

Review Article

The Influence of Nanoparticle on Vaccine Responses against Bacterial Infection

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Nowadays, nanovaccine is considered as an evolving method in the field of vaccination to induce immunity in the human body against various diseases, including bacterial or viral diseases as well as virulent tumors. Nanovaccines are more efficient than traditional vaccines since they could potentially induce both humoral and cellular immune reactions. Various studies have shown that nanoparticles with multiple compounds have been designed as delivery systems or as adjuvants for vaccines. Nanoparticles could function as a drug delivery tool, as an adjuvant to promote antigen processing, and as an immune modulator to induce immune responses. These nanoparticles generate immune responses through activating immune cells as well as through the production of antibody responses. Design engineering of nanoparticles (NPs) used to produce nanovaccines to induce immunity in the human body needs comprehensive information about the ways they interact with the component of immune system. Challenges remain due to the lack of sufficient and comprehensive information about the nanoparticles' mode of action. Several studies have described the interactions between various classes of nanoparticles and the immune system in the field of prevention of bacterial infections. The results of some studies conducted in recent years on the interaction between nanoparticles and biosystems have considerably affected the methods used to design nanoparticles for medical applications. In this review, NPs' characteristics influencing their interplay with the immune system were discussed *in vivo*. The information obtained could lead to the development of strategies for rationalizing the design of nanovaccines in order to achieve optimum induction of immune response.

1. Introduction

Since their discovery by Jenner in 1796, vaccines have been used to eradicate several life-threatening diseases. Traditional vaccines are produced using completely killed or weakened organisms and often generate unwanted side effects [1]. Subunit vaccines consisting of defined components of a particular pathogen are usually very sound with fully prespecified constituents. Nevertheless, in some cases, the immune response induced by subunit vaccines is weak, and these formulations need the booster doses and/or adjuvants to be efficient [2, 3]. Recently, nanoparticles (NPs) have been manufactured as a means of delivery or adjuvants for vaccines. Nanotechnology has helped formulate efficient

vaccine delivery systems capable of amplifying an immune response, leading to the emergence of “nanovaccinology” [4]. Nanovaccines able to transfer only the intended vaccine could also be very helpful in stimulating cellular and humoral immunity to avoid the spread of infections and diseases. Nanovaccines acting as immune modulators could overcome all physiological barriers, including maintaining antigen stability, improving antigen presentation and processing, and enhancing immune responses by presenting vaccines to target cells [5].

The most critical problem in the development of nanovaccines is the lack of sufficient and comprehensive information concerning the *in vivo* nanoparticles' mode of action, which could serve both as a delivery tool and as an

immunomodulatory adjuvant to improve antigen delivery and processing as well as induce immune responses [6]. The elemental attributes of NPs are essential factors affecting the interplay between them and the immune cells, including size, shape, and surface chemistry [7, 8]. Various *in vivo* molecular imaging methods, such as MRI (magnetic resonance imaging), have been widely used to obtain more information about the interplay between NPs and the immune system [4]. Many review articles have focused on the types and physicochemical properties of nanomaterials, but the factors affecting immune responses induced by nanovaccines have not been sufficiently explored [9, 10].

In this study, a summary was provided about the functional considerations of the interplay between NPs and immune cells. An overview was also provided about the role that nanoparticles play (delivery tool, adjuvant, and immune stimulator) and the interaction of various types of NPs with immune cells, with a special focus on bacterial infections. This review also demonstrated the reasonable scheme of vaccine-equipped NPs for achieving optimal stimulation of the body's defense system against infectious diseases. The review then concluded with other possible applications of NPs in nanovaccinology.

2. Nanovaccines and Immune Responses against Bacterial Infections

The lymph node (LN) is a good target for nanovaccines to stimulate immune cells, especially *B* and *T* cells, which are able to eliminate pathogens from the body. The secretion of a specific antibody against nanovaccines depends on the ability of nanovaccines to stimulate T-helper 2 (Th2) cells to produce cytokines and plasma B cells to produce antibodies against the vaccine. At this stage, the generated antibodies in response to nanovaccines are caused by both the nanoparticle itself and the combined antigen. Those nanovaccines which are able to stimulate B cells are distributed into plasma cells and cause them to produce a specific antibody in response to the nanoparticle and NP-combined antigen. A study reported the production of IgM response in an *in vivo* model with the administration of empty Polyethylene Glylated liposomes (PEGLip) [5]. In another study, mice treated with Mesoporous silica NPs showed changes in lymphocyte populations (CD3+, CD45+, CD4+, and CD8+) in the spleen and elevation in serum levels of IgG and IgM [11].

Several types of NPs could stimulate the complement system via one or several pathways. Recent data have suggested that the complement cascade's activation could be harmful. Activation of the complement system causes the nanoparticles to penetrate through the systemic circulation and produce hypersensitivity reactions. Instead of subcutaneous or intradermal administration, the activation of the complement system could improve nanovaccine efficiency and remove pathogens from the body. An article reported hypersensitivity reactions of nanoparticles [12]. Also, discovered that the complement system activation caused hypersensitivity [12, 13].

Different types of NPs could stimulate the body's defense system by elevating the immunogens, inflammatory responses, and cytokines and, subsequently, by provoking cytokine and antibody responses. Cytokines could induce cell-mediated immunity to protect the body against several diseases, but the uncontrolled release of cytokines causes harmful side effects [14].

