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Review

Perspectives on novel vaccine development

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Abstract

Vaccination is a common routine for prevention and control of human and animal diseases by inducing antibody responses and cell-mediated immunity in the body. Through vaccinations, smallpox and some other diseases have been eradicated in the past few years. The use of a pathogen itself or a subunit domain of a protein antigen as immunogens lays the basis for traditional vaccine development. But there are more and more newly emerged pathogens which have experienced antigenic drift or shift under antibody selective pressures, rendering vaccine-induced immunity ineffective. In addition, vaccine development has been hampered due to problems including difficulties in isolation and culture of certain pathogens and the antibody-dependent enhancement of viral infection (ADE). How to induce strong antibody responses, especially neutralizing antibody responses, and robust cell-mediated immune responses is tricky. Here we review the progress in vaccine development from traditional vaccine design to reverse vaccinology and structural vaccinology and present with some helpful perspectives on developing novel vaccines.

Key words: vaccination, vaccinology, novel vaccine development, perspectives

Introduction

Vaccines are used to prevent bacterial and viral diseases through induction of antibodies produced by B lymphocytes and cell-mediated immunity conferred by $CD8^+$ T lymphocytes. Traditional vaccination involves attenuation of pathogens *in vitro* and administration of the attenuated pathogen into human or animal body. With the development of gene engineering, subunit vaccines and DNA vaccines are produced using recombinant proteins expressed *in vitro* or eukaryotic plasmid for protein expression *in vivo*, respectively (Pulendran and Ahmed 2011). Nowadays, as more and more genome information becomes available, reverse vaccinology has been put forward and tested utilizing in silico analysis for selecting an appropriate gene encoding a protein that could be chosen as a potential candidate to induce a protective immunity (Seib et al. 2012, Kanampalliwar et al. 2013). All these strategies in vaccine development attempt to induce protective immune responses with protein antigens. But proteins are complex macromolecules with four structural levels of organization. Misfolded protein would fail to induce the production of an effective immune response. Hence, the needs to experimentally determine the structures of proteins give rise to structural vaccinology, which crys-

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talizes protein structure using X-ray diffraction and nuclear magnetic resonance spectroscopy. More precisely, the basic determinants for inducing an immune response are epitopes (also named antigenic determinants), which bind receptors expressed on B cells (BCR) or T cells (TCR). For the effective induction of an immune response, it is essential for an antigen to contain multiple epitopes that bind B cells, helper T cells, and $CD8^+$ T cells. The epitopes for binding with these cells are referred to as B cell epitope, T-helper epitopes (Th epitopes), and CTL epitopes. Several studies have shown that cross-linking and multivalent display of these epitopes to B cells and T cells could significantly augment the production of a robust protective immune response (Dormitzer et al. 2008, Nuccitelli et al. 2011, Xuan et al. 2011). By presenting conserved epitopes orientationally displayed on a protein scaffold, broadly neutralizing antibodies against HIV and Influenza virus have been produced (Kanekiyo et al. 2013, Thomas and Luxon 2013). Here, we reviewed the development of vaccines from traditional vaccine design to reserve vaccinology and structural vaccinology and presented with our perspectives on novel vaccine development with an emphasis on high-density surface display and immune cell-targeted presentation of B cell epitopes, Th epitopes and CTL epitopes.

