Chapter 4
Immunomodulatory Nanomaterials

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Abstract The immune system provides protection against infections and toxins and serves as a guard for all tissues against pathogens and malfunctioning cells. Modulation of the immune system to use immune cells as therapeutic agents has been an important target for the treatment of many diseases that could not be cured otherwise such as cancer and autoimmune diseases. In addition, modulation of the immune system is crucial for tissue regeneration, since tissue regeneration and wound healing processes consist of a complicated and ordered array of events, a considerable part of which include the involvement of immune cells. Nanomaterials in the form of nanoparticles and nanofibers provide a wide array of tools for modulation of the immune system. Different types of nanomaterials have been developed to be used for effective targeting and treatment of cancer, as vaccines, and for the treatment of autoimmune disorders. In this chapter, different nanomaterials with immunomodulatory effects will be reviewed with an emphasis on cancer, autoimmune diseases, and vaccine development. In addition, future perspectives for developing materials with more refined immunomodulatory characteristics will be discussed.

4.1 Introduction

The immune system protects against infections and toxins, and plays crucial roles in the regeneration of the tissues after injury, including fighting against pathogens that might attack the regenerating tissue; removal of debris of the wounded and scar tissues; and degradation of the materials that are used for regenerative purposes. It is composed of many different types of cells and lymphoid organs that are tightly regulated and plays an invaluable role in the defense against invading microbes (Moon, Huang, & Irvine, 2012). Spleen, nasal-associated lymphoid tissue, Peyer's patches in the gut, and lymph nodes distributed throughout the body are all parts of the immune system and immune cells are generated at the thymus and bone marrow.

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There are two major types of immune response which are innate and adaptive immunity. Innate immunity is nonspecific and acts as the initial defense against invading microorganisms and foreign particles. Macrophages, dendritic cells, neutrophils, and mast cells perform phagocytosis to destroy the foreign cells and release cytokines. Adaptive immunity plays a role as the second line of defense. In that response, antigen presenting cells (APCs) bring specific antigens to highly specialized T cells and B cells. Antigen is recognized and an immune response that is specific to the invading microorganism is elicited and the target is cleared (Hussain, Vanoirbeek, & Hoet, 2012; Norman, 2005).

The immune system is important in the fight against diseases; however, incorrect regulation of the immune response, immunosuppression and immunostimulation may also lead to pathological conditions (Norman, 2005). Immunosuppression is the state in which the immune system functions are decreased and the response is weakened, which may result in invasion by the pathogens or rapid growth of tumor cells. Immunostimulation enhances the immune response, overactivity of which may lead to a strong adverse response and may result in autoimmune disorders. Since immune system is a very complex mechanism and is very tightly regulated, any effect, inhibition or activation of a pathway may cause unexpected side effects to other pathways or different cells or tissues. Thus, the inflammatory agents are used in a controlled manner due to possible side effects (Chou et al., 2013).

Vaccines are great examples of protection against diseases in which the immune system is stimulated to protect individuals from infectious microbial organisms (Pulendran & Ahmed, 2011). Although there are very successful vaccines against various diseases, there is still a need for vaccines against many other severely infectious pathogens, such as HIV, malaria, tuberculosis, and hepatitis C. Current vaccine development approaches are centered on rational design to have more potency and less immunogenicity, and the number and types of vaccine candidates are increasing rapidly (Mamo & Poland, 2012; Oberg, Kennedy, Li, Ovsyannikova, & Poland, 2011; Rappuoli, Mandl, Black, & De Gregorio, 2011).

Immune system may also provide protection from tumors by using cancer vaccines to stimulate the immune system to inhibit tumors (DeMaria & Bilusic, 2019; Hodi et al., 2010; Kalos et al., 2011; Lollini, Cavallo, Nanni, & Forni, 2006; Williams et al., 2011). Cancer immunotherapy is a popular field that aims to develop novel cancer therapy approaches by understanding and utilizing immune pathways (DeMaria & Bilusic, 2019). On the other side of the spectrum, over-stimulation of the immune system may cause autoimmune disorders that require suppression of the immune system (Feldmann & Steinman, 2005).

Immunomodulatory drugs have been highly coveted for balancing the immune system for autoimmune diseases or specifically enhancing certain immune cells for protection against or treatment of cancer or other infectious diseases and for aiding tissue regeneration. However, due to the complexity of the immune system, using a single-agent immunomodulatory drug may result in severe side effects. Nanomaterials are suggested to be ideal for selective delivery of immune regulating molecules.

