 is a caliraxene-based compound and it binds to Galectin-1 at a more distant location within the CRD as compared to anginex (Dings et al. 2012). It down-regulates cancer cell proliferation, invasion, and tumour angiogenesis in a variety of tumour cells (Raymond 2014). Further, designs of more selective peptide inhibitors are required to target individual Galectins and study their effects.

11.8.4 Neutralizing Antibodies in Galectin Inhibition

High degree of similarity amongst the CRDs of different Galectins impedes the development of much specific and effective inhibitors against the aforementioned lectins. As the differential role of different members of the family in mediating a diverse set of patho(physiological) processes become indispensable, it becomes extremely crucial to be able to check its unbridled expressions (Bättig et al. 2004; Nishi et al. 2008). Hence, highly selective and potent inhibitors against Galectins are

the need of the hour. A neutralizing monoclonal antibody against Galectin-1 (F8.G7) efficiently restricted angiogenesis by downregulating VEGFR2 signalling in tumour cells and constrained its metastatic growth as seen in case of mice with Kaposi's sarcoma (Croci et al. 2012, 2014). Apart from being angioregulatory in nature, a state-of-the-art anti human Galectin-1 mAb (Gal1-MAb3) was designed having immunomodulatory effects (Pérez Sáez et al. 2020). Of much interest here, blocking TIM-3, a binding ligand for Galectin-9 has been proven ameliorating in advanced solid tumours and hematologic malignancies. This interaction forms the basis for a crucial immune system check point in charge of T-cell exhaustion and thus establishing an important option for efficient therapeutic intervention in cancer patients (Yang and Hung 2017).

11.8.5 SiRNA-Mediated Inhibition of Galectins

Small interfering RNA (siRNA) regulates the expression of genes, by a phenomenon known as RNA interference (RNAi). Checking Galectin-3 associated activities using siRNA has shown evident results in the mitigation of metastatic spread of tumour cells in human osteosarcoma cell lines (Park et al. 2015). Additionally, using a siRNA-mediated Galectin-1 silencing, van Woensel et al. (2017) has shown restricted angiogenesis and diminished tumour cell development in glioblastoma multiforme disease model. This also had potential immunostimulatory activities in terms of enhanced $CD5^+$ B cell- and $CD8^+$ Tc cell activation, a ray of hope in eliciting superior antitumour response in cancer patients (van Woensel et al. 2017). On the other hand, another alternative approach in designing effective Galectin inhibitors emerged with the discovery of aptamers which are short and specific oligonucleotides used for selective silencing of targeted molecules. Galectin-1 specific aptamers competently blocked interaction with CD45 and rendered affirmative results in terms of suppressed tumorigenic maturation and recovered cell mediated immunity in patients with lung cancer (Tsai et al. 2019). A better understanding of Galectin interactions with its physiologically significant ligands and how it is modulated through different stages of cancer progression is extremely crucial for the development of effectual therapeutic approaches. Moreover, amalgamating different arms of immunotherapy with the contemporary and existing approaches of treating cancer increased the rate of survival amongst myriad of cancer patients.

Galectin inhibitors and anti-Galectin compounds have humongous potential in the field of therapeutics designed against cancer but despite being a field of infinite possibilities, it remains hugely unexplored. However, progress is being made in deciphering the actions of Galectin-targeting compounds, fuelling more research in this field. Using X-ray crystallography and NMR spectroscopy studies, Galectin-3 inhibitors with high stability and specificity have been designed which serves as a breakthrough in this field. With the revelation of these stable structures of Galectin-3 inhibitors, more research is being focused in these areas (Laaf et al. 2019). When administered in combination with chemotherapy or radiotherapy, Galectin inhibitors

improve the treatment efficiency in several neoplasms. It has been proved to be effective in preventing multiple drug resistance (MDR), eliciting the actions of the chemotherapeutics on cancer patients. Increasing the specificity of the Galectin inhibitors would be beneficial to get over the problem faced due to the redundancy of the Galectin structures and the fact that different types of Galectins are secreted within the same tumour. Using an inhibitor to block Galectin's action could be effective when the inhibitor has selective specificity for that particular Galectin over another (Wdowiak et al. 2018). Thus, more research needs to be focused in this area to upgrade the knowledge we have on Galectin targeted therapy.

11.9 Conclusion and Future Perspective: Galectins in Cancer and the Development of Therapeutics

As discussed in the chapter, Galectins are a crucial part of the TME with an array of different modulatory roles like tumour progression, metastasis, angiogenesis, immune response modulation, immune evasion including both pro-tumour and antitumour functions. Galectins govern the oncogenic development through numerous pathways closely intertwined together with each other. Considering their crucial role in different 'hallmarks of cancer', they have undoubtedly emerged as effective biomarker for disease diagnosis and potential target for therapeutic intervention in cancer. Although having shown significant convalescence in patients, the conventional methods of cancer treatment such as surgery, chemotherapy or radiotherapy bear limitations in terms of tumour relapse or falling inadequate at later stages both in solid tumours or leukaemia. Extensive research over the past decades has greatly enhanced our knowledge on different co-dependent glycoconjugates and their ligands interacting at a molecular level in order to dictate the fate of tumour cells. By taking control of a school of physiological and immunological cellular processes during different stages of cancer, Galectins meticulously mould the prognostic behaviour of the transformed cells. To be even more precise, Galectin-1 and -3 have been established as notorious for being the poor prognostic markers in many types of cancer, having a direct or indirect impact on the early tumorigenic transformation or late metastatic events. Hence, targeting Galectins or their cognate glycoconjugate partners, alone or in combination with existing methods of treating cancer, provides a promising vision in designing effectual next-generation therapeutics for cancer treatment in near future. A number of groups have developed Galectin inhibitors, blocking Galectin activity by interacting with their CRDs but whether to target them extracellularly or intracellularly is still a matter for further research. Such inhibitors also face restrictions in terms of their specificity as CRDs of different members of the family share high degree similarity. Another approach here is nano-based inhibitors, which showed much optimistic result in selective inhibition of lectin-glycan interaction exerting antitumour activity. These nanoparticles are multifunctional and very flexible in nature as they can easily be rewired and rendered

