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Review Article

Strategies to Designing Chimeric Recombinant Vaccines

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Abstract
With the advent and development of the science of immunology, molecular biology, microbiology, genetic and biochemistry, human beings embarked on vaccines and deployed them. Since the birth of vaccine by Edward Jenner, there has been great progress in the production of various vaccines against different pathogens and antigens. Due to increased infectious diseases and multi-drug resistant strains, one of the best ways to encounter them is through vaccination. There are various vaccines with some problems which are the result of various mechanisms for the escape of pathogenic microbes from the immune system. Therefore, there is a need for comprehensive vaccines that can provide extensive immune responses. Chimeric vaccines and recombinant chimeric vaccines are developing nowadays and can protect against different serovars. The first recombinant vaccine was introduced in the mid-1970s against the hepatitis B virus (HBV). Recombinant chimeric proteins are developing nowadays that have the advantage of both recombinant and chimeric properties.

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Introduction

Many deaths in the world are caused by infectious diseases and the emergence of multi-drug resistant strains. Therefore, one of the best ways to encounter them is through vaccination. There are various types of vaccines. However, Different vaccines can cause different adverse reactions that are caused by vaccination. The escape from the immune system by pathogens often makes difficult vaccine development.[1] Chimeric proteins carry epitopes from various pathogens, linkers, or adjuvant sequences offer increased immunogenicity for recombinant antigens and can also produce widespread immune responses.[2] Utilization of vaccination in opposition with wide spread diseases has resulted in significant step in the combat against many infectious diseases. Operation of recombinant DNA has led to a new concept in vaccination in which isolated epitopes, capable of stimulating a protective immune responses and avoid undesirable ones, have been identified.[3,4] In this review article, the various aspects of recombinant vaccines are discussed.

Antigen Discovery Technologies

At first glance, the idea of using protein toxins as vaccines against bacterial human diseases seems somewhat of a paradox. However, in some diseases, the severe pathological effects manifested by the causative agents are mediated entirely by protein toxins. Thus, it seems reasonable to expect that if antibodies could be induced against the protein toxin, they should be effective at preventing severe disease. [5]

Along use of antibiotics for treatment of infections, vaccination had the greatest impact on human health in recent history. Millions of deaths from infection diseases are prevented by vaccines in each year. Vaccines also are the cost-effective tools for health improving and saving lives.[6] According to previous experiences, vaccines were developed by understanding of the pathogenesis of infectious agents. Protective antigen may or may not being virulence factor which were selected for vaccine candidates against infection diseases.[7] However, the development and introduction of vaccines against many pathogens remains as a problem because some organisms are more complex in their pathogenicity, great variety and disrupt the human immune system with immune evasion mechanisms. In this case, proper and rapid development are needed for effective vaccines against emerging and reemerging infections.[6,7] During the last decades, the vaccine field was developed by new technologies such as recombinant DNA and chemical conjugation. Recently, new methods and technological advanced

in molecular and cellular genetics, immunology, structural biology, bioinformatics, computational biology, nanotechnology, formulation technologies, and systems biology are used. They are including of vaccine design and antigen discovery methods, including reverse vaccinology, structural biology, and systems biology.[8] The recent approach to antigen discovery is used of bioinformatics tools on whole genomes sequence of microorganisms for vaccines design, which termed "reverse vaccinology".[9] This technology is a genome-based technology that there is a blind method. It can scan the genome and predict the vaccine candidates. This method not only can discover the novel protective antigens but also revealed new virulence factors of several pathogens. The development of genome-based technologies will be increased efficient development of vaccines against many pathogens.[10] *Meningococcus* type B was the first pathogen which applied in reverse vaccinology, the cause of 50% of global meningococcal meningitis.[11] After that, many other bacterial pathogens including group B *streptococcus*, group A *streptococcus*, *Streptococcus pneumoniae*, *Staphylococcus aureus* and *Chlamydia* were applied to reverse vaccinology.[12]

Bacterial Protein Toxin Used in Vaccines

Bacterial toxins are transported across the bacterial membranes through co-translational and post-translational mechanisms to reach their targets. Toxin transport occurs by multiple mechanisms, which have been characterized within Gram Negative and Gram Positive bacteria. Bacterial toxins are a virulence factor of pathogenic bacteria.[13] There are two main toxins in bacteria including endotoxin and exotoxin. Endotoxin