4.2   Nanomaterials and Immune System

Nanotechnology gives the scientists ability to design nanomaterials at different sizes, shapes, chemical and physical properties and composition for use in the treatment of different diseases (Couvreur & Vauthier, 2006; Hubbell, Thomas, & Swartz, 2009; Mamo & Poland, 2012; Moghimi, Hunter, & Murray, 2005; L. Zhao et al., 2014). Nanomaterials have been the subject of research for being used as drug delivery vehicles or adjuvants for vaccines (Hubbell et al., 2009; Kasturi et al., 2011; Moon et al., 2011; Reddy et al., 2007). There is also interest in use of them in diagnostic systems (Cho et al., 2011; Noh, Jang, Ahn, Lim, & Chung, 2011). Moreover, they may be used for the treatment of cancer (Hellstrom et al., 2001; Kalos et al., 2011; Steenblock & Fahmy, 2008) and as delivery vehicles for immunotherapy drugs (Ali & Mooney, 2010; Peer et al., 2007; Svenson, 2012). The shape, size and surface properties of the nanoparticles can be engineered to aid in cell specific responses (Treuel, Jiang, & Nienhaus, 2013). An important advantage of the nanoparticles is their ease of functionalization through adding or removing properties.

Nanomaterials with immunomodulatory properties are highly coveted for regenerative medicine applications to enable the immune cells to work in a controlled manner. Depending on the material that they are made of, nanoparticles might be perceived as foreign materials when they enter the body, eliciting an immune response in the form of either suppression or stimulation (Dobrovolskaia & McNeil, 2007). Immunomodulatory properties of nanoparticles may be used in development of vaccines or anti-allergy drugs (Parween, Gupta, & Chauhan, 2011; Ryan et al., 2007).

There is ongoing research on targeted delivery of nanoparticles to specific tissues and cells for treatment or diagnosis. Nanoparticles should be relatively stable in the body and should not elicit immune response to prevent degradation, but they should also reach the target site and stay there. Nanoparticles may accumulate in the desired tissue through blood vessels or bind to specific biological structures at the target site (Pelaz et al., 2017). Also, nanoparticles may be tailored to prevent nonspecific binding. Recent advances in nanoengineering methods allowed production of nanoparticles that may be used to target diseased tissues, in diagnostic applications or to regulate the immune system to produce vaccines, inhibit tumors or prevent autoimmunity.

In the following sections, we will describe how nanomaterials can be used for different immunomodulatory purposes and for aiding immunotherapeutics. We will also summarize recent developments in the design and implementation of immunomodulatory particles.

4.3   Nanomaterials for Immunotherapy of Cancer

According to World Health Organization data, cancer is the second leading cause of death in the world, and approximately 1 in 6 deaths is caused by cancer. The economic burden of cancer among both developed and under-developed countries is

also devastating, and even though an incredible amount of effort has been spent on cancer research and therapy, the currently available methods are still limited, which causes the high lethality rate of cancer.

Main methods for treating cancer are surgery, radiation and chemotherapy. Immunotherapy has recently been developed and it is suggested to be a promising approach for the treatment of various diseases including cancer, because it regulates the immune system to target and destroy cancer cells and tumors (Song, Musetti, & Huang, 2017). Immunotherapy is the regulation of the immune system (activation or suppression) for treatment of diseases. Immunosuppressive approaches may be used to reduce allergic reactions and reduce excessive inflammation in organ transplants. In contrast, in cancer patients, immune system is aimed to be more active and new methods are developed to activate the system against malignant cells. There are different approaches and materials used in cancer immunotherapy, which is expected to result in more durable antitumor responses and reduce metastasis and recurrence compared to previous treatment methods. Currently, immunotherapy is one of the most highly coveted treatment methods; however, it is also a very expensive treatment method and is not suitable for all cancer types.

4.3.1   Nanomaterials for Cancer Immunotherapy

Tumor microenvironment prevents the activity of the immune system, and tumor cells inhibit the activity of tumor-specific T-cells (Munn & Bronte, 2016; Vasievich & Huang, 2011). New research focuses on regulating the tumor microenvironment by inhibiting immunosuppressor molecules or activating soluble mediators, which in turn may elicit an immune response. Nanomaterials have the ability to activate or suppress immune system depending on their type, size, side groups, and functionalization and use of nanomaterials may reduce these side effects by achieving specific delivery to target tumors. Nanomaterials may be used to selectively distribute immune checkpoint regulators to the tumors to inhibit their growth. For example, PLGA nanoparticles that were conjugated with anti-OX40 mAb resulted in stronger cytokine production and increased overall antitumor cytotoxic cell response when injected into the tumor compared to the administration of free antibody (M. Chen, Ouyang, Zhou, Li, & Ye, 2014). Targeted inhibition of cytokines using various approaches is also important for preventing the immunosuppressive environment of the tumor. Park et al. developed nanoscale liposomal polymeric gels (nanolipogels, nLGs) for co-delivery of IL-2 and small molecule TGF-β receptor-I inhibitor SB505124 for this purpose (Park et al., 2012).