with distinctive biologically relevant properties like composition in the outer functional groups, size, shape, luminescence, magnetism, plasmonic heating or a unique combination of each of them (Thakor and Gambhir 2013). The advancement in theranostics has presented glyconanoparticles as a novel mean to target lectin-glycan interaction much effectively and thereby having a plausible effect in the designing of more potent antitumour vaccines. They are showing prodigious results in the restoration of TME by combating oncogenic, immunogenic suppression, restricting EMT transition, restoring vascular signalling and finally by halting the metastatic growth. Superior understanding of complex structure of glycans, their binding activity with cognate nano-ligands and how they conduct in a closed environment is extremely necessary in order to construct efficient and integrate nanosystems. As the saying goes, put your every nerve and muscle to make things happen, an amicable effort between the fields of glycobiology, nanotechnology and oncology is indispensable to explore this potentially fruitful yet unexplored area of therapeutics presenting a beacon of hope in the treatment of cancer.

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Chapter 12
Lectins in Diagnostic Tools and Therapeutic Agents

Mani Divya, Sekar Vijayakumar, and Baskaralingam Vaseeharan

Abstract Lectins are a collection of assorted proteins of carbohydrate binding capability in nonimmune derivation. This protein can be act together with cell surface and surface glycans. For the reason it possibly stands for an expensive tool intended for disease diagnostic purposes. Immense advancement has been completed in current existence considerate vital part engage in recreation with lectins in numerous natural processes. Lectin could exhibit a range of biological functions including in the development of therapeutic agents and disease diagnostic tools for the treatment managing systems. The present chapter has been constant en route for the precise purpose of lectin-based strategies utilized in the diagnosis and therapeutic implements.

Keywords Lectin · Disease management · Diagnostic tool · Therapeutic agent

12.1 Introduction

To address the present situation in the medical sector, it is clear that the use of slower discovery methods to faster ones is necessary (Alberts et al. 2008). The deficient analytical outcome of these methods results in increased mortality of lives at an earlier stages (Allee 1996). The need for severe diagnostic methods becomes an extremely pressing matter of worry. To overcome this problem, the rapid development of innovative methods in the field of molecular biology, which make use of molecules such as lectin, has provided fresh insights into the structural description of proteins (Alwine et al. 1977). The sophisticated molecular diagnostics techniques are

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used to test deoxyribonucleic acid (DNA), protein, ribonucleic acid (RNA), and other metabolites to detect mutations, changes in biochemical variables, and illnesses, among other things (Bakermans and Madsen 2002).

Lectins are carbohydrates-binding proteins that may be found throughout the environment. Lectin provides a variety of natural activities by being required for carbohydrates. Plant lectins, which agglutinate the foundation of definite animal cells, serve a critical part in the defense against microorganism invasion (Dias et al. 2015). Lectins have recently emerged as the most advanced biological equipment for the development of anti-pathogenic, immunomodulatory, and anticancer medicines. Certainly, the number of reported cancer diagnosis and deaths is continuously rising throughout the globe, with over 18 million new cases and a total of 10 million people have died as a result of cancer (Bray et al. 2018). The fact that has been reported indicates that lectins may be useful in the investigation or treatment of a variety of illnesses. Because of the apoptotic, cytotoxic, autophagic, and antitumor effects resulting from the covering of cells, tissues, and malignant processes, lectins have shown diagnostic and therapeutic utility against cancer (Estrada-Martínez et al. 2017). Lectins are glycan-binding proteins present in cells and on the extracellular surfaces of all living creatures. In 1988, Woodley and Naisbett proposed the concept of lectin-mediated selective medication distribution (Bies et al. 2004).

12.2 Lectins in Diagnostic Tools

In the search for a new clear diagnosis and treatment, a variety of techniques are being investigated. The lectins are utilized in cell biology, biochemistry, and immunology research and cancer diagnostics. They are also employed in cancer testing (Lei and Chang 2009). Lectins distinguish tissue sections, biological fluids, being expensive tools in biotechnology, carbohydrates and glycoconjugates in cells, including pharmacological and diagnostic applications (Lam and Ng 2011; da Silva et al. 2014). The studies that have been carried out using lectins as tools have been resolute on diagnosis due to the incongruity estimation of strapping neoplastic tissues. Diverse tools are being examined in the investigation of a new precise diagnosis and treatment. Assessments exist the condition of research into their possessions for diagnosis and discriminating treatment (Ikeda et al. 2012).

At present, there is modest confirmation on the use of plant lectins as diagnostic agents. Seeing as it is deadly in about 80% of cases due to diagnosis in superior stages (Layke and Lopez 2004). Therefore, it is important to discover more definite markers with well-organized diagnosis in the early stages (Roy et al. 2014).

Several lectins keep on entire as of side to side intestinal tract; they have feasible to be used as diagnostic tools (Ferriz-Martinez et al. 2010). The first is due to their competence to discriminate cancer cells predominantly by the presence of tumor glycosylations (Mody et al. 1995; Gorelik et al. 2001). This could authorize for an enhanced prognosis and diagnosis of cancer (Gupta et al. 2010; Li et al. 2009). The anticancer potential of lectins can be considered from two chief angles: diagnostic

and therapeutic. One of the victorious medical translations of lectin being used in diagnosis tools is *Lens culinaris* agglutinin (LCA). Currently, LCA-based hepatocellular carcinoma (HCC) diagnosis is an FDA-approved HCC clinical diagnosis technique covered by the Japanese Medical Service's health care and utilized by leading cancer treatment centers around the US (Monira et al. 2015; Leerapun et al. 2007).