Traditional vaccinology

The practice of introducing foreign substances into human body for potential protection from pathogen invasion initiated when ancient Chinese tried to intranasally breathe scab powder from mild cases of smallpox infection into kids. This technique substantially reduced morbidity from smallpox infection and spread westwards to Europe where Edward Jenner modified this approach by using cowpox (a disease similar to smallpox, but much less virulent) as a substitution for prevention of smallpox. Afterwards, Louis Pasteur suggested the principle of isolation, inactivation, and injection of the causative microorganism for vaccinations (Serruto and Rappuoli 2006). Pasteur's principles guided the development of many vaccines including killed and live attenuated polio vaccines, MMR vaccine (Measles, mumps, and rubella), and vaccines against diphtheria, tetanus, *Neisseria meningitidis*, and *Streptococcus pneumoniae* (Serruto and Rappuoli 2006, Nossal 2011, Hajj Hussein et al. 2015). Pasteur's principles of vaccination allowed the control and elimination of some of the most devastating infectious diseases such as smallpox. But they are not applicable for developing vaccines against microorganisms that are hard to cultivate *in vitro* or with no obvious protective antigens (Kanampalliwar et al. 2013). In addition, the Pasteur's approach need a long time period during which circulating microorganisms may experience antigenic drift or shift that make them different from the seed microorganism used in vaccine formulation.

Reverse vaccinology

Reverse vaccinology refers to the technique of developing vaccines based on genomic or proteomic information for identification of surface-exposed proteins rather than the direct cultivation of the causative microorganisms *in vitro* (Sette and Rappuoli 2010). Vaccine candidates identified from a pathogen's genome or proteome can then be expressed as recombinant proteins and tested in appropriate *in vitro* or *in vivo* models to assess immunogenicity and immunoprotection (Seib et al. 2012). The effectiveness of reverse vaccinology was firstly evidenced by the development of a universal vaccine against serogroup B *Neisseria meningitidis* (Men B). Starting from the complete genome sequence of Men B, bioinformatics algorithms allowed the prediction of 570 ORFs expressing putative surface-exposed or secreted proteins, 350 of which were expressed in *Escherichia coli* (*E. coli*) and tested for their ability to elicit protective immunity in mice. 28 novel protective antigens were identified using this approach in less than 18 months, surpassing the total number of vaccine candidates identified over the past 40 years by traditional methods (Pizza et al. 2000).

In addition, reverse vaccinology has allowed genome-wide screening of novel vaccine candidates by performing multi-strain genome analyses of different isolates or pathogenic and non-pathogenic strains within the same species or between closely-related species (Tettelin et al. 2005, Lefebure and Stanhope 2007). Multiple genome analysis of Group B *Streptococcus* (GBS) strains identified a core genome shared by all strains and a dispensable genome absent in one or more strains (Tettelin et al. 2005). After testing 589 putative surface-exposed proteins using a mouse maternal immunization/neonatal pup challenge model, four novel protein candidates were identified to be protective, one from the core genome, the other three from the dispensable genome (Maione et al. 2005). Compared with the empirical screening of a few candidates by traditional vaccinology, reverse vaccinology has revolutionized the identification of vaccine candidates, mainly protein antigens, at a time (Donati and Rappuoli 2013, Kulp and Schief 2013). But it is hard for reverse vaccinology to identify other active vaccine compounds including detoxified toxins, lipids and polysaccharides, which often constitute protective antigens of bacterial pathogens.

Structural vaccinology

Using X-ray crystallography and NMR spectroscopy, structural vaccinology allows the atomic resolution of the structures of potential antigens and, through the structure, the rational design of target epitopes to use as vaccine candidates (Serruto and Rappuoli 2006). Nuccitelli et al. (2011) successfully applied structural vaccinology to design a fully synthetic protein with multivalent protection activity against GBS, a microorganism that is classified into 10 capsular polysaccharide serotypes because of the unique antigenicity and structure (Maione et al. 2005). Extensive analysis showed that all GBS strains have one or more of the three pilus islands, PI-1, PI-2a, and PI-2b. The backbone proteins (BP, also named shaft-forming subunit) encoded by pilus island PI-1 and PI-2b were able to induce pilus island-specific protection. But BP-2a encoded by PI-2a had six variants, which only induced variant-specific protection. Nuccitelli et al. (2011) determined the three-dimensional structure of one of the six BP-2a and found that domain 3 (D3) induced protective immunity in mice against lethal challenge with the corresponding GBS strains. Hence, D3 domains from each GBS BP-2a variant were fused into a recombinant construct and expressed in *E. coli*, which could provide protection to all strains expressing a BP-2a variant. The work greatly facilitated the development of a broadly protective pilus-based vaccine against GBS and provided a template procedure to develop vaccines against other bacterial pathogens.