Targeted delivery to tumors may also allow the use of highly potent drugs with reduced side effects. Intratumoral injection of anticancer antibodies from functionalized nanoporous silica inhibited tumor growth more efficiently and at a longer duration than systemic antibody injections (Lei et al., 2010). In another study, anti-CD137 mAb and engineered IL-2Fc fusion protein that were anchored to PEGylated liposomes prevented lethal toxicity and increased systemic antitumor immunity

compared to free anti-CD137 antibodies. In this study, it was also determined that the size of the nanomaterials is important. The nanoparticles that are small enough to reach into the tumor but large enough to join the systemic circulation were found to be highly efficient (Kwong, Gai, Elkhader, Wittrup, & Irvine, 2013). Heo et al. found that after injection with immunomodulating oligodeoxynucleotides or siRNA encapsulated PLGA's in addition to chemotherapy, dendritic cells become active and migrate to the tumor-draining lymph nodes. This combination therapy using immunomodulatory nanomaterials inhibited tumor growth and increased the survival rate (Heo, Kim, Yun, & Lim, 2015).

Cell therapy is an alternative for treatment of many diseases; however, it has major limitations such as loss of function of the transplanted cells. A novel approach uses nanoparticles, created from liposomes and liposome-like synthetic nanoparticles 100–300 nm in diameter with a drug-loaded core and phospholipid surface layer, which carry adjuvants. These nanoparticles continuously stimulate donor cells and were shown to increase the efficiency of tumor elimination (Stephan, Moon, Um, Bersthteyn, & Irvine, 2010).

4.3.2   Cancer Vaccines

There are two types of cancer vaccines, therapeutic and prophylactic. Prophylactic vaccines are used to prevent cancers such as the hepatocellular carcinoma secondary to hepatitis B virus and squamous cell carcinoma secondary to human papillomavirus (HPV) (DeMaria & Bilusic, 2019). Therapeutic vaccines, on the other hand, are aimed to treat cancer.

Tumor cells express a variety of antigens, some of which are specific to them, and are not produced by healthy cells. In earlier studies, the cancer vaccines were developed with whole-cells together with adjuvants to target tumor cells. However, because of a need to develop more potent vaccines with fewer side effects, current research focuses on antigens that are specific to tumors (Herlyn & Birebent, 1999). Some of these targets are products of mutated oncogenes (p53, ras, PSA, GP-100, MART-1, B-raf). For cancer to occur, the cells have to have many mutations and escape through many checkpoints, thus every tumor has a different composition. Therefore, a personalized approach may be more effective for cancer treatment, and new developments in genomics allow scientists to determine the specific mutations in the patients. Together with the developments in nanotechnology, this information may be used to customize a specific therapeutic approach for each patient. Initial clinical trials of personalized cancer vaccines have shown the feasibility, safety, and immunotherapeutic activity of targeting individual tumors (Sahin & Türeci, 2018).

Cancer vaccines may not only target different antigens and immune adjuvants, but also use different vaccine platforms, such as peptides/proteins, whole tumor cells, recombinant vectors, dendritic cells (DCs), gangliosides, and genes (DeMaria & Bilusic, 2019). Cancer cells may also be coated with nanoparticles conjugated with tumor antigens to elicit an immune response (Fang et al., 2014). There are

already approved cancer vaccines for treatment against early-stage bladder cancer, mCRPC, and metastatic melanoma, such as TheraCys® and TICE®, PROVENGE, and IMLYGIC®, and several new vaccines are undergoing clinical trials.

Cancer vaccines are promising; however, their impact in metastatic carcinomas is not very high. Cancer vaccines need to work in synergy with other therapeutic approaches to inhibit local (tumor-site) immune response and act as an immunosuppressive agent while stimulating antitumor response. Nanoparticles are very important in that respect, since they may be tailored for different functions.

There is an increased focus on vaccine development by using nanoparticles and nanotechnology methods. Tailored nanoparticles may have fewer side effects and can be more effective. Nanoparticle use in vaccines may protect antigens, allow for more specific targeted delivery and result in sustained slow release. The nanoparticle vaccine field is developing very rapidly and promising; however, there are some problems that need to be addressed, an important one of which is the lack of understanding of the behavior of nanoparticles in the body, when they are used as a shuttle system or an adjuvant in vaccines (L. Zhao et al., 2014).

Nanomaterials may be used for encapsulating antigen and adjuvants, protecting them from degradation and to increase the efficiency of T-cell response (Irvine, Hanson, Rakhra, & Tokatlian, 2015; Irvine, Swartz, & Szeto, 2013; Zhu, Zhang, Ni, Niu, & Chen, 2017). Some carbon-based nanomaterials, such as carbon nanotubes and graphene, might affect immune cells by specifically activating them and initiate an antitumor immune response (Orecchioni et al., 2014; Pescatori et al., 2013; Xu et al., 2013). In one example, PC7A nanoparticles were shown to deliver tumor antigens to cytosol and activating the stimulator of interferon genes (STING) pathway (Luo et al., 2017).

4.4   Nanomaterials for Development of Vaccines

Vaccines are microbial antigens or attenuated/killed microbes administered together with an adjuvant to induce antigen-specific immune responses that result in long-lasting immune memory against specific pathogens. In autoimmune disorders, the vaccine should work in an opposite manner: to inhibit immune responses against self-cells without affecting the capability of the immune system to act against foreign organisms or materials or cancer cells (Clemente-Casares, Tsai, Yang, & Santamaria, 2011).