Lectins have also being investigated to see whether they may help identify ovarian cancer. Two FDA-approved glycoprotein biomarkers for ovarian cancer are cancer antigen 125 (CA125) and human epididymis protein 4 (HE4). *Artocarpus integrifolia* agglutinin (AIA), *Amaranthus caudatus* agglutinin (ACA), *Ulex europaeus* agglutinin I (UEA-I), and *Griffonia simplicifolia* agglutinin are all examples of lectins (GSA I) (Wu et al. 2008; Chen et al. 2013). Moreover, AAL and MAA-II could potentially be used for the verdict of colorectal cancer as well the swot up of disease progression. In summary, the use of lectins for cancer diagnosis, imaging and treatment has received a lot of consideration along with researchers (Coulibaly and Youan 2017). Lectins are useful instruments for cancer detection and prognosis since they are used to investigate the glycan profile of transformed tissues. The carbohydrate antigen sialyl Lewis a (CA19-9) is also utilized as a blood tumor marker (Kannagi 2007). As analytical instruments for glycans and their relevance in detecting pathogens and diagnosing diseases, electrochemical lectin-based biosensors are more attractive (Coelho et al. 2017).

These findings illustrate the consequence of lectin-based recognitions to realize immune mechanisms and diagnostic tools for immunological disorders and diseases. Such valuable diagnostic applications draw attention to the need for expansion within reaches into lectin specific ligand communications (Ambrosi et al. 2005). Furthermore, the prospect impending of lectin-based analysis and treatment has been discussed. The escalating accessibility of simpler equipment and process for lectin analysis involves in glycoconjugates quality to tumor cells. Plant lectins used to monitor tumor-specific glycoconjugates (Mody et al. 1995).

Such equipment is at the present in employ for diagnosis into numerous quantifiable conditions and larger than every grade harmonize the statistics get hold of by immunohistochemistry and immunocytochemistry by way of monoclonal antibodies (Gabius and Gabius 1993). The interactions between lectin and objective ligands include a wide variety of hydrogen bonds, including hydrophobic and nonpolar van der Waals interactions.

For instance, *Polygonatum cyrtonema* lectin (PCL) and erstwhile *Galanthus nivalis* agglutinin (GNA) interrelated lectins given that a range of lectins cooperates with fatal sugars on glycoproteins and glycolipids by way of elevated explicit. It possibly will assist in exemplifying cell facade modifications in cancer. They can be useful in demonstrating cancer-related cell surface changes.

The lectin LCA is well associated with serum thyroglobulin (Tg), a thyroid cancer biomarker that is present in both benign and malignant diseases (Kanai et al. 2009). The above case suggests that by attacking specific differentially glycosylated isoforms, lectins may be constructive in identifying tumor markers in addition to discriminating among cancer and non-malignant cells. Owing to the extent of

Table 12.1 Various types of lectins utilized as diagnosis of several diseases

S. no	Types of lectin	Disease diagnostic purposes	References
1.	*Lens culinaris* agglutinin	Commercial clinical tool AFP-L3 serum concentration utilized for hepatocellular carcinoma	Bialecki and Di Bisceglie (2005)
2.	*Pinellia ternata* lectin (PTL)	Diagnosis of ovarian cancer, breast cancer and pancreatic cancer	Miyoshi et al. (2012)
3.	*Allium chinense* lectin (ACL)	Diagnose proliferation effects and apoptosis	Xiao et al. (2015)
4.	Concanavalin A lectin (Con A)	Inhibit tumor nodule formation	Liu et al. (2009)
5.	Mulberry leaf lectin (MLL)	Apoptosis induction	Deepa et al. (2012)
6.	Soybean lectin (SBL)	Inhibition of tumor growth, apoptosis, and autophagy induction	Panda et al. (2014)
7.	Mistletoe lectin (ML)	Inhibition of metastasis	Pryme et al. (2006)
8.	*Sophora flavescens* lectin (SFL)	Inhibition of tumor growth	Shi et al. (2014)
9.	*Aleuria aurantia* lectin (AAL)	Diagnosis and predicted prognosis of pancreatic cancer	Miyoshi et al. (2012)
10.	*Aleuria aurantia* lectin (AAL)	Thyroid cancer	Zhao et al. (2013)
11.	Phytohemagglutinin lectin (PHA)	Colorectal cancer	Movafagh et al. (2011)
12.	*Sambucus nigra* agglutinin/Elderberry lectin (SNA)	Prostate cancer	Pihikova et al. (2016)
13.	Lectins (from leek, stinging nettle, and HHA)	SARS-CoV (in vitro)	Keyaerts et al. (2007)

glycosylations, lectins have different attachment templates to cancerous tissues. As a result, they're used not only as screening instruments but also as anticancer agents (Bhutia et al. 2019). Lectins, having the assets of recognizing detailed carbohydrate moieties of glycoconjugates, have to turn into a valuable tool for recognition of new cancer biomarkers in complex bodily fluids and tissues (Hashim et al. 2017). Lectins from plants and fungi are very useful for cancer diagnosis and treatment. In several malignancies, using lectins to target T and Tn markers has shown to be a useful approach for cancer detection and prognosis (Desai 2000). Peanut (Peanut *Arachis hypogaea* agglutinin-PNA) and horse gram (DBA from *Dolichos biflorus*) colonic mucosa lectin binding patterns (Fucci et al. 1993). Several kinds of lectins are used to diagnose different diseases (Table 12.1).