Besides identification of protective domains from the antigen side, structural vaccinology starts to use broadly neutralizing antibodies (bNAbs) isolated from infected patients or animal models to design epitope-scaffold immunogens that can accurately mimic the viral epitope structure and induce potent neutralizing antibodies. These epitope-scaffold immunogens show high binding affinity with bNAbs *in vitro* and are efficient at eliciting the production of bNAbs *in vivo*. Using the crystal structure of a neutralizing antibody motavizumab (mota) in complex with its epitope from the respiratory syncytial virus (RSV) fusion (F) glycoprotein, Correia et al. (2014) designed epitope-scaffold immunogens with a helix-turn-helix conformation using a computational method which allowed *de novo* folding and design of scaffold proteins stabilizing the functional motifs of the identified epitope. The designed epitope scaffold immunogens all had high binding affinity with mota and immunization of macaques with multivalent particles of these immunogens induced neutralizing antibodies with titers comparable to those induced by natural human infection. More surprisingly, macaque monoclonal antibodies (17-HD9 and 31-HG7) possessed neutralization potencies similar to mota and structurally targeted the predefined epitope with high precision.

Perspectives on novel vaccine design

The induction of neutralizing antibody responses and robust cytotoxic T lymphocyte (CTL) responses depends on the effective presentation of B cell epitopes or T cell epitopes to B cells and T cells. Epitopes, either linear or conformational, are the target for binding to BCR (B-cell receptors), TCR (T-cell receptors), antibodies and cytotoxic T lymphocytes. The identification of B-cell epitopes that induce production of neutralizing antibodies, Th epitopes and CTL epitopes lays the basis for developing novel vaccines. We hypothesize that immunogens containing the appropriate assembly of these epitopes should be highly efficient at inducing protective immune responses. These immunogens can be prepared by many expression systems including *E. coli*, yeast cells, insect cells, mammalian cells, and Drosophila S2 cells, and purified through Ni-NTA affinity chromatography, ion exchange chromatography, and size exclusion chromatography. They can also be prepared by chemical conjugation of the epitopes to scaffold proteins which have been trimmed to be non-immunogenic in host body. High-density surface display and immune cell-targeted delivery of these immunogens are two ways that can be manipulated to accurately initiate immune responses and induce protective immunity.

High-density surface display of epitopes

Precise initiation of B cell and T cell activation is the key for development of novel vaccines and production of effective immune responses. B cells are activated after binding extracellular pathogens through BCR. Epitopes cross-linked or displayed with high-density on the surface of the immunogens can efficiently stimulate the activation of B cells and promote antibody responses. Surface-display of multiple epitopes using virus-like particles or nanoparticles with pathogen-mimicking features can greatly help improve immunogenicity of antigens and facilitate BCR recognition and B cell activation. Kanekiyo et al. (2013) fused the viral haemagglutinin of A/New Caledonia/20/1999 (H1N1) with ferritin to form nanoparticles composed of 24 identical polypeptides. Immunization with this influenza nanoparticle vaccine elicited both haemagglutination inhibitory antibodies and neutralizing antibodies, and protected ferrets from an unmatched 2007 H1N1 virus challenge. Moon et al. (2012) produced lipid-envel-

oped PLGA nanoparticles displaying a malaria antigen and elicited antibodies with significantly higher titers and more balanced Th1/Th2 responses against Plasmodium vivax compared with soluble protein mixed with adjuvant. Ding et al. (2017) conjugated the capsid protein of porcine circovirus type 2 onto gold nanoparticles which rendered the neutralizing epitopes exposed on the outer surface and a decoy epitope buried within the inner surface. Immunization of mice with this nanoparticle-based vaccine induced the production of significantly higher levels of neutralizing antibodies and activation of $CD8^+$ T cell responses.