The size of the particle effects the time required for drainage of that particle into the lymph node (Manolova et al., 2008). When antigens were covalently bound to nano-beads, the immune response differed with respect to the size of the nano-beads (Fifis et al., 2004). Nanoparticle size is also important in delivery. Amorphous silica nanoparticles that are greater than 100 nm have more difficulty in entering into cytosol compared to smaller nanoparticles (between 70 and 10 nm) (Hirai et al., 2012).

Dendritic cells ingest nanoparticles at varying efficiency. The rate of uptake differs by size, charge and hydrophobicity. Nanoparticles bigger than 500 nm are ingested at low rates (Foged, Brodin, Frokjaer, & Sundblad, 2005). Nanoparticles with positive charge are ingested more efficiently compared to neutral or negatively charged particles (Wischke, Borchert, Zimmermann, Siebenbrodt, & Lorenzen, 2006).

An advantage of nanoparticles is that they may be functionalized through addition of receptors on their surface to increase efficiency of delivery. Use of Fc receptors to deliver antigens to human dendritic cells have been suggested to be successful, by using intact antibodies or engineered fragments (Cruz et al., 2011; Mi et al., 2008).

Another advantage of nanoparticle vaccines is that they enter the APC via phagocytosis, unlike soluble antigens which enter by micropinocytosis. Thus nanoparticle vaccines present antigens more efficiently, which may also result in stronger immune responses (H. Shen et al., 2006).

Delivery of vaccines is another important subject. Several nanocarrier systems have been investigated for vaccine delivery, such as liposomes. Liposomes are important because of their adaptability and flexibility for use in different applications (Schwendener, 2014). Liposomes may be used to encapsulate different types of nanoparticles. Hydrophilic particles may be carried inside the liposome, while hydrophobic particles may be incorporated into the lipid bilayer. Liposomes may also be adjusted regarding their components, size and charge.

As a delivery system, nanoparticles may activate immune system by directly delivering antigen to the immune system cells or perform targeted delivery (Girija & Balasubramanian, 2018; Mody et al., 2013). To function as immunomodulators, nanoparticles may be tailored to activate immune pathways which might then enhance or inhibit antigen processing and immunogenicity. Gold and silica nanoparticles have been analyzed for their potential for use as cargo delivery system (Brito & O'Hagan, 2014; Shah, O'hagan, Amiji, & Brito, 2014). They may be tailored to strongly bind to antigens, co-deliver adjuvants and multi-epitope antigens into lymphoid organs and into antigen-presenting cells (Zhu et al., 2017). The antigens can be attached to nanoparticles by encapsulation, conjugation, or adsorption (L. Zhao et al., 2014). Adsorption is not very strong, and relies on electrical charge or hydrophobicity, which may lead to rapid dissociation of the antigen and the nanoparticle in vivo. Encapsulation and chemical conjugation results in stronger binding of the nanoparticle to the antigen (Pati, Shevtsov, & Sonawane, 2018). The antigen is released when the carrier particle is ingested and degraded by the cell. In chemical conjugation, antigen is coupled irreversibly to the nanoparticle (Andersson, Buldun, Pattinson, Draper, & Howarth, 2019). New research may focus on soft-matter nanoparticles that are based on emulsions which work as adjuvants when they are given into the body independently of the antigen, that is, no attachment (Morel et al., 2011; O'Hagan, Ott, & Van Nest, 1997). Further studies are required to fully understand the effects of these molecules on each other and to the immune system.

4.4.1 Nanoparticle Interactions with Antigen Presenting Cells

For the vaccines to work efficiently, antigens must be delivered to antigen presenting cells, dendritic cells and macrophages, so that these cells are activated and an immune response is elicited (Jones, 2008; Reddy, Swartz, & Hubbell, 2006). Interactions of these cells with nanoparticles and mechanisms of delivery into the cells are important for rational design of vaccines (Kumari & Yadav, 2011). Also, scientists have to be aware of the possible changes to the nanoparticle behavior with the changing size, shape, side-group, and overall functionalization (Khong et al., 2018; Xiang et al., 2006). For example, PLGA particles that are 300 nm in diameter were transported into the cells at a higher rate compared to 1, 7 and 17 μm particles (Joshi, Geary, & Salem, 2013). Internalization of positively charged polystyrene nanoparticles to the dendritic cells was also higher, possibly due to the interactions with the anionic cell membranes (Foged et al., 2005; Y. Shen, Hao, Ou, Hu, & Chen, 2018). However, it was not shown that this increased internalization is correlated with more potent immune response.