The ability of lectins to selectively attach to particular carbohydrates enables them to be used the same as diagnostic apparatus; instances of disparity gratitude include: Mistletoe lectins' damaging behaviors are inhibited by the B-attachment

chains to carbs (MLs, ML-I, ML-II, and ML-III). Gal—1,2Gal—allyl and Gal—1,3Gal—allyl shielded cells against ML-I cytotoxicity 60 and 30 times better than D-galactose, respectively. With further proof, it was discovered that skewed glycosylation influences tumor immune monitoring, malignancy potential, and prognosis, and that it could be used to create new cancer diagnostic methods (Magalhães et al. 2017).

Among various lectins like glycoprotein patterns of human melanoma cells (MAA: *Maackia amurensis*), an increased incidence of division N- oligosaccharides metastatic sites (*Sambucus nigra* and PHA: *Phaseolus vulgaris*) was identified by studies (Litynska et al. 2001). UEA-I (*Ulex europaeus* I), DBA (*Dolichos biflorus*) PNA, LCA (*Lens culinaris*), STL (*Solanum tuberosum*), WGA (*Dolichos biflorus*) PNA, LCA (*Lens culinaris*), STL (*Solanum tuberosum*), UEA-I (*Ulex europaeus* I), and WGA (Dolicho) (Garbor et al. 1998).

The diagnostic approaches were validated using a variety of urothelial bladder cancer models, including animal and in vitro models. As a consequence, careful consideration must be paid to variations in carbohydrate compositions and the lectins that result. Since a growing abundance and form of carbohydrate alterations have been discovered in bladder cancer, lectin-based examinations are one of the burgeoning innovative predictive and analytical tools binding amongst organisms (Zupancic et al. 2014). In view of the fact that, growing quantity and sort of carbohydrate modifications in bladder cancer have been resolute, lectin-based analysis is a method of budding inventive projecting, diagnostic tools (Fig. 12.1).

As monoclonal proteins, clinical micro lectins are often used in diagnostic microbiological applications. Because they have a wide range of specificities and molecular weights, they are useful tools in a variety of situations. Presently, lectins are roughly engaged in glycobiology study for noticing diagnosis, and mapping central neuronal pathways (Annuk et al. 2001). However, an innovative inquiry will roughly have to seem at secluded and proficient drug delivery system advance for lectins, most of their use and amplify the chances for their clinical translation.

12.3 Lectins as Therapeutic Agents

The therapeutic agent has to be sheltered from contamination in the first place, and it must also be free of the DDS inside the objective site. Targeted therapeutics delivered to specific locations through direct and reverse drug delivery systems (DDS) have much compensation above non targeted therapeutics (Minko 2004). Plant sections include lectins that attach to a variety of glycans. The specificities included possible therapeutic and medical diagnostics relevance from various living organisms for their existence of bioactivity, as well as testing pharmaceutical properties and medicinal plant protection. The use of nutraceuticals to obtain more favorable treatment results while experiencing fewer side effects than with conventional medicinal drugs. Medicinal chemicals that have previously been linked to DDS employ an ecological spacer, such as the tetrapeptide Gly-Phe-Leu-Gly, which

Fig. 12.1 Lectin as diagnostic tools

is then degraded by cathepsin B enzyme activity which enabling the therapeutic substance to be released (Kopecek et al. 2000, 2001; Lu et al. 2002).

Lectins are substances with therapeutic or medicinal use. Researchers now have access to a limited quantity of data on *Agaricus bisporus* lectins and lectin-like proteins. Although, *A. bisporus* lectin (ABL) has been found in many samples, there are currently no medicinal medicines produced from ABL on the market. Only one lectin (ABL, GenBank ID XP 006455249) has been categorized in the Genbank database since the genome of *A. bisporus* was sequenced in 2012 (GenBank ID XP 006455249) (Morin et al. 2012). The mannose-binding protein (Abmb) of *A. bisporus* was recently discovered. Its detection contributes to the body of knowledge and piques concern in mushroom-derived proteins with therapeutic potential (Ismaya et al. 2020). Lusvarghi and Bewley (2016) report that the structural and biochemical swot up established approach of act and facilitated creation of Griffithsin (GRFT) analogs, that may direct to resistance, and in vitro and preclinical results that sustain the therapeutic prospective of this lectin. Modern explorations have originated strong anticancer properties of numerous lectins as reported in the literature.

Mutually apoptosis and autophagy are significant features to manipulate the accomplishment of lectin chemotherapy. Lung, epidermal keratinocyte, thyroid, fibroblast, intestine, kidney, colorectal, melanocyte, lymphoid, cortex, liver,

mesenchyma, and spinal mesenchyma have also been investigated for their possible function as anticancer agents (Yau et al. 2015). Depending on the lectin used, autophagy, including apoptosis, can be caused by a variety of pathways and mechanisms. It's worth remembering that certain galectins, such as galectin-1 and -3, are considered to have both anticancerous and pro cancerous effects. As a result, more research is needed in order to make effective and accurate treatment guidelines (Fu et al. 2014). According to Yau et al. (2015), lectin analysis has helped in the detection of their beneficial assets for cancer care. To be eligible for cancer management, not entire proteins in a group of lectin would ultimately persuade apoptosis.

Many lectins have shown promise as biomarkers for early on finding with malignant enlargement otherwise as an inducer of autophagy. Perceptibly, galectin-1 has a wide display of activities, depending on the cell type, spatiotemporal context and availability of counterreceptor(s), in terms of suited glycosylation and protein expression. Ideally, lectin therapy would thus spatially be strictly confined. The line of research toward this goal, initiated to be explored already two decades ago, is the application of the lectin by injection (Smetana et al. 2013). Mistletoe lectins I (ML-I) is a strong lectin anticancer that prevents very effective protein production (Mody et al. 1995). In European countries, mistletoe lectin extracts (ML-I, ML-II, and ML-III) have been used as an experimental augmentation for breast cancer treatment (Heinrich et al. 2005). ML-I may be categorized as a lectin that fulfils the requirements for the second pathway since it has been used to treat breast cancer and has been proven to be cytotoxic (Thies et al. 2005).