T cell epitopes including Th epitopes and CTL epitope are short peptides derived from intracellular protein degradation and presented in complex with a diverse array of MHC molecules onto the surface of dendritic cells and macrophages. Peptides derived from extracellular antigens are presented to $CD4^+$ T cells in complex with MHC-II molecules, while peptides derived from intracellular antigens are presented to $CD8^+$ T cells in complex with MHC-I molecules. In addition, peptides from extracellular antigens can be presented to $CD8^+$ T cells in complex with MHC-I molecules, a process called cross-presentation which is necessary for elicitation of cytotoxic T lymphocyte (CTL) responses (Cintolo et al. 2012). Although the accurate use of T cell epitopes is critical to elicit an appropriate immune response and the treatment of allergy and tumor, efforts now are mainly focused on the prediction and characterization of T cell epitopes due to the fact that multiple factors determine the ability of a peptide to elicit a T cell response (Trolle and Nielsen 2014, Oyarzun and Kobe 2015, Prickett et al. 2015).

Immune cell-targeted vaccine delivery

For the effective induction of immune responses, antigens need to be trapped and processed initially by dendritic cells and macrophages. These immune cells reside mainly in secondary immune organs including the spleen and the lymph node. Vaccines fused with moieties for binding with these immune cells can be easily recognized and processed because of their preferential accumulation at immune organs. Vaccines targeting Peyer's patch M cells and dendritic cells have been developed and tested in terms of immunogenicity and induction of protective $CD8^+$ T-cell responses and anti-tumor immunity. Being needle-free, delivery of vaccines via the mucosal routes allows antigens to interact with the mucosa-associated lymphoid tissue (MALT) to induce IgA response, systemic IgG response, and CTL response which might work together to clear pathogens invading the body via mucosal surfaces (Shakya et al. 2016). Shima et al. (2014) developed an effective antigen-delivering protein, anti-GP2-Streptavidin, which can carry biotinylated antigens to M cells by targeting GP2, a specific antigen-uptake receptor expressed on M cells. Immunization of mice with biotinylated *Salmonella enterica* serovar Typhimurium lysate conjugated with anti-GP2-Streptavidin induced *S.* typhimurium-specific fecal IgA response and protected mice from virulent *S.* typhimurium infection. Although M cell-targeting can be achieved using M-cell-specific lectins, microbial adhesins or immunoglobulins, there are challenges in the delivery of antigens into the mucosa including low pH, gastric enzymes, and the fact that M cells occupy only 10% of the intestinal lymphoid follicle surface area in humans and mice (Vyas and Gupta 2007, Jung et al. 2010, Kim et al. 2010).

Dendritic cells are the most efficient antigen-presenting cells and initiators of immune responses which activate pathogen-specific T and B lymphocytes and cells of the innate immune system (Martinon-Ego and Berthier 2000, Buckwalter and Albert 2009). Fossum et al. (2015) targeted antigens to the chemokine receptor Xcr1 using the only known chemokine ligand for Xcr1, Xcl1, and showed that Xcl1-HA fusion vaccines enhanced T-cell responses and mediated full protection against viral challenge. Besides, DC-targeted vaccines have been developed as immunotherapeutics against cancer, autoimmune diseases and infectious diseases, all of which require T cell immunity (Chen et al. 2016). However, the successful development of DC-based vaccines still needs to consider more factors that may limit their use in clinical trials including lineage, activation state and expression of specific markers in most common species.