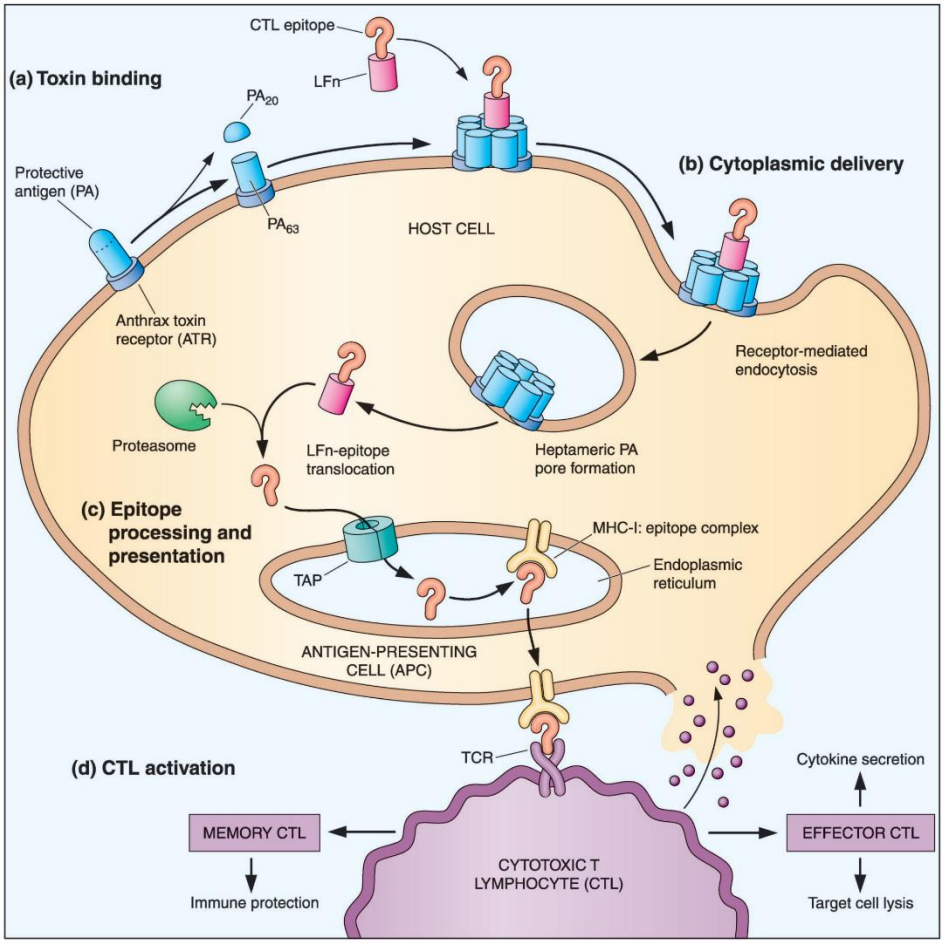

Figure 1. Model for Anthrax Toxin-Mediated Delivery of Epitopes to Stimulate Cytotoxic T Cells. (a) Toxin binding. Protective antigen (PA) binds to its cellular receptor, anthrax toxin receptor (ATR), expressed on host cells. Proteolytic cleavage of PA generates PA63. PA63 then oligomerizes and is able to bind a recombinant fusion protein containing the PA-binding domain of lethal factor (LFn) and a cytotoxic T-cell (CTL) epitope. (b) Cytoplasmic delivery. After LFn fusion protein binding, the entire complex is endocytosed via receptor-mediated endocytosis. Following endosome acidification, a heptameric PA pore mediates translocation of the LFn-epitope fusion protein into the host cytoplasm. (c) Epitope processing and presentation. Once in the cytosol, the fusion protein is processed by the proteasome into peptides. The peptides are then transported into the endoplasmic reticulum (ER) by the antigen-processing (TAP) complex, where they bind nascent MHC class I molecules (MHC-I). The resulting MHC-I: peptide complexes are transported to the cell surface via the secretory pathway. (d) CTL activation. Antigen-presenting cells (APC) that display a peptide epitope can be recognized by epitope-specific T cell receptors (TCR) on circulating CTL. This results in CTL activation and differentiation into memory and effector populations. Effector CTL lyses APC and secrete cytokines that activate other components of the immune response. Memory CTL remain in the host for extended periods of time and rapidly proliferate to provide effector functions following subsequent exposure to the antigen.[18]

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Adjutants

Vaccination is the best method for preventing the effects of infectious diseases in humans and animals. Due to the weakness of antigens in stimulating the immune system, Adjuvants were developed to potentiate the weak antigen.[19] The appropriate adjuvant vaccine is selected based on the nature of the antigen, the type of response required, the method of delivery and stability of vaccine (Figure 2)[13] Adjuvants are classified based on their physico-chemical properties and mechanism of action.[20] The main groups of adjuvants can be in the form of inorganic compounds, bacterial products, and oil emulsions, immunological and mucosal adjuvants.[21] The best known of mineral compounds are salts Aluminum (alum) and calcium phosphate. Alum component adjuvants are the most widely used adjuvants.[22] The oil emulsions of adjuvants, Freund's adjuvants, including complete adjuvants Freud (CFA), Freund's incomplete adjuvant (IFA) and MF59 are the strongest stimuli and reinforcement Immune system.[23] Some bacterial components such as endotoxin and flagella can induce strong immune responses. Lipopolysaccharide as a bacterial product can strongly stimulate and activate innate immune cells such as macrophages and other antigen-presenting cells.[24] Flagellin is a major protein component of the Gram-positive and negative bacterial flagellum that can be detected by the cell surface receptors that TNF-α is produced following this identification.[25]

Mucosal Vaccine

In the era of the revolution in developing vaccination against infectious diseases, mucosal vaccine was considered as one of the most cost effective and preferable options. Nasal, oral, ocular, gastrointestinal, rectal and vaginal tissues are the most important organs covered by mucosal layer. It is critical

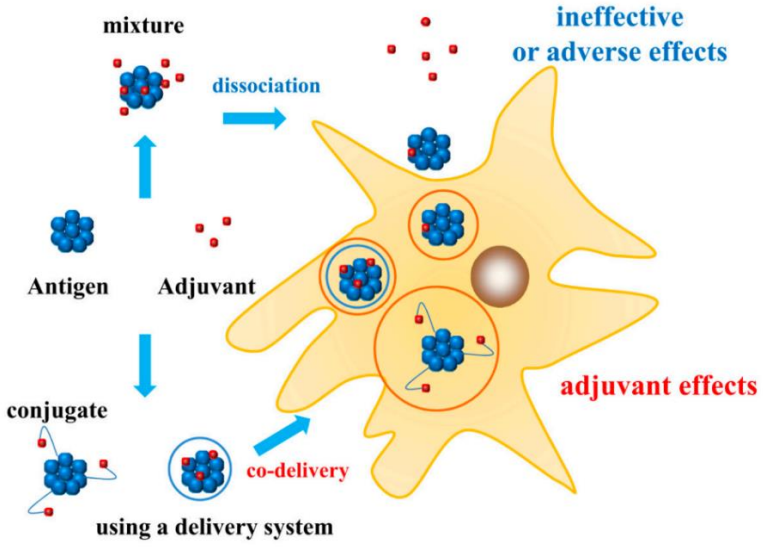

Figure 2. Different Interactions between Antigens and Adjuvants may Induce Different Effects.[26]