Mistletoe (*Viscum album* L.) extracts (VAE) and *V. album* agglutinins have anticancer properties (VAA) VAE and VAA have been extensively researched as anticancer or adjuvant medicinal drugs (Kienle et al. 2011). Several plant lectins have been revealed to activate cell demise in cancer cells, implying that they may be used to treat cancer (Monira et al. 2009). Glycoconjugates with an abnormal glycan composition are expressed and/or secreted by cancer cells (Kjeldsen et al. 1988). Lectins are non-immune proteins or glycoproteins that have a specific preference for the carbohydrate moiety of glycoconjugates (Goldstein et al. 1980). Lectins tend to be promising therapeutic and diagnostic strategies for cancer treatment, as discussed in this chapter. Likewise, some animal experiments have shown that lectins may be effective as therapeutic agents (Fig. 12.2).

To analyze the structural alterations of glycans and to screen and assess the glycosylation outlines of therapeutic proteins, Pažitná et al. (2020) utilized lectin-based microarrays to evaluate the structural modifications of glycans and the glycosylation outlines of therapeutic proteins. The researchers at Wu et al. (2019) discovered that lectins play a variety of natural functions, as well as having insecticidal, antibacterial, and anticancer characteristics. The oncolytic vaccinia virus vector—*Aphrocallistes vastus* lectin (oncoVV-AVL) has a substantial anticancer impact in the mouse model of colon cancer and liver cancer, according to in vivo studies. This swot up may reveal a new method to use the marine lectin AVL in oncolytic viral treatment that is now possible. In the meantime, Li et al. (2018) discovered that the presence of a gene encoding the *Tachypleus tridentatus* lectin (TTL) in a

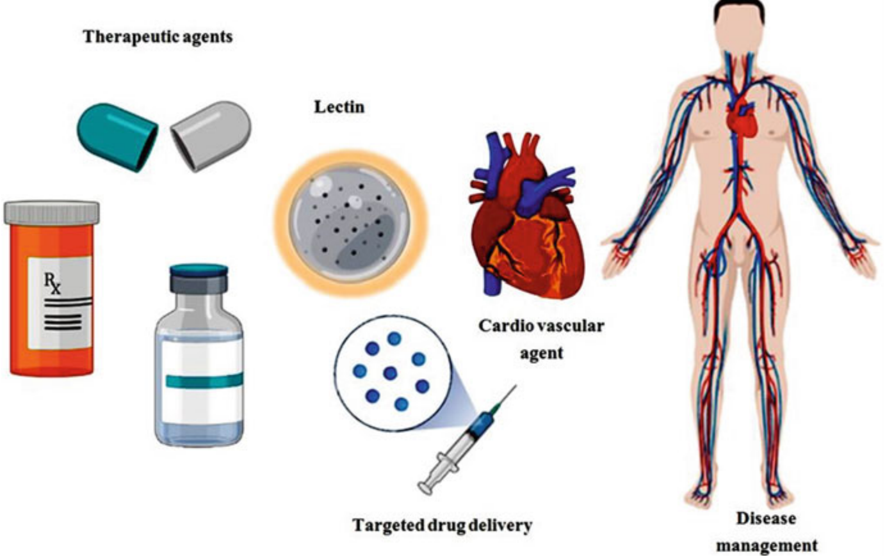

Fig. 12.2 Lectins utilized as therapeutic agents for various systems

hepatocellular carcinoma mouse representation increased the therapeutic effect of an oncolytic vaccinia virus, indicating that lectin genes may enhance the therapeutic effect of oncolytic viruses.

12.4 Conclusion

To summarize, this chapter shows the main role of lectins as instruments for swotting up interactions in the treatment of chronic diseases. The recent emergence of molecular biology has transformed the field of illness diagnostics, allowing for the development of new techniques for accurate detection and identification. These molecular-based diagnostic techniques are becoming more important in health care, particularly in the early stages of diagnosis to provide appropriate therapy. These new insights highlight the importance of this model as a diagnostic tool for protein functioning, as well as the versatility of the lectin as a programmable model with which to experiment. These lectins have been extensively studied and are excellent instruments for a variety of preparative and diagnostic procedures. In the medical and research fields of systems, biological materials for instrumental methods such as lectin-based medicinal agents and diagnostic instruments are most often employed in medical and research fields of systems. They are limited to the most commonly used methods for computing lectin interactions, which are the most often used techniques.

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Conflict of Interest The authors declares no conflict of interest.

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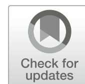
Chapter 13
Modern Approach in Lectin-Based Nanomedicine



Arivarasan Vishnu Kirthi, Loganathan Karthik, and Babu Gajendran

Abstract The era of nanotechnology has led to the development of the science of miniaturized medicines which has helped the human individual to great extent. The presence of the nanomedicine in the scientific world has steered the human populace to a better life with an advent of precision medicines. In this, the glyconanotechnology has been playing a pivotal role in areas like biological sensing, theranostics, drug delivery and imaging. The presence of carbohydrate as the targeting agent or capping agent has delivered the development of versatile and working model of newer species of nanoparticles called glyconanoparticles. In this, we have tried to give a brief but precise nature and properties of the glyconanoparticles and its application. This chapter also details about the dimensions of glyconanoparticles which can be used in the near future.