The use of nanovaccines to accelerate the uptake of antigen by dendritic cells is considered a reasonable way for antigen presentation. The targeted delivery of nanovaccine to Antigen-presenting cells (APCs), especially DCs, is employed to stimulate adaptive immune responses and improve antigen intracellular uptake and processing. The use of MHC class I and II molecules for antigen presentation stimulates CD4+ and CD8+ *T*-cell mediated immune reactions [15]. A study reported that a nanoemulsion stimulated Th1 and Th17 cells, which could be significant in vaccination for cellular immunity [16].

Toll-like receptors (TLRs) on immune cells could elicit immune responses specific for each antigen through recognizing TLR ligands in nanoparticles. The use of poly lactic-co-glycolic acid (PLGA) NPs conjugated with modified TLR elicits considerably more responses compared to the use of antigen or antigen + TLR ligands in the absence of PLGA NPs [17]. Carbon nanotubes decrease phagocytosis against *Listeria monocytogenes* [18]. The use of nanotoxoid against pathogenic *Pseudomonas aeruginosa* in a pneumonia model was shown to stimulate robust humoral reactions, particularly phagocytosis [19].

Hepatitis B virus core protein coupled with *Mycobacterium tuberculosis* 10 (CFP-10) antigen was shown to generate a significant antigen-specific immune response against tuberculosis (TB) by Th1 and the secretion of IFN- γ [20].

3. Parameters Affecting Immune Responses against Bacterial Infections

Although the interplay between NPs and the body's defense system is not well understood, their effect is anticipated to be expanded by developing an efficient vaccine formulation against diseases. Nanovaccines characteristics, including chemical composition, particle size, surface charge, hydrophobicity, shape, administration route, and their formulations, determine the type of immune responses elicited (humoral vs. cellular) [6, 9, 21]. These parameters could influence their uptake by cells; in addition, the induced immune reactions are also crucial in dictating cellular fate. Studies on the immune responses elicited by nanovaccines against bacterial infection are mentioned in Table 1.

3.1. Size. The size and adequate control of nanoparticles' size are very influential in the interaction between NPs and the immune system. Several studies have reported that adjuvant nanoparticles size might affect the type of immune responses elicited. Nevertheless, the optimum size of nanovaccines employed against microbial agents is a critical factor [61]. Typically, nanoparticles are defined as particles with a size of

TABLE 1: Applications of nanovaccine against bacterial infection.

Bacteria	Type of antigen in vaccine	Nanoparticle composition	Diseases	Outcomes	Ref.
<i>Mycobacterium tuberculosis</i>	Esat-6 three T cell epitopes (Esat-6/3e) and fms-like tyrosine kinase 3 ligand (FL) genes (termed Esat-6/3e-FL)	Chitosan	Tuberculosis	(i) Eliciting higher levels of IFN- γ and IL-12 (ii) Increasing the expression levels of T-bet mRNA and protein (iii) Inducing a stronger Th1 response (iv) Remarkably increasing immunological and defensive effects (v) Increasing the number of CD3+ cells	[22]
<i>Mycobacterium tuberculosis</i>	Mycobacterium lipids	Chitosan	Tuberculosis	(i) Inducing the production of Th1 and Th2 cytokines (ii) Inducing the production of higher levels of IgG, IgG1, IgG2, and IgM (iii) Remarkably inducing the activation of $\gamma\delta$ -T cells in the lymph nodes (iv) Substantially increasing CD8+ cells rather than CD4+ cells (v) Presence in both the lymph nodes and spleen cells	[23]
<i>Mycobacterium tuberculosis</i>	New DNA plasmid encoding eight HLA-A * 0201-restricted T-cell epitopes	Chitosan	Tuberculosis	(i) Maturation of dendritic cells (DCs) (ii) Inducing the secretion of high levels of IFN- γ by T cells	[24]
<i>Vibrio cholerae</i>	OmpW protein	Chitosan	Cholerae	(i) Increasing IgG and IgA immunoglobulins levels	[25]
<i>Vibrio cholerae</i>	LPS	Chitosan	Cholerae	(i) Inducing high levels of IgG and IgA antibodies in the sera and lavage fluid	[26]
<i>Shigella flexneri</i>	MxiH antigen	Chitosan	Shigellosis	(i) Improving the secretion of IgG and IgA (ii) Increasing the levels of IL-4 and IFN- γ	[27]
Enterohemorrhagic <i>E. coli</i> (EHEC)	rEIT (EspA, Intimin, Tir)	Chitosan	Enterohemorrhagic <i>E. coli</i> (EHEC) infections	(i) Decreasing symptoms associated with EHEC infections (ii) Prompting robust humoral and mucosal immune reactions (iii) Protecting mice from live EHEC O157:H7 (iv) Reducing adhesion properties of <i>E. coli</i> O157:H7 in vitro	[28]
<i>Escherichia coli</i> O157: H7	EspA (E), intimin (I), Tir (T) and Stx2 toxin	Chitosan	Haemorrhagic colitis (HC) and haemorrhagic uremic syndrome (HUS)	(i) Decreasing the adhesion ratio of pre-treated <i>E. coli</i> O157:H7 (ii) Significantly reducing bacterial colonization and excretion (iii) Inducing robust humoral and mucosal responses	[29]

TABLE 1: Continued.