ADE versus ADCC

Besides neutralization, antibodies, whether neutralizing or not, can eliminate antibody-coated pathogens through antibody-dependent cell-mediated cytotoxicity (ADCC) performed by $CD8^+$ T cells and NK cells, and through opsonization performed by phagocytes. Both ADCC and opsonization contribute to host defense against microbial infections. However, antibody responses against certain determinants of some viruses can actually facilitate viral infection and exacerbate disease, a phenomenon known as antibody-dependent enhancement of viral infection (ADE) (Morens et al. 1987, Osiowy et al. 1994, Qiao et al. 2011). ADE is a main obstacle for developing vaccines against viruses including dengue virus, yellow fever virus, respiratory syncytial virus, and porcine reproductive and respirato-

ry syndrome virus (Morens et al. 1987, Osiowy et al. 1994, Yoon et al. 1996). These viruses preferably choose to replicate in macrophages and other immune cells, and can cause persistent infections. Possible mechanisms for ADE has been related to antibody subtypes, Fc receptors, complement receptors and CD4 molecules (Takada and Kawaoka 2003, Qiao et al. 2011). To avoid the side-effect of ADE, induction of robust CTL responses becomes the priority for developing vaccines against these viruses. The assembly of Th epitopes and CTL epitopes into viral vectors including recombinant adenovirus, vesicular stomatitis virus, and replicating cytomegalovirus might provide a solution. Another strategy is to develop vaccines that can induce antibody responses against a known neutralization epitope to avoid the production of disease-enhancing antibodies.

For successful vaccinations, adjuvant is usually needed in the vaccine formulation. Adjuvants help augment vaccine efficacy in two ways: one is the sustained release of antigen to persistently stimulate the activation of T cells and B cells; the other is to stimulate the production of certain cytokines that facilitate initiation of an effective immune response. The use of TLR agonists or other stimulatory molecules including cell targeting moieties or other biologically active mediators in vaccination have been shown to produce long-lasting antibodies with higher titers (Brito et al. 2013, Smith et al. 2013). In addition to efficacy, safety is also a concern in the development of novel vaccines. High purity of vaccines with complete removal of cell debris and genetic materials is required. Immunogenic foreign substances that are not needed in vaccine formulation may elicit unwanted immune response and interfere with the production of specific immune responses against vaccine component.

Conclusion

Traditional vaccinations with live attenuated vaccines have eliminated several devastating pathogens such as smallpox. But this method is ineffective for producing vaccines against pathogens that are hard to culture *in vitro* and it is time-consuming to identify surface-exposed protective protein antigens. Besides, persistent antigenic drift or shift as demonstrated by influenza virus poses inherent challenge for the use of these traditional vaccines due to differences between the seed strain in vaccine formulation and the circulating isolates. With the advent of reverse vaccinology, identification of novel vaccine candidates has been revolutionized based on multi-genome analysis or proteomic analysis. More and more protective antigens are being identified at a time, yet how to efficiently stimulate the immune system to respond to these antigens needs critical thinking based on knowledge from immunology. The elicitation of an effective immune response needs the precise initiation of B cells and T cells by epitope binding. Structural vaccinology is now able to locate and design the exact epitope that binds BCR or TCR. The three-dimensional structures of antigens or antigen-antibody complexes is now helping the development of epitope-based vaccines. High-density surface display of epitopes using nanoparticles or scaffold protein and immune cell-targeted vaccine design are helpful strategies for the effective presentation of these epitopes to antigen-presenting cells, B cells, and T cells. Besides, the delivery and release of vaccines into target sites is also a pursuit in novel vaccine development. More vaccines can be produced by targeting Peyer's patch M cells and dendritic cells to induce protective $CD8^+$ T-cell responses.

Antibody-dependent enhancement of viral infection hampers the development of vaccines against certain pathogens. The induction of CTL responses using viral vectors and the development of vaccines to elicit antibody responses against a known neutralization epitope might be possible solutions to avoid the production of disease-enhancing antibodies.

In conclusion, future vaccine development can greatly benefit from reverse vaccinology and structural vaccinology by identification of more protective antigens and determination of the structural basis for antigen-antibody interactions. Effective presentation of B cell epitopes, Th epitopes, and CTL epitopes using nanoparticles or scaffold proteins will gain more concern from the scientific community and be the pursuit of future vaccine development.

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