Keywords Carbohydrate · Nanoparticle · Quantum dots · Sensing · Glucose

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13.1 Introduction

A nanoparticle is an intriguing construct that could be manipulated/altered/designed so that different parts can be attached with specific functions. This property of nanoparticles has been well studied and documented in the scientific community. Its main advancement was found to be in drug delivery. The notion of drug delivery via nanoparticles can be functionalized with different advantages, that can be listed to improved drug's half-life, PK-PD profiles, to be able to target the specified target cells or tissues and lowering drug adverse effects, to deliver drugs individually or in combination, to deliver drugs by crossing the biological barriers, to be utilized in diagnostic, imaging and therapeutic in a single delivery of the nanoparticles. Nanomedicine and nano-delivery systems are a moderately new however quickly evolving science where materials in the nanoscale range are utilized to fill in as a method for diagnostic tools or to deliver therapeutic agents to targeted sites in a controlled manner in a controlled way (Moghimi et al. 2005; Russell et al. 2020). Nanotechnology offers different advantages in treating ongoing human illnesses by site-specific and target-situated delivery of exact medications. Two main types of targeting are employed by the researchers, which can be divided into passive and active targeting (Bertrand et al. 2014). This method is mainly employed in the drug delivery strategies to enhance the intracellular concentrations of drugs in cancerous tissues/cells while avoiding the toxic effects on the non-target cells (Bertrand et al. 2014; Falagan-Lotsch et al. 2017). It is known that the nanoparticles are bound to the specific receptors and are then endocytosed by the cell, at the same time avoiding the P-glycoprotein—one of the drug resistance mechanisms. Also, the nanoparticles provide advantages but still, they come with drawbacks which can be stated as the limitations in bioavailability, unstable while in the circulatory system and toxic effects.

Lectin is a group of specialized glycoproteins (carbohydrate and protein complex), which do not react to the immune system and its cellular architecture. These groups of proteins can be found in the sugar complexes either reversibly or non-covalently to the proteins and lipids alike. They are highly specific due to the presence of a unique and specific polypeptide moiety (Campbell 1999; Mathiowitz et al. 1999). These proteins were first described by Hermmann Stillmark in his thesis, where he described the agglutination activity of ricin isolated from the castor seeds. This resulted in huge attention and research during the 1960s with the research of simple and complex carbohydrates and cellular characterization discovery (Campbell 1999; Klain and Jaeger 1990; Nilsson 2007; Sierra and Martínez-Álvarez 2020). In 1972, Sharon and List published a list of various lectins which paved the pathway for the modern era of lectinology (Sharon and Lis 2003). From then onward, many have published their list of databases, some of which are very much in detail (Damodaran et al. 2008; Lawrence et al. 2007). Some of the carbohydrate and its derivatives have been shown in the Fig. 13.1.

D- Gluconic Acid

Lactobionic acid

Lactonic (di-acetylated) Sophorolipids

Fig. 13.1 Examples of carbohydrates used for direct synthesis of glyconanoparticles

13.1.1 Types of Glyconanoparticles

Up until this point, three primary various kinds of nanoparticles are functionalized with carbohydrates that have been accounted for. In other words, they are gold glyconanoparticles, Glyco-quantum dots, and magnetic glyconanoparticles (Fig. 13.2).

13.2 Gold Glyconanoparticles

The gold nanoparticles utilization was first reported in the 1970s with the advent of an immune-gold stained procedure for electron microscopy (Polak et al. 1984). From that point, the molecules are to be targeted using the gold nanoparticles for visualizing the cellular organelles using electron microscopy. These system have been used for the chemical characterization of interactions among the

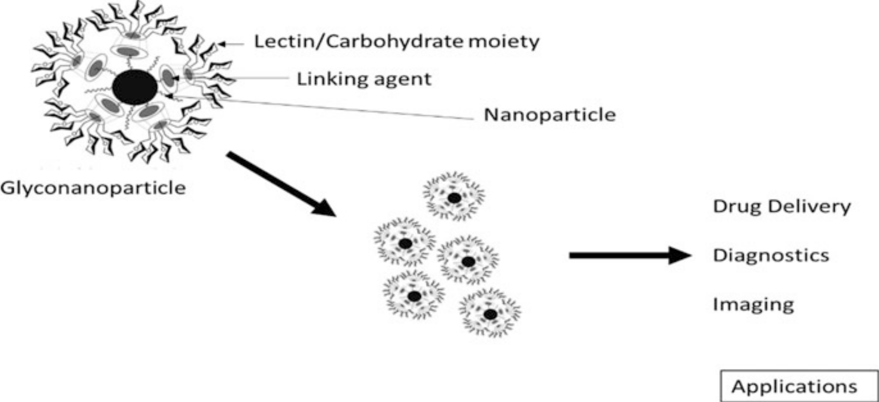

Fig. 13.2 Glyconanoparticle and applications

carbohydrate-carbohydrates and carbohydrate-protein moieties from the pathogen (Marradi et al. 2010).There are many reports on the use of gold nanoparticles along with proteins and nucleic acids (Cigler et al. 2010; Ghosh et al. 2008; Liu and Liu 2019; Zhang et al. 2019). But the first report of the carbohydrates along with nanoparticles was reported only in 2001(de la Fuente et al. 2001).The cholera toxin-induced aggregation of lactose-stabilized gold nanoparticles was demonstrated as early as 2007 by Schofield et al. (2007). The gold nanoparticles were synthesized along with glucose, lactose and maltose, and were functionalized with 3D polyvalent carbohydrate display and globular shapes (Barrientos et al. 2003). This methodology allowed the preparation of nanoparticles along with carbohydrate molecules which were biologically ideal and significant for research purposes. The successful reduction of the progression of an experimental cancer metastasis was carried and reported by Barrientos et al. (2004). This affirms the budding utilization of glyconanoparticles to be used in medicine. Gold glyconanoparticles coupled to listeriolysinO 91–99 peptide was used as the adjuvant for cancer therapy. This method prevented the progression of melanoma since it stimulated the innate immune responses, which is similar adjuvants carry out by promoting cytotoxic immune responses inducing again the antigen presentation and apoptosis in the cancer cells (Calderon-Gonzalez et al. 2017). Further, similar glyconanoparticles was shown to improve the cellular uptake and tumor targeting. Also, it inhibited the glucose transporters in the cancer cells and prolonged blood circulation with both renal and hepatobiliary clearance pathways among the cancer cells (Wang et al. 2019). In another work by the core–shell glyconanoparticles for galectin-3-targeted, trigger-responsive combination chemotherapy showed a great work against MDA-MB 231 cancer cells (Balakrishnan et al. 2020).