In addition, nanoparticles have been increasingly used to deliver not only antigen of interest but also co-adjuvants, such as poly(I:C), CpG and MPL (De Temmerman et al., 2011; Hafner, Corthésy, & Merkle, 2013). However, more work is needed in nano-vaccine research to overcome challenges including synthesizing nanoparticles that are cheap, uniform, and consistent, that are functionalized with desired properties, ability to target the particle to desired location, with fewer side effects. Thus, rational design of these nanoparticles with respect to the disorder is imperative. New technologies such as micro- and nanofluidics are also important for more efficient analyses of the effects of nanoparticles on different cell types (Hong, Lu, Liu, & Chen, 2019).

4.4.2 Polymeric Nanomaterials for Vaccine Development

Synthetic polymers have different compositions and properties and some of the most widely used ones to prepare nanoparticles are poly(D,L-lactide-co-glycolide) (PLG) (Kim et al., 1999; Köping-Höggård, Sánchez, & Alonso, 2005; C. Thomas, Rawat, Hope-Weeks, & Ahsan, 2011), poly(D,L-lactic-coglycolic acid) (PLGA) (Demento et al., 2012; Lü et al., 2009; Manish, Rahi, Kaur, Bhatnagar, & Singh, 2013; Silva et al., 2013), poly(g-glutamic acid) (g-PGA) (Akagi, Baba, & Akashi, 2012; Akagi, Kaneko, Kida, & Akashi, 2005), poly(ethylene glycol) (PEG) (Köping-Höggård et al., 2005), and polystyrene (Kalkanidis et al., 2006; Minigo et al., 2007). PLG and PLGA nanoparticles have high biocompatibility and biodegradability which are needed for use in drug delivery (D'souza et al., 2014; Danhier et al., 2012).

Importance of polymeric nanoparticles comes from their ability to deliver antigens to target cells or provide sustained release of their cargo antigens. These polymeric

nanoparticles entrap antigens for delivery to certain cells or sustain antigen release by virtue of their slow biodegradation rate (Danhier et al., 2012). PLGA nanoparticles were shown to shuttle antigens against *Plasmodium vivax* with monophosphoryl lipid A as adjuvant (Moon, Suh, et al., 2012), hepatitis B virus (HBV) (C. Thomas, Rawat, et al., 2011), *Bacillus anthracis* (Manish et al., 2013), and model antigens such as ovalbumin and tetanus toxoid (Demento et al., 2012; Diwan, Tafaghodi, & Samuel, 2002).

Cationic alginate-polyethylenimine (PEI) nanogels have been proposed to be used as a vaccine delivery system (Li et al., 2013). Compared with the empty nanogels, nanoparticle loaded nanogels enhanced vaccine-induced antibody production more efficiently, showing the potential of this approach to be used to enhance vaccine-elicited humoral and cellular immune responses.

4.4.3 Metal Nanoparticles

Metal nanoparticles are widely used for different applications. They have well developed and controllable synthesis methods with precise sizes and shapes, and they can also be readily functionalized (Kalkanidis et al., 2006; L. Zhao et al., 2014).

Gold nanoparticles have been used in several studies for vaccine development (L. Zhao et al., 2014). The size of gold nanoparticles usually ranges from 2 to 150 nm, and they may be given spherical, rod or cubic shapes (Gregory, Titball, & Williamson, 2013; Niikura et al., 2013). In one study, the highly conserved extracellular region of the matrix 2 protein of influenza A virus was conjugated to gold nanoparticles to treat influenza. Intranasal administration of this nanoparticle system induced matrix 2 protein specific IgG serum antibodies (Tao, Ziemer, & Gill, 2014). Stone et al. fabricated gold nanorods which were attached to the respiratory syncytial virus by covalent binding of a viral protein. This gold nanorod construct contained the major protective antigen of the virus, the fusion protein (F), and was able to successfully induce immune response (Stone et al., 2013). In another study, gold nanoparticles were attached to a synthetic peptide resembling foot-and-mouth disease virus protein, with sizes ranging from 2 to 50 nm. These nanoparticles activated the antibody response at different strengths (Y. S. Chen, Hung, Lin, & Huang, 2010). Xu et al. conjugated the surface of the gold nanorods with cetyltrimethylammonium bromide (CTAB), poly(diallydimethylammonium chloride) (PDDAC), and PEI. PDDAC- or PEI-attached gold nanoparticles with DNA stimulated stronger immune response compared DNA only approach (Xu et al., 2012).

Surfaces of gold or iron oxide may also be functionalized by coating them with sugar molecules (glyconanoparticles). Carbohydrates and other molecules may be attached to metal nanoparticles, and the core may be filled with magnetic or fluorescence molecules (Marradi, Chiodo, García, & Penadés, 2013).

Some inorganic materials such as silica-based nanoparticles are biocompatible and are being investigated as nanovaccine constituents. Mesoporous silica nanoparticles

may be tailored for targeted release of antigens by changing properties such as the shape, size and surface functionalization (Manzano et al., 2008). These mesoporous silica nanoparticles can carry more cargo compared to solid silica nanoparticles, and could be regulated to release the cargo in a controlled manner by changing mesoporous structures. Mesoporous silica nanoparticles are promising candidates for use in vaccines due to their controlled-release abilities.