Bacteria	Type of antigen in vaccine	Nanoparticle composition	Diseases	Outcomes	Ref.
<i>Acinetobacter baumannii</i>	Outer membrane protein	Chitosan	Nosocomial infections	(i) Increasing the concentration of cytokines (IL-2, IL-6, IFN- γ) and antibodies titer (ii) Increasing cellular and humoral immunogenicity and stimulating a balanced Th1/Th2 response (iii) Increasing total leukocytes and differential leukocytes	[30]
<i>Campylobacter jejuni</i>	pCAGGS- <i>flaA</i>	Chitosan	Traveller's disease Guillain-Barré syndrome (GBS)	(i) Improving serum levels of anti- <i>C. jejuni</i> IgG and intestinal mucosal IgA antibodies (ii) Declining bacterial expulsion	[31]
<i>Salmonella</i>	Outer membrane proteins (OMPs) and flagellin (F) protein	Chitosan	Salmonellosis	(i) Upregulating TLRs, and Th1 and Th2 cytokines mRNA expression (ii) Improving immune reactions, systemic IgY and mucosal IgA antibodies reaction in chickens (iii) Decreasing problems associated with <i>Salmonella</i> load in vaccinated chickens	[32]
<i>Salmonella enterica</i> serovar <i>enteritidis</i>	Outer membrane proteins (OMP) and flagellin (FLA)	Chitosan	Salmonellosis	(i) Inducing substantial immune responses (ii) Ag-specific splenocyte multiplication (iii) Improving systemic antibody response and the frequency of IFN γ -producing <i>T</i> cells (iv) Upregulating mRNA levels of TLR 2 and TLR 4 as well as IL-4 and IL-10 cytokines	[33]
<i>Chlamydia trachomatis</i>	Recombinant MOMP DNA (DMOMP)	Chitosan	Sexually transmitted infection	(i) Improving in vivo presentation and expression of DMOMP in mice (ii) Stabilizing and protecting against enzymatic digestion	[34]
<i>Clostridium tetani</i>	Tetanus toxoid (TT)	PEG-PLA	Tetanus	(i) Inducing a long-lasting antibody (IgA, IgG levels) response	[35]
<i>Chlamydia trachomatis</i>	A peptide obtained from the recombinant major outer membrane protein (rMOMP) of <i>Chlamydia trachomatis</i>	PLGA	Sexually transmitted disease	(i) Increasing CD4+ and CD8+ <i>T</i> cells number (ii) Inducing higher serum levels of IgG, IgG2a (Th1), and IgG1 (Th2) (iii) Increasing CD4+ and CD8+ <i>T</i> cell subsets number (iv) Inducing more cytokines and chemokines: rMOMP-specific interferon-gamma (Th1) and interleukin (IL)-12p40 (Th1/Th17) than IL-4 and IL-10 (Th2) cytokines	[36]

TABLE 1: Continued.

Bacteria	Type of antigen in vaccine	Nanoparticle composition	Diseases	Outcomes	Ref.
Group A <i>Streptococcus</i>	A vaccine candidate based on lipopeptide (LCP-1)	PLGA	Group A <i>Streptococcus</i> (GAS) infections	(i) Enhancing antigen trapping and APCs (ii) Maturation (iii) Improving the levels of J14-specific salivary mucosal IgA and systemic IgG antibodies titers	[37]
<i>Pseudomonas aeruginosa</i>	Detoxified LPS (D-LPS)	PLGA	Nosocomial infections	(i) Stimulating humoral immune response (ii) Declining the spread and proliferation of bacteria and killing opsonized bacteria	[38]
<i>Bacillus anthracis</i>	Protective antigen domain 4 (PAD4)	PLGA	Anthrax	(i) Increasing IgG1 and IgG2a responses (ii) Producing a Th1/Th2 response and a Th2 response by PAD4 (iii) Producing high levels of IL-4 and IFN- γ	[39]
<i>Brucella melitensis</i>	Oligopolysaccharide (OPS)	PLGA	Fever of Malta	(i) Elevating the total IgG and IgM antibody titers (ii) Stimulating well-ordered opsonophagocytosis of <i>Brucella</i> (iii) Exhibiting a high level of protection in challenge assay compared to other groups	[40]
<i>Pseudomonas aeruginosa</i>	Exotoxin A	PLGA	Cystic fibrosis	(i) Adequate immunogens for inducing humoral and cellular immunogenicity (ii) Reducing bacterial load in the spleens after challenge (iii) Substantially inducing higher secretion of INF- γ , TNF- α , IL-4, and IL-17A cytokines as well as IgG responses	[41]
<i>Brucella abortus</i>	rL7/L12 ribosomal protein	PLGA	Brucellosis	(i) Producing particular <i>Brucella</i> Ag-specific humoral and cellular reactions (ii) Inducing high IgG antibody titers, IgG1 as the predominant subclass (iii) Identifying a mixed Th1/Th2 response based on IgG1/2a ratio (iv) Recording Th1 cytokines particularly IFN- γ (v) Stimulating inflammatory responses necessary for combating <i>Brucella</i> infection (vi) Decreasing CFU of splenic bacteria	[42]
<i>Burkholderia Cenocepacia</i>	OMP antigen derived	Nanoemulsion	Cystic fibrosis	(i) Inducing the secretion of higher levels of IgG and mucosal IgA	[43]

TABLE 1: Continued.