The competence of glyconanotechnology additionally permits the control of both the organic shell and the metallic center to get nanobioconjugates with electrochemical properties. A plenty of potential applications in biomedicine and material science can be predicted utilizing these new biomaterials.

13.3 Glyco-Quantum Dots

The quantum dots are nanomaterials which have gained notoriety especially from the medicine world. Their addition to the nanoparticle's regimen with their unique properties which includes optical properties make them as a great fluorescent probe. The quantum dots are nanoscopic spherical shaped semiconductor nanoparticles with a diameter from the 2 to 10 nm. The alterations in the particles size range have exposed the researchers with drastic variations in the optical absorption, excitation energy and electron hole pair recombination. This variation in the physicochemical properties can be controlled using the protocol changes made during the synthesis stage, which also is dependent on size, shape, impurities, and crystallinity (Jacak et al. 2013; Jesus and Penadés 2005; Molaei 2020).

Novel phases are being created with the intend to make glyconanomaterials to perform robotic investigations of carbohydrate interactions just as for clinical applications which includes the diagnostics and sensors. The addition of glycans/lectins and carbohydrate moieties onto the Quantum particle generally includes the two categories. The synthetic conjugation method employs direct attachment of mono, oligosaccharides onto the quantum particles with linkages so that to fix the moieties in place. Many have reported this kind of conjugation technique for the synthesis of glyco-quantum particles. Han et al. (2010) have reported the synthesis of the quantum dots with immobilized carbohydrate molecules. The magnetic graphene quantum dots and cyclodextrin decorated chitosan was immobilized on to the palladium, which was experimented for the hydrogenation catalyst (Esmaeilzadeh et al. 2020). The CA 15-3 antigen was immobilized on thiolated graphene quantum dots for accurate quantification of the level of breast cancer specific protein CA 15-3 in serum was reported (Hasanzadeh et al. 2018). In another category, the protocol for the synthesis of phosphorylcholine self-assembled monolayers (Betanzos et al. 2009) (SAMs)-coated quantum dots displaying various glycans such as Lewis^{x}, sialyllewis^{x} was reported (Ohyanagi et al. 2011). In recent times, these quantum dots/particles have gained attention from glycans or lectins as tool to study the biological processes. The CdSe/ZnS QDs with long decay times were modified with aminophenylboronic acid (APBA) to achieve QD-APBA conjugates, which act as glucose nanosensors, which detected intracellular levels of glucose in cancer cells (Ripoll et al. 2019). Chitosan/carbon quantum dot/aptamer complex were synthesized and were used as drug delivery system for cancer cells (Zavareh et al. 2020). With the advancement of glyco-quantum dots, the methodology has been additionally reached out to comprehend the vital jobs of glycans on the cell surfaces. The rise of glyconanotechnology and the imaging ideas have opened up another detecting cycle, which the current biomarkers think that it's hard to address.

13.4 Magnetic Glyconanoparticle

The magnetic glyconanoparticles can be used to identify and characterize the cells and their types/subtypes based on the interactions related to the carbohydrates-receptor moieties. The nanoparticles have increase surface areas, which makes it a possible notion to affirm the attachment of the difference as well as many sugar molecules to be attached to them. This makes it a great possibility to enhanced avidity with the sugar molecule via multi-valency of carbohydrate-binding. This work was made possible by El-Boubbou et al. (2010), where they synthesized magnetic glyconanoparticles and to use them to make a nanosensor to detect and differentiate cancer cells and make a profile of their carbohydrate-binding abilities using magnetic resonance imaging. Neto et al. (2020) reported the synthesis and utilization of magnetic nanoparticles which were coated with galactose, glucose, maltose and sucrose and their application in the 3D mammalian cell culture, *E. coli* and in 3D array technology. The gentamicin-loaded magnetic gelatin nanoparticles used for the treatment of osteomyelitis as targeted therapy. As per the study, the ex vivo and in vivo studies showed that the abscess began to heal and the integrity of periost and bone began to reconstruct after six doses (Ak et al. 2021).

13.5 Applications of Glyconanoparticles in Nanomedicine

13.5.1 As Lectin-Based Drug Delivery

The administration of the drugs to the patients is undertaken via the oral and intravenous routes for delivery of drugs for systemic disease treatment. This involves the delivery of the drug to the non-target tissues and organs; therefore, the amount of the drug which is required to be reached to the affected part of the organ/tissue only receives traces of the drug administered. Many researchers have worked hard on the notion of the targeted approach of the drug delivery to the affected tissue/organs and also lowering the drug concentration at the non-target areas and a higher concentration at the required part of the organs, and also lowering the occurrences of the side-effects to the patients (Alruwaili et al. 2021; Bae and Park 2011; Reis et al. 2010; Richardson 2012).

The utilization of carbohydrates in drug delivery is primarily focused on the targeting process. The targeting notion is divided into (1) Active targeting and (2) Passive targeting, both approaches have employed the help of carbohydrates (Reis et al. 2010).