4.4.4 Carbon-Based Nanomaterials

Carbon nanoparticles have been studied for their use in vaccine delivery (Gregory et al., 2013; T. Wang et al., 2011). Particles which were 450 nm in size and with 50 nm mesopores on the particle surface were produced. These pockets would be used to transport protein antigen, protecting the antigen and allowing oral administration (T. Wang et al., 2011). They are tolerated well inside the body and were given different shapes including mesoporous spheres (Bianco, Kostarelos, & Prato, 2005; Gupta et al., 2015). Carbon nanotubes are pure carbon molecules and are generally 0.8–2 nm in diameter with a length of 100–1000 nm (Parra, Abad-Somovilla, Mercader, Taton, & Abad-Fuentes, 2013; Villa et al., 2011).

4.4.5 Biological Materials for Vaccine Development

Proteins, peptides, carbohydrates, nucleic acids, and liposomal systems have all been proposed to be used for developing effective vaccines for various purposes. They can be used in the form of either nanoparticles and nanofibers or hydrogels. Recently, nanoparticles are proposed to be synthesized by self-assembling of proteins that assemble to form higher level quaternary structures. By using protein structure information, self-assembling nanoparticles that resulted in stronger immune response than influenza vaccines were designed (Kanekiyo et al., 2013). In this study, the viral hemagglutinin was genetically fused to ferritin, which is a protein composed of 24 identical polypeptides that can self-assemble into spherical 10 nm particles. This nanoparticle vaccine elicited hemagglutination inhibition antibody titers that were more than tenfold higher compared to the traditional vaccine. The advantages of protein nanoparticles in vaccine development include having highly organized structures and symmetry, biodegradability, and tailorability at three different interfaces and size (Neek, Kim, & Wang, 2019).

Vault nanoparticles are self-assembled to form an ellipsoid that contains an empty region inside (Buehler et al., 2014). Recent work which focused on the use of vault nanoparticles to carry antigens showed that these nanoparticles elicited potent immune response, that may also be useful in cancer vaccines (Kar et al., 2012). A new class of monodispersed, self-assembling vault nanoparticles comprises a protein shell exterior with a lipophilic core interior and these recombinant vaults

contained a small amphipathic α-helix obtained from hepatitis C virus. This design resulted in a small area in which lipophilic compounds would be encapsulated and delivered to target cells (Buehler et al., 2014).

Liposomes are spherical structures composed of phospholipid bilayers with a core that may be used for antigen delivery. Liposomes are biodegradable, and range from 20 nm to 1 μm in size. Liposomes may be used as delivery systems for vaccines by encapsulation, adsorption, or surface coupling of antigens (Giddam, Zaman, Skwarczynski, & Toth, 2012). Liposomes do not have immunostimulatory or immunosuppression activity, so they are combined with adjuvants for vaccine development. An in-depth analysis of liposomal interaction with the immune system would allow scientists to design more potent vaccines. Liposomes may be used to deliver DNA vaccines or virosomes that contain viral envelope glycoproteins (Glück, Moser, & Metcalfe, 2004; Khatri et al., 2008). Liposomes also allow the use of intranasal approach for delivery of vaccines (S. Sharma, Mukkur, Benson, & Chen, 2009).

Recombinant vaccines using proteins have very little toxicity but their potency is not high. In one study, an interbilayer-cross-linked multilamellar vesicle system was produced by cross-linking headgroups of adjacent lipid bilayers. These vesicles internalize antigens in the core and immunostimulatory adjuvants on the vesicle walls. These vesicles were shown to have potency, eliciting very strong endogenous T-cell and antibody responses (Moon et al., 2011).

4.5 Nanomaterials for the Treatment of Autoimmune Diseases

Autoimmune disease is the name of around 80 disorders that share a common etiology: an immune attack on the body's own cells. There are self-reactive B-cells and T-cells that recognize and attack the self-antigens. During T-cell development, self-reactive cells are normally deleted, and problems in removal or inhibition of these self-reactive cells may lead to autoimmunity (Notarangelo, Gambineri, & Badolato, 2006).

The prevalence of autoimmune diseases is increasing in industrialized countries, which may be due to environmental changes, such as air quality. There are no successful cures for these diseases. The disease progresses slowly and organ and tissue damage occur before diagnosis. To battle against autoimmune diseases, inhibitors against immunostimulatory molecules may be used, such as monoclonal antibodies or small receptor blockers. These inhibitors downregulate or result in the degradation of immunostimulatory agents.