Bacteria	Type of antigen in vaccine	Nanoparticle composition	Diseases	Outcomes	Ref.
<i>Bacillus anthracis</i>	Recombinant <i>Bacillus anthracis</i> protective antigen (rPA)	Nanoemulsion	Anthrax	(i) Secreting high serum levels of antibodies neutralizing lethal toxin in mice and guinea (ii) Elevating the expression of IFN- γ , TNF- α , and IL-2 (iii) rPA-NE based antigen-specific Th1-type polarization of cellular responses (iv) Eliciting high serum levels of anti-PA IgG and bronchial anti-PA IgA. (IgG2a and IgG2b were more than IgG1)	[44]
Group A <i>Streptococcus</i>	Lipid-core peptide-1	Nanoliposome	GAS infections	(i) Inducing higher titers of Ag-specific mucosal IgA and systemic IgG	[45]
<i>Helicobacter pylori</i>	Fusion peptide <i>CtUBE</i> of cholera toxin B subunit and <i>Helicobacter pylori</i> urease B subunit epitope	Liposome	<i>H. pylori</i> infection	(i) Elevating the serum levels of specific anti-urease IgG and mucosal IgA (ii) Elevating the levels of IFN- γ	[46]
Avian pathogenic <i>Escherichia coli</i> (APEC)	Inactivated avian pathogenic <i>E. coli</i> vaccine (APEC)	Liposome	Avian colibacillosis	(i) Elevating mucosal and serum antibodies (ii) Decreasing bacteria in blood	[47]
Group A <i>streptococcus</i>	Lipopeptide	Liposome	GAS infections	(i) Mediating both mucosal and systemic immunity, IgA and IgG (IgG1 and IgG2a) (ii) Inducing prolonged immunity with high levels of antibodies (IgA and IgG) detected even five months' postvaccination	[48]
<i>Yersinia pestis</i>	Formaldehyde-killed whole cell KWC <i>Y. pestis</i>	Liposome	Plague	(i) Increasing IgA and IgG levels in mucosal secretions (ii) Increasing specific cells secreting antibody in the lungs (iii) Increasing Ag-specific proliferative responses and IFN- γ -producing cells (iv) Improving the spleens of mice immunized against an intranasal <i>Y. pestis</i> challenge	[49]
Enterohemorrhagic <i>Escherichia coli</i> (EHEC)	Putative outer membrane protein (<i>LomW</i>) and (<i>EscC</i>) structural type III secretion system protein	Gold nanoparticles	Enterohemorrhagic infection	(i) Elevating IgG and IgA titers in serum and feces, respectively (ii) Decreasing the attachment to human intestinal epithelial cells (iii) Inducing Ag-specific bactericidal properties in serum, engaging the classical complement pathway	[50]
<i>Francisella tularensis</i>	Glycosylated protein complex	Gold nanoparticles	Tularemia	(i) Increasing the protection and high specific antibodies titers	[51]

TABLE 1: Continued.

Bacteria	Type of antigen in vaccine	Nanoparticle composition	Diseases	Outcomes	Ref.
<i>Burkholderia mallei</i>	<i>B. thailandensis</i> E264 lipopolysaccharide (LPS) with <i>Hc</i> fragment of tetanus toxin	Gold nanoparticles	Glanders	(i) Increasing the secretion of IgG1, IgG2a, and IgM in mice combating with <i>B. mallei</i> (ii) Enhancing exposure of LPS to B memory cells	[52]
<i>Clostridium tetani</i>	Tetanus toxoid	Gold nanoparticles	Tetanus	(i) Significantly inducing higher mucosal response following oral administration	[53]
<i>Clostridium botulinum</i>	Recombinant binding domain <i>BoNT/E</i>	Gold nanoparticles	Botulism	(i) Inducing high titers of antibody and immune response	[54]
<i>Pseudomonas aeruginosa</i>	<i>N</i> -terminal domains of <i>P. aeruginosa</i> flagellin	Gold nanoparticles	Nosocomial infections	(i) Eliciting higher titers of anti-flagellin (1–161) antibodies	[55]
<i>Staphylococcus aureus</i>	Bacterial extracellular vesicles (EVs) coating indocyanine green (ICG)	MSN	<i>S. aureus</i> infections	(i) Stimulating DCs maturity and enhancing the proteasome-dependent antigen presenting pathway through simplifying endolysosomal escape, promoting proteasome activity, and upregulating MHC-I expression (ii) Promoting CD8+ <i>T</i> cell responses while promoting CD4+ <i>T</i> cell responses and humoral immunity (iii) Facilitating vaccine delivery from the injection site into lymph nodes	[56]
<i>Vibrio cholerae</i>	Recombinant cholera toxin subunit B	MSN	Cholerae	(i) Significantly inducing mucosal immune responses (ii) Inducing more efficiently whole immune responses in challenge trials	[57]
<i>Mycobacterium avium subsp. paratuberculosis</i>	Whole cell lysate (<i>PAN-Lysate</i>) and culture filtrate (<i>PAN-Cf</i>) of <i>M. paratuberculosis</i>	Polyanhydride nanoparticles (PAN)	Johne's disease (JD)	(i) Elevating the levels of Ag-specific <i>T</i> cell responses postimmunization (ii) Increasing the number of CD8+ <i>T</i> cells secreting cytokines (IFN- γ , IL-2, TNF- α)	[58]
<i>Salmonella enterica</i> serovar <i>enteritidis</i>	Outer membrane proteins (OMPs) and flagellar (<i>F</i>) protein	Polyanhydride nanoparticle (PAN)	Salmonellosis	(i) Eliciting OMPs-specific IgG responses and the production of Th1 cytokine IFN- γ in the serum (ii) Increasing CD8+/CD4+ cell ratio in the spleen, promoting OMPs-specific lymphocyte multiplication (iii) Increasing the genes expression of TLR2 and -4, TGF- β , and IL-4 cytokines	[59]
<i>Streptococcus pneumoniae</i>	Pneumococcal surface protein A (<i>PspA</i>)	Polyanhydride nanoparticle (PAN)	Pneumonia	(i) Inducing an anti- <i>PspA</i> antibody with high titer and high avidity	[60]

10–100 nm and in some cases, up to 1,000 nm, which are used in nanovaccine design. It has been reported that the smaller their particle size, the greater their efficiency in passing antigens through existing barriers. The transdermal

route of administration is considered as a practical choice for immunization. The ability of nanoparticles to cross physiological barriers is related to their particle size. Small NPs may translocate more easily through the lymphatics and

assemble in dendritic cells in the lymph nodes (LNs) compared to larger NPs. Generally, because of their size similarity to pathogens, NPs could be readily identified and efficiently captured by cells via the cellular endocytosis process.