Mucosal Vaccine

In the era of the revolution in developing vaccination against infectious diseases, mucosal vaccine was considered as one of the most cost effective and preferable options. Nasal, oral, ocular, gastrointestinal, rectal and vaginal tissues are the most important organs covered by mucosal layer. It is critical to develop strategies for counteracting the infectious agent at these surfaces considering that many infections are initiated at mucosal sites.[27] Mucosal vaccination is involved the administration of immunogen at mucosal sites leading to stimulating humoral and cellular Immunity in systemic and mucosal process to create durable protection.[27] Secretory IgA (SIgA) and the cell-mediated mucosal immune response are the effector mechanisms for mucosal immune response.[28] Some licensed mucosal vaccines currently were used such as *Salmonella Typhi* (Vivotif, Ty21A) and *Vibrio cholera* (Dukoral, ORC-Vax, and Shanchol). They had efficacy more than 50%. An overview of chimeric vaccines was adapted to bacterial infectious diseases in the last decade which could induce mucosal immunity. A chimeric protein composed of F1/V antigen of *Yesinia pestis* was expressed in *Salmonella* vaccine vector and administrated to mice orally.[29] Serum IgG1, IgG2a and copro-IgA Ab titers were elevated as well as IFN-γ and IL-4 that showed the efficacy of *Salmonella*-(F1_V) Ags vaccine in mice that were challenged with *Y. pestis.*[30] Another parallel studies were designed for multiple antigen peptide (MAP) including three B, one T-cell epitopes of F1 antigen and Six protective epitopes of V antigen entrapped in PLGA (polylactidecoglycolide) microspheres to showing of protection in experimental animals. The significant peak antibody titer for IgG and mucosal sIgA of mice after intranasal immunization highlights the importance of MAP in stimulating mucosal and systemic immune responses.[31] Nasal administration of chitosan-based vaccine consists of intimin and Tir of EHEC indicated stimulation of specific immune responses (IgG and IgA) against fused antigen in mice model.[32] This nasal nanovaccine induced mucosal Immunity toward systematic immune responses and imparts protection to *E. coli* O157:H7 adhering to mucosal surfaces. Furthermore, a plant-derived edible chimeric EspA, Intimin and Tir was injected subcutaneously and orally to mice and then challenged with *E. coli* O157:H7. Induction of humoral and mucosal immune responses in orally immunized mice showed a significant IgG and IgA responses compared to control group.[33]

The cell surface antigen I/II (Ag I/II) and glucosyltransferase enzyme of *Streptococcus mutans* are colonization factors have been implicated in the initial attachment to saliva-coated tooth surfaces. A genetic chimeric protein consisting of the two virulence adhesions injected throw intranasal route in mice model and the potential of immunostimulatory effects evaluated. The results indicated that serum IgG (notably IgG1 and IgG2a) as well as salivary IgA and sIgA in vaginal samples increased significantly and in the next step oral administration of mice with *S. mutans* reduced colonization level in immunized mice. So this chimeric protein predicted to appropriate vaccine candidate for dental caries.[34] Accordingly, in another study, Ag I/II was fused to A2 and B subunits of cholera toxin (as an adjuvant), then was administrated to mice for the induction of immunity pathway assay. The results of flow cytometry of intestinal cells showed that the chimeric protein could take up by mucosal dendritic cells (DCs) in Peyer's patches and mesenteric lymph nodes effectively. The interaction of DCs with Th1 and Th17 in mesenteric lymph nodes can stimulate immune mechanisms to reduce colonization and protect from *S. mutans* induced dental caries.[35]

Recombinant and Recombinant Chimeric Vaccines

Pursuing ways to go through steps to control and prevent infectious diseases by vaccines dates back to Edward Jenner era when he inoculated a boy with cowpox to immunize him against smallpox.[36] Afterwards Louis Pasteur and other scientists extended the perspective of vaccination by using live attenuated and killed or inactivated vaccines. With the progression of vaccine technology, other forms of vaccines have emerged. Recombinant vaccines are among the most promising options. The first recombinant vaccine was introduced in the mid-1970s against hepatitis B virus (HBV). In this approach the gene that encodes the antigen of interest is cloned in a host. Recombinant technology is growingly being tested for other viruses like noroviruses and parvoviruses (Figure 3). Bacteriology also benefits from the results of this new vaccine technology; in that the purified proteins of pertussis toxin and filamentous haemagglutinin (HA) made up a new form of pertussis vaccine without the side effects of inactivated whole-cell pertussis vaccine.[37] Epitope enhancement greatly helped to improve the immunogenicity and immunodominancy of the recombinant protein. Chimeric sequences in the case of HIV envelope protein can induced broadly cross-reactive cytotoxic T lymphocyte (CTL) that recognized multiple strains of HIV.[38] Recombinant chimeric proteins are developing nowadays that have advantage of both recombinant and chimeric properties. The conserved moieties among serovars are gathered together and generate a chimera that can protect against different serovars.[39] This type of proteins shows great potential to act as a new generation of vaccines. Such constructs showed effective outcomes against visceral leishmaniasis and dengue virus.[40] In the latter case the chimeric recombinant protein was shown to induce neutralizing antibodies to all four dengue serotypes and could induce cell-mediated immune responses to dengue non-structural proteins.[41] In a recent attempt to design a vaccine against brucellosis three immunodominant and immunoprotective antigens including trigger factor (TF),