Scientists have tried to develop techniques to transport anti-inflammatory drugs to target immune cells in affected tissues and inhibit or limit their pathological effects. For the treatment of autoimmune diseases, it would be extremely important to inhibit the T-cells that attack the body's own tissues, but not effect T-cells in other tissues. A wide and very strong immunosuppression would result in infections. In the immune system, T-cells play a very critical role in the defense against diseases or degradation of tumor cells. However, they may be responsible from self-tissue

damage in autoimmune disorders. Thus, it is imperative that T-cell activity is regulated to develop successful strategies against autoimmune diseases. This may be done by converting T-cells into other types, redirecting their program, such as conversion of effector cells to regulatory T-cells (Moon, Huang, & Irvine, 2012; O'Shea & Paul, 2010; Rose, 2016). Nanoparticles would be very important in these types of altering function activities. By specifically tailoring the abilities of the drugs, nanoparticles may regulate these cells (Park et al., 2011). Metal nanoparticles, liposomal systems, biomaterial-based nanomaterials and carbon-based nanoparticles have been widely studied for this purpose.

4.5.1 Carbon-Based Nanoparticles

Carbon nanotubes are formed by carbon atoms that arrange forming a two-dimensional hollow cylinder. Carbon nanotubes were shown to induce systemic immunosuppression in mice (L. A. Mitchell et al., 2007; Leah A. Mitchell, Lauer, Burchiel, & McDonald, 2009; Tkach et al., 2011; X. Wang, Podila, Shannahan, Rao, & Brown, 2013).

Fullerene is also formed by carbon atoms and forms a closed structure that may be a hollow sphere, ellipsoid, tube, or many other shapes and sizes. It has anti-inflammatory and antioxidant effects (Magoulas et al., 2012). Fullerenes inhibit the allergic response against Ag-driven type I hypersensitivity by decreasing the level of reactive oxygen species (ROS) (Ryan et al., 2007). Fullerene derivatives may defend against oxidative stress in ischemia-reperfused lungs (Y.-W. Chen, 2004). Fullerenes were also shown to inhibit the development of arthritis in a rat model (Yudoh, Karasawa, Masuko, & Kato, 2009). In another study, hydroxylated fullerenes inhibited neutrophil function in fathead minnows (Jovanović, Anastasova, Rowe, & Palić, 2011).

4.5.2 Metal Nanoparticles

Gold nanoparticles have diverse properties and their size can be tailored to modulate their immune response and biodistribution. In gold nanoparticles, gold core is inert and nontoxic, and the nanoparticles can be manufactured with a very wide size distribution ranging from 1 to 150 nm and can be easily synthesized by various methods (C. P. Sharma, 2010). Twenty-one nanometer spherical gold nanoparticles caused no apparent organ or cell toxicity in mice, but resulted in inhibition of inflammatory effects (H. Chen et al., 2013). Five and 15 nm gold nanoparticles reduce pro-inflammatory responses induced by interleukin-1 (Sumbayev et al., 2013).

Iron oxide nanoparticles have also been used for immunomodulatory purposes. When the mice were exposed to ovalbumin and to varying doses of iron oxide submicron- or nanoparticles, allergic response was significantly inhibited.

Interestingly, low doses of submicron particles had no significant effect on the allergic response while the same dose of nanoparticles had an adjuvant effect on the response to ovalbumin. This study clearly showed that the particle dose and size affect the allergic response (Ban, Langonné, Huguet, Guichard, & Goutet, 2013). Administration of iron oxide nanoparticles (58.7 nm) also suppressed T-helper 1 cell-mediated immunity in ovalbumin sensitized mice (Shen, Liang, Wang, Liao, & Jan, 2012). Compared to single instillation, repeated instillations resulted in a reduction of inflammatory cell numbers in both bronchoalveolar lavages and pulmonary parenchyma (Ban, Langonné, Huguet, & Goutet, 2012).

Cerium oxide nanoparticles have the potential to reduce reactive oxygen species production and may be used to battle chronic inflammation (Hirst et al., 2009; Schanen et al., 2013). Also, cerium oxide nanoparticles (5–8 nm) protected the cardiac progenitor cells from H_2O_2-induced cytotoxicity (Pagliari et al., 2012).

Quantum dots are artificial semiconductor particles that are a few nanometers in size and their distinctive conductive properties are usually determined by their size. Cadmium telluride quantum dot nanoparticles suppressed the immune responses of macrophages to *Pseudomonas aeruginosa* by reducing NO, TNF, KC/CXCL-1, and IL-8 levels (Nguyen, Seligy, & Tayabali, 2013). Immunosuppression was also observed in Juvenile rainbow trout. When Juvenile rainbow trout were exposed to 5, 10, and 20 nM cadmium tellurium quantum dots, each form of dots resulted in a different pattern of gene expression and lowered fish immune response (Gagné et al., 2010). Sub-toxic levels of cadmium telluride quantum dot nanoparticles were also shown to suppress immune responses against bacteria in macrophages and epithelial cells (Nguyen et al., 2013).

4.5.3 Polymeric Nanoparticles

The size, shape and material of polymeric nanoparticles have also been shown to be important for suppression of immune responses. Twenty nanometer polystyrene particles decreased the efficiency of dendritic cells to degrade soluble antigens, without affecting their ability to induce antigen-specific CD4+ T-cell proliferation. Thousand nanometer polystyrene particles did not have such effects, while 20 nm particles accumulated in the lysosomes. Size-dependent accumulation of particles in lysosomes modulates dendritic cell function through impaired antigen degradation (Seydoux et al., 2014).