Several studies have reported that only 40 nm particles could deeply cross epidermal barriers and interact with APCs such as epidermal Langerhans cells [8]. Particles smaller than 500 nm (mainly 40–50 nm) are better cleared from the bloodstream and could better cross the extracellular matrix and reach the lymph nodes. These particles are taken up by DCs (cells recognizing, trapping, and presenting antigens) to activate the CD8+ T cell-mediated (cellular) immune response. Nanoparticles larger than 500 nm induce CD4+ T cell-mediated (humoral) immune response. Hence, the optimum size of nanoparticle in nanovaccines for eliciting proper immunity has been reported to be from 40 to 50 nm [7].

The size and distribution of NPs are effective factors in their uptake by APCs. Nanoparticles with 10–100 nm size are better taken up by DCs to provoke immunity, while larger NPs (>100 nm) are scavenged by macrophages, and smaller NPs (<10 nm) are evacuated into the blood capillaries [10].

Some nanoparticles by themselves are able to directly stimulate the secretion of cytokines by immune cells. Nanoparticle size has been reported to be a factor directly affecting the secretion of various cytokines by immune cells. Several studies have shown the effects of the smaller size of nanoparticles as adjuvants on stimulating higher antibody (IgG, IgG1, IgG2a, IgA, and IFN- γ) responses compared to larger nanoparticles [62–64].

Several studies have tried to explore the association between particle size and immune reactions, reporting equal efficiency for nano- and micro-sized particles. A study appraised the effect of physical features of particulate vaccine carriers on stimulating immune responses, including size and shape. The results showed the effect of NPs size and shape on stimulating immune reactions associated with total IgG antibody titer and subtypes of Th1 and Th2. Small particles (193 nm) with spherical shape elicited strong Th1 and Th2 mediated reactions compared to other particles; also, they elicited a Th1-biased reaction and significantly elevated the levels of Th1 cytokine (IFN- γ) as well as IgG total, IgG-1, and IgG-2a antibodies, while rod-shaped particles (1530 nm in length) elicited a Th2-biased reaction against ovalbumin (OVA). On the other hand, larger rod-shaped NPs increase slightly more potent IgG-total and IgG-1 antibodies and lowered IgG-2a titers compared to smaller rods with no significant difference in IgG-2a counts [21]. These results suggested that 40 nm gold nanoparticles (AuNPs), 20 nm AuNPs, and rod-shaped NPs (40 nm \times 10 nm) were more effective in inducing antibody responses and APCs uptake compared to other NPs types [65].

Jiang et al. proved that engineered NPs of particular sizes and gold-silver NPs covered by antibodies could selectively induce the activation of membrane receptors. The results showed that nanoparticles with 2–100 nm size could alter signaling methods; for example, cell death and 40–50 nm

nanoparticles induced the most significant effect. These findings indicate that stimulation of membrane receptors and, following that, protein diffusion is influenced by NPs size [66].

A review study reported that the cytotoxicity and differences in the kind of cell death pathway of modified gold NPs depended on their size. Also, 1.4 nm NPs caused rapid cell death by necrosis within 12 h. In contrast, 1.2 nm NPs caused programmed cell death by apoptosis, and 1–2 nm NPs were highly toxic compared to smaller or larger ones [67]. The results of another study showed that cellular uptake of NPs was wrapped by cell membrane tension with different degrees of wrapping, and some were endocytosed under the influence of particle size. The optimal NPs size for endocytosis is 25–30 nm [68]. Elbakry et al. demonstrated that changes in the NPs size could cause significant changes in the number of antigens delivered per cell. They revealed that less internalization was done with increasing size [69]. Another study found that uptake of silver NPs and quantum dots by APCs stimulated the secretion of inflammatory mediators regardless of the particle size, including TNF- α , MIP-2, and IL-1 β [61]. Morishige et al. demonstrated that silica NPs size could affect inflammatory responses [70]. They reported that outer membranes of *Escherichia coli* and 30 nm gold NPs caused rapid stimulation and maturation of dendritic cells (DCs) in the lymph nodes; as a result, immunization using these NPs induced long-term and robust antibody responses [71].

The results of the aforementioned studies show the important role of size in modulating immune responses and subsequently in the design of nanovaccines. It is a factor that should be considered in the development of efficient nanovaccines to induce more potent immune responses. However, reports on the association of particle size and immune responses are contradictory in comparison with the impressive results on the usefulness of particles' small size in inducing immune responses. It is recommended that the association between particle size and both humoral and cellular immune responses induced by NPs be comprehensively evaluated in future studies.