The pharmacokinetic and pharmacodynamic properties of the API is primarily dependent on the pharmacokinetics of the carrier substance. There are molecular forces that make the drug to be present in the carrier molecule enclosing the drug in a supramolecular aggregate. The notion of the targeted approach in drug delivery is appealing in many applications which include a varied collection of diseases. The

targeted approach of the nanoparticles for the delivery of the drugs at the requisite site of the tissue/organs. The very targeted molecule/or the stimuli for the release of the payload/drug is the glucose molecule. The nanomaterials can be made to be sensitive to carbohydrates and being responsive in the presence of glucose, these were used to limit the diabetic disorders and deliver the active pharmaceutical ingredient in the appropriate dose and time. Many researchers have used this molecule for their release of the drug at the site required. For example, some of the carrier molecules where the glucose are used as stimuli include the boronic acid-derived polymers (Tang and Chen 2020), chitosan-g-polyethylene glycol monomethyl ether nanocomplex (Liu et al. 2019), Hyaluronic Acid-coated calcium carbonate NPs (Liu et al. 2017), carboxymethyl chitosan-phenylboronic acid-L valine nanoparticles (Li et al. 2017).

13.5.2 As Detection and Imaging Tools

The proteins are processed after their translation via post-translational modification in the endoplasmic reticulum and the Golgi apparatus. With the addition of the glucose/or carbohydrate molecules, and this process is modulated by the glycosyltransferases and glycosidases. The variation of carbohydrate moieties to be attached is followed by the amino acid sequence of the core protein, concentration of the nucleotide sugar moieties and the expression of the enzymes in the ER/Golgi complexes. There are two main types of protein glycosylation, N- and O-linked glycosylation (Stanley 2011). The changes or modifications are found in the glycosylation of the proteins expressed in the cancerous cells and are commonly called tumor-associated glycans or glycoproteins. They are mostly involved in tumor development and cancer progression. These glycoproteins are used as biomarkers for cancer detection. The most common ones are the mucins and are often considered as the targets for cancer progression and treatment. Some of the examples are MUC1, MUC4, MUC13, MUC16, MUC5AC, MUC6, and many more (Bhatia et al. 2019; Dhanisha et al. 2018; Hollingsworth and Swanson 2004; Kufe 2009). An antibody/glycoprotein/lectin sandwich assay was developed for screening potential markers of pancreatic cancer (Li et al. 2009). They used the biotinylated lectins which included *Aleuria aurentia* lectin, *Sambucus nigra* bark lectin, *Maackia amurensis* lectin II, *Lens culinaris* agglutinin, and Concanavalin A. The utilization of the differential glycosylation patterns detected on high-throughput lectin glyco-antibody microarrays is a promising biomarker approach for the early detection of pancreatic cancer. Similarly, many of the research articles in the scientific literature confirm the utilization of glycosylation markers in cancer detection. Some of the examples are alpha-fetoprotein (Lai et al. 2017; Mohammadinejad et al. 2020; Peracaula et al. 2008; Zhao et al. 2020), Human epidermal growth factor receptor 2 (Ranganathan et al. 2020; Shen et al. 2018; Wolff et al. 2018), beta-subunit of hCG (Rizwan et al. 2019; Xia et al. 2017).The silver and gold nanoparticles were synthesized by stabilizing them with a 2-mercaptoethyl α-D-mannopyranoside to

induce the aggregation due to the interaction among the mannose and Concanavalin A for colorimetric assays (Schofield et al. 2006). Similarly, the glyconanoparticles containing zinc (II)phthalocyanine and an anticancer drug, doxorubicin which was used as Chemo-photodynamic Combination Therapy. This combination showed good colloidal stability, pH-responsive drug release, and generation of singlet oxygen when exposed to light (Dag et al. 2021).The glucose modified carbon quantum dot containing 2,2,6,6-tetramethyl-piperidinooxy (TEMPO) for targeted bimodal MR/optical imaging of tumor cells. This enhanced cellular internalization of CQD-TEMPO-Glu in cancer cells through GLUT mediated endocytosis (Lu et al. 2018).

Betanzos et al. (2009) have coupled QDs with *Escherichia coli* lipopolysaccharide and studied their binding onto the surface of the cells, after addition of these conjugates to cultured mouse monocytes as a model. Similarly many have reported the glyco-quantum dots for cellular imaging (Rees et al. 2020a), bioanalysis and imaging (Rees et al. 2020b), glucose nanosensor made up of CdSe/ZnS QDs (Ripoll et al. 2019). The effect of chitosan modified magnetic nanoparticles were investigated for its biological durability, cytotoxicity, and MRI image contrast. The magnetic particle fluid based on the chitosan modified Fe_3O_4 nanoparticles had great potential for enhancing the image contrast in image diagnosis by MRI magnetic resonance imaging (Le et al. 2020). Many of the magnetic nanoparticles with carbohydrate-based nanoparticles have been studied (Jeon et al. 2018; Jun et al. 2007; Yallapu et al. 2011).

The living world includes animals and plants that can synthesize and also utilize the carbohydrates that get releases carbon dioxide and water after/and during their metabolism processes. Also, these carbohydrates are widely present on the cellular surfaces playing a pivotal role in many of the physiological reactions, ranging from adhesion, cellular differentiation, growth inhibition, cellular recognition and other cell tissue interactions (Chen et al. 2016). These interactions also include the interaction between the normal cell and the cancerous cells, pathogens and other antigenic substances. And this can be utilized for disease detection and its targeted treatment via drug delivery agents. One of the detections of pathogenic antigen and antibodies are focused on the detection of the diseases. There are examples of detection-based sensors found in the scientific literature.

13.6 Conclusion

The utilization of nanotechnology in biology and medicine has started to become more of a reality. The conjugate formation of the biological molecules with that of the nanoparticles has been used to explore the recognition of target cells and tissues. The special physicochemical properties of nanoparticles like magnetism and its optical effects are of particular interest to be applied in sensitive probes in basic sciences, which has led to the development of biosensors. The advances of nanomedicines had led to the real-time detection of pathogens and microorganisms.

Conflict of Interest The authors have no conflicts of interest to declare.

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