When dendritic cells (DCs) were treated with model biomedical poly(vinyl alcohol)-coated super-paramagnetic iron oxide nanoparticles (PVA-SPIONs), they were observed to exhibit decreased antigen processing capacity and CD4+ T-cell stimulation capacity (Blank et al., 2011).

Inert 50 nm polystyrene nanoparticles were also shown to inhibit allergic lung inflammation by modification of pulmonary dendritic cell function (Hardy et al., 2012). Particles composed of PLGA or PEG did not result in production of pro-inflammatory cytokines or inflammasome activation in macrophages. When instilled into the lungs,

particle composition and size may increase the number and type of innate immune cells in the lungs without triggering inflammatory responses (Roberts et al., 2013). In another study, polystyrene or biodegradable poly(lactide-co-glycolide) microparticles encapsulating encephalitogenic peptides (500 nm diameter) were observed to induce long-term T-cell tolerance and ameliorated experimental autoimmune encephalomyelitis (Getts et al., 2012).

4.5.4   Biomaterial-Based Nanoparticles

Peptide, carbohydrate, nucleic acid, and liposomal formulations have also been used for immunomodulation for the treatment of autoimmune diseases. Nanoparticles carrying disease-related peptide-major histocompatibility complexes were shown to reduce polyclonal autoimmunity by activating the selective expansion of memory-like autoregulatory CD8+ T-cells (Clemente-Casares et al., 2011). These antigens interacted with CD8+ T-cells only in diseased but not healthy individuals, thus these nanoparticles coated with any relevant pMHC may be used as vaccines to inhibit polyclonal autoimmune responses in a disease and organ-specific manner (Tsai et al., 2010).

Liposomal encapsulation of glucocorticoids both enhances their efficacy in the treatment of encephalomyelitis and alters their target cell specificity and their mode of action compared to free glucocorticoids (Schweingruber et al., 2011). A study with folate-targeted nanoparticles showed promising results in the treatment of inflammatory arthritis (T. P. Thomas, Goonewardena, et al., 2011). Liposomal glucocorticoids inhibited proinflammatory macrophage functions and upregulate anti-inflammatory genes, but had little effect on T-cell apoptosis and function.

Injection of DNA plasmids encoding immunomodulatory proteins (OX40-TRAIL), and a cationic lipid was shown to ameliorate experimental autoimmune encephalomyelitis (Yellayi et al., 2011). Controlled release of immunomodulating peptide antigens from PLGA suppressed production of inflammatory cytokines and helped to reduce dosing without increased frequency (H. Zhao, Kiptoo, Williams, Siahaan, & Topp, 2010). A study in siRNA silencing in inflammatory monocytes to suppress expression of the chemokine receptor CCR2 showed that this approach prevented accumulation of inflammatory monocytes and their differentiation into highly activated antigen-presenting macrophages at the sites of inflammation. This therapeutic approach reduced inflammation in atherosclerotic plaques, decreased infarct size after coronary artery occlusion, prolonged survival of pancreatic islet allografts after transplantation, and suppressed tumor growth (Leuschner et al., 2011). Polylactide-cyclosporine A nanoparticles were produced at sub-100 nm sizes and narrow particle size distributions, and released cyclosporine A continuously for targeted immunosuppression. This study showed that poly-lactide-cyclosporine A nanoparticles were internalized into the dendritic cells with a continuous release to the culture medium, suppressing proliferation of T-cells without any systemic release of the drug (Azzi et al., 2010).

In another study, when leukemia inhibitory factor-loaded nanoparticles were directed to T-cells, vascularized heart grafts survived longer, Foxp3+ cells were induced and Th17 cells were expanded. These results show that engineered nanoparticles regulate immune pathways to elicit wanted response, thus enabling a new therapeutic approach for autoimmune disorders (Park et al., 2011).

4.6 Conclusions and Future Perspectives

Strategies to develop therapies based on inherent properties of different types of nanoparticles, which may be tailored for different diseases, should also employ methods to understand the mechanisms of the immunomodulatory action of these nanoparticles. There are many different types of nanoparticles with various chemical structures and sizes, and they can be functionalized with different side units. In addition to the size, particle composition, surface chemistry, ability to bind to plasma proteins, and drug excretion time and route is important for the immunomodulatory characteristics of the nanomaterials. These responses may be beneficial or harmful depending on the disease type. Research on immunomodulating nanomaterials to fight against cancer focuses on avoiding side effects and enhancing the tumor degrading ability of these nanoparticles. On the other hand, nanoparticles should have immune suppressing ability to treat autoimmune disorders.

It is extremely important that the correlation between the nanoparticle properties and immune response is elucidated for development of treatment and diagnosis methods in medicine. However, further detailed studies are required for tissue regeneration and diagnosis, prevention and treatment of diseases through immunomodulatory nanoparticles.

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