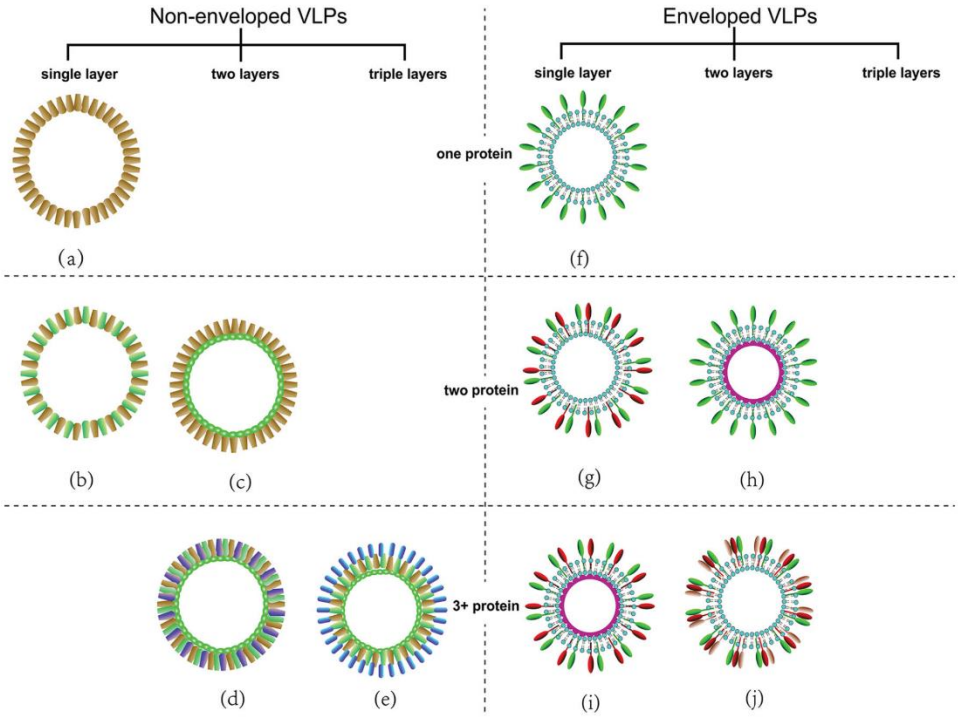

Figure 3. Classification of Recombinant Protein-Virus-Like Particles (VLPs). Particles are assembled by one or multiple proteins building single or multilayered structures. Both lipid enveloped and non- enveloped VLPs can be used for antigen presentation and packaging of DNA, proteins or small molecules. (a) The single layered non-enveloped VLPs assembled by one protein; (b) The single-layered nonenveloped VLPs assembled by two proteins; (c) Two-layered non-enveloped VLPs assembled by two proteins; (d) Two layered non-enveloped VLPsassembled by multiple proteins; (e) The triple-layered VLPs assembled by multiple proteins; (f) Single-layered VLPs consisted of one protein; (g)Single-layeredVLPsconsistedoftwoprotein; (h) Two-layeredVLPsconsisted of two protein; (i) Two layered VLPs consisted of multiple proteins.[20]

Omp31 and Bp26 were fused to produce a chimera. Mice infected with this recombinant chimeric protein showed increased levels of antibodies against the protein.[42] Similar studies are under way to introduce new recombinant chimeric vaccine candidates for other pathogens.[39,43,44] As time pass this new field of vaccination gain more attention to act as alternatives to traditional vaccines. The following will be explained a number of recombinant vaccines against certain pathogens.

Chimeric Recombinant Vaccines against Staphylococcus aureus

Staphylococcus aureus (*S. aureus*) is an important human pathogen that causes a range of clinical symptoms such as skin infection and soft tissue.[45,46] Many notable virulence factors attribute to the pathogenesis of staphylococcal infections, surface-associated adhesions, secreted toxins, iron acquisition-associated proteins and factors that enhance immune evasion.[47,48] The epidemiology of disease caused by *S. aureus* is under the influence of rapid antibiotic resistance. Some strains are resistant to first-line antibiotics.[49] The vaccine is a great way to reduce the disease, mortality and economic impact associated with Staphylococcal infections. Vaccinations with killed bacterial cells or bacterial products have not always resulted in protection against new infections or have not elicited heterologous protection.[50] A successful vaccine of *S. aureus* should be able to prevent infection Strains with a wide range of genetic fields.[51] For the good protection the humoral immunity alone is not useful against *S. aureus* infections.[52] In vaccination stimulation of cellular responses are more useful compared with humoral responses alone.[53] Potential candidates for development of an effective *S. aureus* vaccine are IsdB and ClfA. All strains of *S. aureus* express these two superficial proteins. The new chimeric vaccine was designed as $IsdB_{151-277}ClfA_{33-213}$ (IC).[54] IsdB (an iron-regulated surface protein) of *S. aureus* that plays a key role in heme iron acquisition.[55] Clumping Factor A (ClfA) is a superficial protein bound to fibrinogen *S. aureus* that is an antiphagocytic factor.[56] IC is a potential vaccine candidate for the fight against *S. aureus* sepsis and pneumonia.[54] TARP (Target of RNAIII activating protein) is a highly conserved protein among staphylococcal strains. TRAP is a master regulator of virulence in *S. aureus* and regulates the pathogenesis of *S. aureus.*[57] One study showed that the fusion protein tIsdB-TRAP had a much heavier immunity than IsdB or TRAP alone.[51]