3.2. Surface Charge. Surface charge is another factor that plays a vital role in the interaction of NPs with immune cells. The cellular uptake of NPs is significantly affected by their surface charge. The surface charge of NPs could stimulate their interaction with cells and cellular uptake; nanoparticles could interact nonspecifically with oppositely charged binding cells, while nanoparticles without charge may not be able to interact with nonspecific cells. Interestingly, the positive surface charge of NPs increases their cellular uptake compared to neutral or negative surface charge [72]. It has been found that NPs positive surface charge is one of the parameters affecting their accumulation in the bloodstream. It induces hemolysis and platelet aggregation compared to the neutral or negative surface charge of nanoparticles [73].

NPs surface charge plays a role in elevating the expression of DCs surface markers, eliciting the secretion of cytokines, and stimulating inflammatory responses. Few

reports have suggested that positively charged NPs are more probable to cause inflammatory reactions [74, 75]. It has been speculated that NPs positive surface charge is crucial for the activation of the complement system [6]. Negatively charged AuNPs have been shown to induce higher cytotoxicity and immunological responses than AgNPs. Perhaps anionic AuNPs may more easily adsorb nonspecific serum proteins on their surface and penetrate cells through the endocytotic pathway [76]. In a study, pulmonary immunization with ovalbumin (OVA) incorporated NPs with positive charge induced strong antigen-specific *T*-cell multiplication and enhanced the expression of surface MHCII and cytokines compared to anionic nanoparticles or soluble OVA. These findings showed the significance of the surface charge of NPs used in nanovaccines against pulmonary diseases [77].

To design innovative nanocarriers as vaccine delivery systems, it should be taken into account that physical attributes, such as particle shape and size, affect the interaction of NPs with immune cells. An investigation showed that negative NPs were ideal carriers and could be used as a transdermal drug delivery carrier in the design of vaccines [78].

3.3. Shape. The shape of nanoparticles is another factor affecting the stimulation of immune responses. If the nanoparticle is spherical, phagocytosis happens more efficiently compared to when nanoparticles have other shapes such as disc, rod, or elliptical shapes [6]. Some studies have shown that NPs shape significantly affects the interplay between NPs and immune cells, such as macrophages, complement proteins, DCs, and *T*-cells [79].

Kumar et al. showed that small particles (193 nm in diameter) with spherical shape induced a Th1-biased immune reaction, while rod-shaped particles (1530 nm in length) induced a Th2-biased immune reaction against OVA [21]. In the design of nanoparticles for use in nanovaccine, it should be considered that the shape of NPs plays an essential role in their interaction with APCs [5]. A study found that the uptake of nanorods by macrophages occurred more efficiently than that of nanospheres [80].

The effect of NPs shape on the stimulation of the innate immune system has also been proven [81]. Therefore, for the design of nanovaccines, it should also be considered.

3.4. Hydrophobicity. The effect of hydrophobicity on the stimulation of immune responses has been reported in some studies [82]. An increase in the hydrophobicity of polymer nanoparticles causes higher levels of expression of costimulatory markers (CD86) in DCs [83]. Several studies have reported that hydrophobic particles compared to hydrophilic ones, display higher immune responses [1]. In a study, hydrophobicity was shown to have no significant effect on the cellular uptake of nanoparticles. In contrast, higher levels of hydrophobicity caused cell membrane damage and induced autophagy [84]. Moyano et al. demonstrated a direct association between hydrophobicity and immune responses [82]. Hydrophobic poly (styrene) nanoparticles are highly

taken up by the M-cells surface of the Peyer's patches and promote opsonization by APCs [85].

Therefore, according to some studies on the design of nanovaccines, hydrophobicity has a vital role in nanoparticles absorption and immune response induction.

3.5. Formulations of Nanovaccines. In nanovaccine formulations to strengthen the immune system, the association between antigen and nanoparticle has a fundamental role. The binding of NPs to antigens could be achieved via different ways such as chemical connection/conjugation, simple mixing, encapsulation, or adsorption. In the encapsulation method, both antigens and NPs precursors are blended with each other during the synthesis; in the chemical conjugation method, a chemical connection is created between antigen and NPs surface. These two methods are used to provide a robust interplay between antigen and nanoparticle. Immunization with encapsulated nanoparticles is done effortlessly because of the protection of antigen against enzymatic and hydrolytic degradation. It has been found to be efficient with a single dose due to the slow release of antigen. The physical adsorption method creates a weak link between NPs and antigens and requires a simple chemical conjugation to prevent rapid separation of antigens from NPs *in vivo* [1]. In simple mixing, a minimum link is created between NPs and antigens before being given to the host [86]. A study showed that chemical conjugation was more useful for peptide antigen delivery than simple mixing due to the induction of higher systemic and mucosal antibody titers [87].

Mesoporous silica nanoparticles (MSNs) are recognized as a novel carrier that ensures the controlled release of vaccine while maintaining chemical stability [88].

3.6. Route. Administration routes of NPs could affect the induction of various immune responses. The choice of NPs administration route quantitatively and qualitatively affects the robustness of immune responses. Many of the nanovaccines are noninvasive and multiinjection [89]. NPs administration routes include oral, nasal, intramuscular, subcutaneous, transmucosal, ocular, pulmonary, intravenous, vaginal, and patches. In intravenous administration, nanoparticles could be captured by circulating macrophages or hepatic macrophages known as Kupffer cells. In oral administration, NPs are captured through GIT (gastrointestinal tract) by intestinal epithelial cells in the mucosa, M-cells, and cells of the Peyer's patches; small NPs are better taken up compared to large ones, but a high concentration is required for a vaccine to achieve adequate efficacy. The intradermal administration is a possible choice for vaccinations that require the antigen to be released in a controlled and gentle manner from the formulated nanoparticle, resulting in a long-lasting effect. The ability of NPs to cross the epidermal barrier is related to their particle size [8, 9]. Newman et al. proved that particles of similar size, administered through various routes, were captured by diverse APCs, resulting in the induction of various immune responses [86].