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), an essential housekeeping enzyme in the survival of bacteria, has been investigated to be associated with pathogenicity and adherence in *S. aureus.*[58,59] *S. aureus* had two conserved

proteins with GAPDH activity, GapB and GapC which produce strong humoral and cellular immune responses in mice.[50,60] tIsdB-TRAP from *S. aureus* could raise the potential cross-protective role of GapC against *S. aureus*. The immunogenicity of a multi-antigen chimeric vaccine against *S. aureus* named GIT (GapC $_1$-tIsdB-TRAP) is protective.[61]

Pagibaximab® is a mouse chimeric monoclonal antibody against lipoic acid that is used for clinical use and reduces the incidence of bacterial *S. aureus* in premature infants.[62] IsdA (iron-regulated surface determinant A) adhesion is vital for *S. aureus* colonization on human nasal epithelial cells and plays an important role in iron absorption and resistance to human skin defenses. The results showed that a cholera toxin A2/B (CTA2/B) chimera containing IsdA can induce significant IsdA-specific Th2-type humoral and cellular responses when delivered intranasally to mice and for development of a mucosal vaccine against *S. aureus* is effective.[63] Bacteriophage endolysins present as a potential antimicrobial. Streptococcal λSA2 endolysin endopeptidase domain fused to staphylococcal cell wall binding domains from either lysostaphin (λSA2-E-Lyso-SH3b) or the staphylococcal phage K endolysin, LysK (λSA2-E-LysK-SH3b) are chimeric, which reducing the *S. aureus* bacterial load induced bovine mastitis.[64]

One of the main bacterial superantigens is Staphylococcal Enterotoxin B (SEB) that exerts profound toxic effects upon the immune system, leading to production of information.[65] Blocking the SEB connection to each of the receptors prevents the formation of the MHC II-SEB-TCR complex and inhibits the superantigenic action of SEB.[66]

Chimeric human-mouse antibodies directed against different neutralizing epitopes of SEB synergistically repressed its activation of human T-cells.[67] P128 is a bacteriophage derived staphylococcal cell wall-degrading enzyme. This chimeric protein developed to reduce MRSA-colonized patients and *S. aureus* nasal colonization. P128 consisting of the lethal activity of the phage tail-associated muralytic enzyme of Phage K and SH3b (staphylococcal cell wall targeting-domain) of lysostaphin.[68] rSip-ClfA, a novel chimeric based on B cell epitope against mastitis caused by *S. agalactiae* or *S. aureus* would be an effective vaccine candidate.

The Sip (surface immunogenic protein) from *S. agalactiae* and A protein of *S. aureus*, named ClfA (clumping factor A) protein. Two fragments containing B cell epitopes, one each from Sip and ClfA make a fusion gene and production of a recombinant fusion protein named rSip-ClfA.[69]

Chimeric Recombinant Vaccines against Neisseria gonorrhoeae

A 1995 World Health Organization report estimated that there were 62.2 million cases of the sexually transmitted infection gonorrhea worldwide.[70] *Neisseria gonorrhoeae* (the gonococcus, or GC) remains an important disease. Still relatively common in the US, with over 300,000 reported cases annually, and probably as many that are not reported, it is much more common in Africa and in many other parts of the less-developed world.[71] Furthermore, it has been shown that coinfection with *Neisseria gonorrhoeae* and human immunodeficiency virus (HIV) can increase the risk of transmission of HIV.[71]

This disease is a silent killer of the unborn, due to ectopic pregnancy. Some might view GC as just a minor infection, and one that is acquired by personal choice. Certain GC strains were capable of infecting the urethra, pharynx, and cervix; the infectious dose was high for the pharynx and cervix, but for the male urethra the required inoculum was about 1×10^4 colony forming units (CFU), essentially the same as for human urethral infection.[72] Initiation of a second infection by the same strain 1 week after termination of first infection required an infectious inoculum about 1000-fold greater.[72] the only GC capable of infection were of the PorB1B serovar class, which were able to bind chimp complement four binding protein (C4bp), rendering them phenotypically serum resistant.[73] *Neisseria gonorrhoeae* (*N. gonorrhoeae*) remains a major global public health concern. *N. gonorrhoeae* may be incurable due to resistance to all available antimicrobial classes for treating infections.[74]

Lipooligosaccharide (LOS), a part of the outer membrane, facilitates evasion of gonococcal killing by the alternative and classical pathways of complement and may also enhance bacterial resistance to killing by cationic peptides. A chimeric molecule (FH/Fc fusion protein that possesses bactericidal activity) comprising FH domains 18–20 fused to mouse IgG2a Fc mediates complement-dependent killing of sialylated gonococci FH18–20 also binds to select host glycosaminoglycans to limit undesirable complement activation on host cells.[75,76]

A study showed that chimeric vaccin comprised of gonococcal transferrin binding protein (Tbp) and cholera toxin B subunit (Ctb) can prompt serum bactericidal, growth-inhibiting antibodies in the vaginal environment and acquire protective antibody responses in mice.[77]

The transferrin binding proteins (TbpA and TbpB) comprise the gonococcal transferrin receptor and are considered potential antigens for inclusion in a vaccine against *Neisseria gonorrhoeae*.[78] The gonococcal transferrin binding proteins, TbpA and TbpB, have generated particular interest as vaccine antigens because they are ubiquitously expressed among clinical isolates, they exhibit low strain-to-strain variability, and they are not subject to high-frequency antigenic or phase variation.[79-81] In spite of their expression in vivo, it was shown that antibody responses to the transferrin binding proteins resulting from natural infections were weak in the serum and nonexistent in vaginal washes and seminal fluid.[82]

Intranasal immunization with the gonococcal transferrin-binding proteins TbpA or TbpB, or both, elicited bactericidal

immune responses; TbpA stimulated more broadly cross-reactive antibodies than did TbpB.[83,84] Immunization of mice with genetic chimeras that fused parts of TbpA and TbpB stimulated production of vaginal antibodies that inhibited growth in vitro.[85]

Chimeric Recombinant Vaccines against Neisseria meningitides

Neisseria meningitides (*N. meningitides*) is a pathogenic member of the *Neisseriae* family, which normally colonizes the throat and nasopharynx. This colonization may result in invasive disease. In general, most meningococcal polysaccharide vaccines are weak immunogens in neonates and fail to induce immunological memory in people of different ages.[86] Factor H binding protein (fHbp) is as a major factor of *N. meningitides* that attaches to the human complement factor H (fH) is a promising vaccine antigen and this compound increases the survival of the organism in serum.[87] One of the limitations of fHbp as a vaccine candidate is the antigenic alteration because the antibodies against fHbp in the antigenic variant 1 (v.1) group do not defend against strains that express the protein v.2 or v.3. Epitopes are expressed in all three groups by recombinant chimeric proteins including the A domain, a part of the B domain of a v.1 protein and the carboxyl-terminal of the B and C domains of a v.2 protein.[88] The murine IgG1 mAb (6E3) that was able to recognize the two main antigenic variants of NadA on the surface of strains expressing NadA variants 1 and 2/3.

Variable areas of the murine mAb 6E3, protective, were mixed to human IgG3 firm areas.[89] NID is a chimeric protein vaccine candidate against *N. meningitides* consisting of MID (Moraxella IgD-binding protein) that a well characterized trimeric autotransporter and targets the IgD of B cells and NadA is an oligomeric outer membrane protein of *N. meningitides.*[90] NadA was merged with the IgD-binding region of MID that would target B cells.[91] A chimeric molecule that includes human FH domains 6 and 7 fused to human IgG1 Fc can attach to meningococci and effectively blocked FH binding to bacteria, increase complement deposition, Direct Kill by complement and defend infant rats against meningococcal bacteremia. Thereby development of FH/Fc chimeric proteins that fuse different microbial binding domains of FH with Fc as adjunctive immunotherapeutics against microbial infections.[92] A chimeric vaccine named as NHBA-FP that comprised the recombinant neisserial heparin binding antigen (NHBA) and a periplasmic protein, GNA1030. NHBA-FP is a useful vaccine due to bactericidal activity, induce a high-avidity IgG response and complement deposition onto NHBA-expressing strains of *N. meningitides.*[93]

Chimeric Recombinant Vaccines against Yersinia pestis

Yersinia pestis is the agent of bubonic and pneumonic plague in the human. According to history, this organism has been the cause of over 200 million human deaths from pandemics. But, today, reported cases of *Y. pestis* infection is decreased in the world wide, because rapid treatment with antibiotics is effective and can prevent mortality rates.[94] According to Centers of Disease Control (CDC) data, *Y. pestisis* considered a Category a bioterrorism agent. Despite the data, development of a protective vaccine against infection disease due to this bacterium is needed.[95] Until now, there is no licensed vaccine available against plague for general populations. Currently a formalin-killed whole cell vaccine is used for military personnel and high risk people. But, it has been reported that this vaccine is only effective against bubonic plague and it has not protection against the pneumonic type of infection.[96,97] In other hand, a live attenuated vaccine has been use which it is highly protective, but the safety of this strain still remains elusive.[98] By using the recombinant DNA technology, immunodominant and protective antigens can be easily identified and selected for development of subunit vaccines. The advantages of these vaccines are reducing the risk factors and adverse effects associated with live and kill whole cell vaccines.[99]

According to literature, *Y. pestis*, mainly have two virulent factors, capsular F1 and the low calcium response LcrV antigens. It has been demonstrated that, these virulence factors are immunodominant and protective against *Y. pestis's* infections.[100] A pioneer study has been showed, vaccination with recombinant F1 failed to protect mice against bubonic plague.[101] This failure was happen due to existence of some F1-negative *Y. pestis* virulent strains. In case, vaccines based on F1 are not effective against plague.[102] But immunization with recombinant LcrV subunit vaccine provided protection in mice against bubonic and pneumonic plague.[102,103] By using recombinant vaccines technology, combination of recombinant F1 and LcrV antigens provide greater protection in comparison to either F1 alone or LcrV alone.[104,105] Also, immunization with F1 and LcrV antigens adjuvanted with alum provide good mice protection against plague.[106,107] According to chimera vaccines technology, when bacterial enterotoxins, including cholera toxin (CT) and *E. coli* heat-labile toxin (LT), can induce both systemic and mucosal immune responses against subunit vaccine candidates.[108-110] In this regard, addition of CT and LT to LcrV and F1 recombinant subunit vaccine has been demonstrated to enhance IgA induction conferred by F1 and LcrV subunit vaccines separately.[111, 112] Also, the effects of these toxins are induction of cellular responses that also are a key component of protection.[111,113] A study was conducted for evaluation of CT chimeras containing the LcrV antigen from *Y. enterocolitica* and *Y. pestis* (LcrV-CTA2/B) as vaccine candidate.[114] They are found many advantages of this vaccine including, the induction of both cellular and humoral responses, cross

protection against *Y. enterocolitica*, fewer side effects and can be delivered mucosally.