The intramuscular administration could be performed indirectly on dendritic cells. Several studies have reported that PEGylation of liposomes could increase the amount of lymphatic uptake in LNs in large quantities via the subcutaneous administration route compared to other routes [9].

The use of patches is a noninvasive approach. They could be delivered to the follicles of hair via the skin to reach DCs and then to LNs to provoke humoral and cellular immune responses. A study reported that intranasal administration of nanovaccine induced a more robust CD8+ T cell-mediated response than intraperitoneal administration [11].

It has been reported that after subcutaneous administration, the amount of small liposomes coated by PEG reaches the highest levels in LNs compared to intravenous (iv) and intraperitoneal (IP) administration [90].

A study reported that the immune effects of hypersensitivity responses of Titanium dioxide nanoparticles (TiO₂ NPs) might be observed following parenteral or dermal administration compared to oral gavage [91]. The results of a review study indicated that oral administration of Silver Nanoparticle (AgNP) caused changes in microbiota and modulated immunological homeostasis of the intestine and gut tract [92]. The results of another study showed the stimulation of cytokine secretion following oral administration of Poly Lactic-Co-Glycolic Acid (PLGA) NPs [93]. One study demonstrated that PLGA NPs coupled with OVA + monophosphoryl lipid A (MPLA) elicited high IgA and IgG titers [94]. The results of a study showed that polyethyleneglycol-poly (lactic) acid (PEG-PLA) nanoparticles could cross the rat nasal epithelium and produce high antibody levels as well as long-term immunity [95]. In an investigation, mice were immunized via oral, nasal, and intraperitoneal administration routes with tetanus toxoid (TT NPs) loaded by adsorption. Oral and nasal administration increased serum levels of IgG and IgA antibodies. In this review, nasal administration was more useful for vaccination in mice than the oral one. Nasal administration induced higher antibody levels with lower doses [96]. Intravenous administration of Ag-NPs induces suppression of immune responses [97]. Conway et al. reported that oral administration of (pertussis toxoid) PTd + (filamentous haemagglutinin) FHA-encapsulated PLGA NPs induced Th2 cell-mediated and antibody responses against a respiratory disease caused by *Bordetella pertussis* [98].

Intramuscular and subcutaneous administration of zein nanoparticles induced a Th2-biased immune response and induced high IgG antibody titers. IgG antibody was more produced via intramuscular injection than via subcutaneous injection [99]. A study reported that the mixture of N-trimethyl chitosan (TMC/antigen) and THC nanoparticles as an immune potentiator could induce immune responses and DCs maturation after intradermal administration. Another study reported that thiolated N-Trimethyl Chitosan-Hyaluronic acid (TMC/HA) NPs filled with OVA enhanced serum level of IgG after subcutaneous administration [100].

3.7. Types of Nanoparticles against Bacterial Infections. Synthetic Polymers, Inorganic nanoparticles, Copolymer Micelles, Liposomes, Emulsions, Metals, and Immune Stimulating Complexes (ISCOMs) are promising nanoparticle technology that could cause strong immune responses [9].

Liposomes are considered one of the most common nanocarriers studied for drug delivery/nanovaccines against infectious diseases, such as tuberculosis. They are able to optimize humoral and cellular immunity, specifically by monocytes and macrophages, by triggering innate immunity via uptake and stimulating APCs and subsequently by triggering adaptive immunity. Liposome formulations increase potency and reduce toxicity; they are suitable delivery carriers for nanovaccines since they could entrap somewhat significant amounts of antigen payload and could be used to overcome the reduced immunogenicity of subunit vaccines. Besides, liposomes are composed of unsaturated and saturated lipids, which prompt Th2 and Th1 cell-mediated immune responses. The unique feature of liposomes is the association of liposomal size with immune reactions. In general, 100 nm liposomes induce the activation of Th2 cell-mediated reactions and higher interferon γ (IFN- γ) levels and IgG2a/IgG1 ratios compared to liposomes with 400 nm size or more, inducing the activation of Th1 cell-mediated reactions [101, 102].

OVA-encapsulated galactosylated liposomes as delivery carriers considerably elicit higher levels of IgA and IgG antibodies after mucosal administration. Various types of liposomes include conventional liposomes, surface-modified liposomes, immunoliposomes, cationic liposomes, bilosomes, virosomes, niosomes, and pH-sensitive fusogenic liposomes. Conventional liposomes are a weak nanovaccine delivery system because of the drawback of instability in plasma. Immunoliposomes with surface-linked antibodies make it possible to present encapsulated vaccines to the target immune cells. Cationic liposomes are composed of cationic lipids. The interaction between positively charged liposomes and negatively charged APCs membranes causes liposomes to be more readily taken up by cells and allows a long-term antigen uptake by augmenting the contact between ligands and liposomes surface. This phenomenon is considered one of the advantages of using cationic liposomes as vaccine carriers. Many studies have reported that cationic liposomes induce more durable antigen-specific immune responses than other liposomes. Cationic liposomes elicit cytokine production and upregulate DCs surface markers expression. The cationic liposomes as nanocarriers have been shown to boost Th1 and TH17-mediated immune responses. They process antigen via antigen presentation pathway MHC class I and endocytic escape property. Induced secretion of cytokines by cationic liposomes has also been reported to increase inflammatory responses [101].

In a study, the impact of liposomes charge on the efficiency and depot effect of vaccines was evaluated after intramuscular administration. In this study, Ag85B-ESAT-6 and CTH1 protein antigens were encapsulated in liposomes for *M. tuberculosis* (TB disease) and *Chlamydia*. Cationic liposomes allowed long-term trapping of antigens by APCs

compared to neutral ones. They also elicited Th17 (IL-17) mediated responses and CD4+ T mediated coexpression of IFN- γ and TNF- α , leading to a remarkable decline in infection [102].

Cationic liposomes provoke Th1 (IFN- γ) and Th17 (IL-17) mediated immunity. pH-sensitive fusogenic liposomes are stable at pH 7.4 with the ability to fuse their membranes with APCs membranes. They initiate to mix the lipids of both membranes, transport the antigens directly into the cells' cytosols via MHC-I presentation pathway, and begin intracellular processes. These nanoparticles could protect entrapped antigens against the extracellular environment [103]. Watarai et al. found immune responses in chickens after intraocular administration of 3-methylglutaryl-ated poly (glycidol) (MGLuPG)-modified pH-sensitive fusogenic polymer liposomes coupled with *Salmonella enteritidis* antigen. In this study, a remarkable antigen-specific preventive immunity was found in the chicken sera and intestines [104]. Bilosomes are nonionic surfactant vesicles for oral immunization, which could incorporate bile salts in their formulation. Bilosomes used as adjuvants increase immunogenicity significantly. Bilosomes formulations could substantially increase the amount of antigen presented to the Peyer's patches and mesenteric LNs and elicit a long-lasting immunity against fatal diseases compared to free antigen. A study showed that bilosomes could become constant antigens of tetanus toxoid (TT), diphtheria toxoid [105]. An optimized bilosome formulation was shown to promote antigen uptake in the Peyer's patches. The results showed that bilosomes as adjuvants significantly increased immunogenicity through drug delivery, for example, bovine serum albumin (BSA) and cholera toxin subunit B [106].

Niosomes are surfactant-based vesicles similar to liposomes, which possess nonionic amphiphiles into closed bilayers, promoting the effectiveness of vaccines with a constant and higher stimulatory effect on antibodies and lymphoid cells. It has been reported that the use of lipopolysaccharides (LPS) into PLGA NPs loaded with antigen increases LPS-induced NALP3 inflammasome. They also lead to Caspase 1 activation to improve proinflammatory cytokine IL-1 β secretion [107]. In some studies, chitosan NPs have been presented to enhance neutrophils and macrophages' performance and elicit cytokine secretion [70]. A study showed that the interaction of amorphous silica NPs with endothelial cells causes monocytes to adhere to endothelial cells, beginning with inflammatory processes [108]. Babin et al. concluded that TiO₂ induced the degranulation of neutrophils and subsequently the secretion of various proteins [109]. A study demonstrated that rapamycin (RPM)-loaded PLGA NPs reduced the expression of ICAM-1 (intercellular adhesion molecule 1) in dendritic cells. This nanoparticle displayed an immunosuppressive response in the body [110]. DNA-based chitosan nanovaccines were shown to significantly induce immunologic effects such as Th1 reactions and CTL (Cytotoxic T lymphocyte)-mediated immune responses in mice (Mtb H37Rv) with *M. tuberculosis* [22]. Wang et al. showed that oral administration of BSA-loaded carbon NPs significantly

induced mucosal IgA antibody reactions and Th1 and Th2 mediated reactions [111]. A review study showed that metallic NPs as adjuvants induced cellular immune responses, especially Th1 and Th17 [112]. The use of outer membranes of *E. coli* and gold NPs (BM-AuNPs) was also shown to significantly induce specific T-cell responses (Th1 and Th17) and higher production of IFN- γ and IL-17 against bacterial infection [71].

These nanoparticles generate immune responses through activating immune cells (such as TH1 and TH2) and inducing cellular responses, as well as through inducing the production of antibodies (such as IgA and IgG) and inducing antibody responses; however, their mechanisms are different depending on the formulation and method of nanoparticles release.

4. Conclusion

In the last decades, nanovaccine has been shown to play an important role in promoting human physical abilities by interacting with the immune system components in different ways; however, these interactions are not well understood. Studies have shown that the possibility of interaction between NPs and different components of the immune system is very high. These interplays could activate or disrupt the performance of the immune system or may lead to unpredictable alterations in the function of various immune cells. It has been well documented that NPs properties (size, shape, hydrophobicity, and surface charge), the type of nanovaccine, and the route of administration are important factors that significantly affect the interaction between NPs and immune cells and the type of immune responses elicited against bacterial infections. Therefore, more research is recommended to be performed to know other important parameters, the ways NPs interplay with the immune system, and how to develop novel approaches for enhancing the efficacy and safety of vaccination. New strategies are urgently needed to struggle with bacterial infections in developing countries, from evolution to immunity. Limited applications of existing therapeutic methods and the evolution of coinfections and resistant pathogens highlight the need for advanced vaccination strategies. These problems could be eliminated by designing and engineering new vaccines. Future research should focus on determining the mechanisms and the ways NPs interact with various components of the immune system. Herein, nanoparticles' characteristics affecting their interactions with the immune system were discussed, especially against bacterial diseases. Obtaining new information about the interplay between NPs and the immune system makes it possible to design novel vaccines with the ability to efficiently modulate immune responses.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

SB-J and BB drafted the manuscript. SB-J and BB contributed to data interpretation. SB-J and BB discussed and revised the manuscript. All authors reviewed the manuscript. All authors read and approved the final manuscript.

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