Chimeric Recombinant Vaccines against Clostridium perfringens

Clostridium perfringens is an anaerobic, Gram positive, spore forming pathogen which cause many types of infections in humans and animals.[115] This organism is classified into 5 different toxin types, Type encode alpha toxin, type B encode alpha, beta and epsilon toxins, type C encode alpha and beta toxins, type D encode alpha and epsilon toxins. Also, enterotoxin can produced by any toxinotype.[116] This organism generally can causes two types of infections in human and animals, including acute soft tissue infections like cutaneous abscesses, necrotizing muscular infections and gas gangrene.[117] Next type is diarrhea, food poisoning and enteritis.[118,119]

Control and prevention of this organism is very complicated due to lack of proper vaccine and this limitation may increase the rates of morbidity, mortality among human and animals. Vaccines design and production of this organism is very difficult because it's not cost benefit, time-consuming and dangerous processes due to the necessary detoxification, purification and antigen concentration stages.[120,121] In other hand toxigenic strains must selected for producing high titers of toxins.[122] In this regard the use of recombinant vaccines against infections due to this organism has yielded promising results in animal species.[123-126] Therefore this approach is considered a more stable, high-yielding process with superior biosafety; thus, recombinant proteins may be an alternative way for the prevention of clostredial infections.[127] There are many studies were conducted for recombinant one subunit toxin of *C. perfringens* as vaccine candidates. For example Lobato et al., were evaluated the potency of a *C. perfringens* type D epsilon toxoid expressed in *Escherichia coli* which tested in goats, sheep, and cattle.[124] Their reports showed the epsilon toxoid vaccine is adequate for immunization of ruminants against enterotoxemia. In another study, Brown et al., used recombinant epsilon toxin against enterotoxaemia in mice model.[128] Their data showed recombinant epsilon toxin is a good candidate against enterotoxemia. One subunit recombinant toxin as vaccine candidate against *C. perfringens* is encounter to major problem because this organism has multivirulence factors. So, development of vaccines against one toxin is not recommended. Therefore, by using structural biology for designing of new ways for vaccine development, new field of science is emerged termed 'structural vaccinology'.[129] This approach works by identification of protective domains/epitopes in the immunogenicproteins of a pathogen or multiple pathogens. Multiple epitopes or domains are designed and constructed synthetic protein chimeras comprising two or more such domains.[8,130,131] By using this strategy Shreya et al., evaluated immunization with recombinant bivalent chimera C-terminal binding regions of alpha toxin and enterotoxin against alpha toxin and enterotoxin of *C. perfringens* type A in murine models[129] and reported a considerable protection against its infections. In another study, a trivalent recombinant vaccine against the three major *C. perfringens* toxins including alpha, beta, and epsilon in cattle, sheep, and goats was developed.[132] It has been showed this trivalent vaccine is effective in generating protective antibodies and, thus, may represent an interesting alternative for the prevention of *C. perfringens*-related intoxications in farm animals.

Chimeric Recombinant Vaccines against Mycobacterium

In the recent years, DNA vaccination has emerged as an influential approach in the investigation for a more efficacious vaccine against *tuberculosis* (TB). The antigens encoded by the 6 kDa early secretory antigenic target (esat-6),[133] and antigen 85A (ag85a) genes from *Mycobacterium tuberculosis* (*M. tb*) are identified to exert protective responses against *tuberculosis* in animal models. Yan Liang and his colleagues have constructed a chimeric DNA vaccine from two copies of the esat-6 gene inserted into the ag85a gene from *M. tb* and treated BALB/c mice with this chimeric vaccine after infecting with one of two, *M. tb* H37Rv or a clinical multi-drug resistant TB isolate. In the first trial, for evaluating adjunctive therapeutic effects of Ag85A/ESAT-6 chimeric DNA, in female BALB/c mice aged between six to eight weeks, have been infected intravenously with MDR-TB HB361. In the second trial, for further evaluation the therapeutic effects of Ag85A/ESAT-6 chimeric DNA, and to assess the effects of Ag85A/ESAT-6 chimeric protein enhancement, female BALB/c mice with similar age with the first trial group were infected intravenously with *M. tb* H37Rv. In their study, they concluded that ESAT-6 chimeric DNA is not appropriate vaccine in both groups,[134] but another study reported that, the humoral immunity against the ESAT-6 antigen extensively improved in the mice primed with chimeric DNA vaccines, HG856K or HG856A, pursued by boosting with ESAT-6 or ESAT-6/Ag85A mixed proteins.[135] In 2016, Ping et al., reported that a chimeric DNA vaccine HG856A encoding *M. tb* immunodominant antigen Ag85A and two copies of ESAT-6 has been showed efficient protection against *M. tb* challenge infection and significantly increased the immune protection prepared by BCG vaccination in *M. tuberculosis*-infected mice.[136] On the other hand, the immunodominant antigens of *M. tb* such as TB10.4, Ag85B and TB10.4-Ag85B chimeric protein expressed in *Escherichia coli* and purified in considerable quantities of soluble antigens is effective in generating immunological reaction against *M. tb*.[137] Moreover, in 2011 S-S Ahn et.al designed all TB antigens as a chimeric

combination with Flt3-L to boost antigen-specific T-cell immunity consequent to vaccination in a mouse model. According to this study, F-Mtb32 DNA vaccine is the mainly successful protective immunity that represses bacterial growth in the active or latent status of *M. tb*.[138] The MPT64 recombinant TB antigen expressed by *Bacillus subtilis* spores has been reported as important for protecting against TB disease.[139]

The chimeric vaccine, expressing HSP65 and combined T cell epitopes has been created by Haifeng Gao and his collogues and immunized mice with DNA vaccine three times by injecting ECANS. According to their result, DNA vaccine with ECANS be capable of effectively inducing boosted specific cellular immune respond to PPD.[140] Also related research reported that MPT64 protein filtrated from mycobacterial culture has been expressed as a chimeric protein combined to one of three variants of the ubiquitin protein (UbG, UbA, and UbGR) identified to differentially influence the intracellular processing of the co-expressed antigens. The DNA vaccine that fused with destabilizing ubiquitin molecule (UbA or UbGR) change the host response towards stronger Th1-type immunity that differentiated by low definite antibody levels, high figures of IFN-g-secreting cells, and important in resistance to a tuberculous threat.[141]

Ying Xu et.al, were designed and constructed recombinant BCG expressing chimeric protein Ag85BN–ESAT-6–Ag85BC (rBCG-AN-E-AC). Then it's the immune response was compared to that protein with that to rBCG expressing the Ag85B–ESAT-6 fusion protein (rBCG-A-E) and BCG. Their research results indicate that this rBCG-AN-E-AC strain enhances the Th1 cell-arbitrated response and might serve as a possible vaccine against *M. tb*.[142] In the same way vaccination with sAg85A plasmid DNA co-expressing wild-type, other than the mutated caspase gene, has been come out with efficient potential in protecting mice against M. tuberculosis challenge, as showed by diminishing bacterial replication and prolonged survival.[143] Research conducted by Hui Li et al., assessed the immunogenicity and protective effectiveness of Mtb8.4/hIL-12 chimeric gene vaccine. The secretion of more of Th1 cytokines induced by Mtb8.4/hIL-12 chimeric gene vaccine, but not IL-4 and boosted CTL activity. Finally, they found that mice immunized with Mtb8.4/hIL-12 chimeric gene vaccine had fewer and smaller tubercles than control groups.[144] *Mycobacterium bovis* antigens known as MPB83 has been expressed as a chimeric protein fused to one of the two, b-galacotosidase, outer membrane lipoprotein OMP19 or periplasmic protein BP26 in gram-negative *Brucella abortus* S19, in BALB/c mice immunized with the recombinant S19 strains carrying the genes coding for the heterologous antigens in replicative plasmids, showed equally specific INF-g production in response to MPB83 stimulation. The report showed that B. abortus S19 is a suitable applicant for the expression of M. bovis antigens mutually correlated to the membrane or cytosolic fraction and maybe it will grant the root for a combined vaccine for bovine *brucellosis* and *tuberculosis*.[145]

Chimeric Recombinant Vaccines against Shigella

Shigellae cause brutal illness in endemic countries, particularly in kids. Many novel vaccines trial has been carried out with candidate vaccines against Shigelloses, but still no one successful on use. In 2015 research conducted on the novel vaccination found that *Shigella dysenteriae* bioconjugate vaccine (GVXN SD133) constructed from the polysaccharide component of the *Shigella* O1 lipopolysaccharide, conjugated to the exotoxin protein A of *Pseudomonas aeruginosa* (EPA) has been shown a satisfactory safety profile vaccine.[146] Another study reported that SC599 vaccine a live automated *Shigella dysenteriae* 1 strain by deletion of invasion, iron chelation, and shiga toxin A subunit genes has been used as vaccine for inducing significant IgA and IgG LPS-specific ASCs and antibody responses that might confer protection against the majority severe Shigellosis in human.[147] For inducing local or systemic immunity inactivated whole-cell vaccines have been orally administrated and its safety has been evaluated. In this phase-1 trial, whole-cell vaccines showed immunogenic and protective feature in animal studies and well tolerated.[148] There is not research and has not been reported about chimeric recombinant vaccines protecting against shigellosis, but Enterohemorrhagic *Escherichia coli* (EHEC) which produces Shiga toxin (Stx) causes prodromal hemorrhagic enteritis one of the most epidemic forms of Hemolytic-uremic syndrome.[149-151] Recently a new immunogenic that depend on the B subunit of Shiga toxin 2 (Stx2B) and the enzyme lumazine synthase from *Brucella* spp. (BLS) (BLS-Stx2B) has been developed. Before matting, BALB/c female mice have been immunized with BLS-Stx2B. In the titers of anti-Stx2B antibodies in sera and fecal extracts, dams and pups existed in more, and pups is important in protecting against a lethal dose of systemic Stx2 injection up to two to three months postpartum and also maternally transferred immunity expanded an active and specific immune response that defended them against a successive challenge with intravenous Stx2. Finally, they concluded that maternal immunization with BLS-Stx2B is incredibly efficient at encouraging the transfer of specific antibodies, and put forwards that pre experience of adult females to this immunogen might defend their offspring throughout the early stage of life.[149] Other study conducted in 2013 by Marı´a P. Mejias et.al., were designed and constructed a novel immunogen by inserting the B subunit of Stx2 at the amino termini of *Brucella* spp. They found that, chimera demonstrated mice developed strong ability to stimulate a long-term humoral immune response, that can neutralize Stx2 and its variants. According to their research results, this new immunogen

signifies a hopeful candidate for vaccine development with wonderful protective capacity against hemolytic uremic syndrome Stx-producing *E. coli*.[150]

Chimeric Recombinant Vaccines against Vibrio cholerae

Shortly after the discovery of the causative agent of cholera attempts have been started to find practical and acceptable interventions to control the episodes of cholera. Access to safe drinking water, improved sanitary and hygienic practices, education and better surveillance systems has led to decline of cholera burden. Vaccines are also progressively recommended as a preventive intervention approach that is complementary to other actions for endemic or at risk countries.

Despite several attempts to develop an effective vaccine to control cholera in endemic regions or for travelers, the issue has remained unsolved. Dukoral®, ORC-Vax and mORC-Vax, Shanchol, Euvichol®, Vaxchora and Cholvax® are among the Killed oral cholera vaccines (OCVs) that are currently available. Short lived protection and limited efficacy especially in children under 5 years of age, the need for multiple booster doses, high-cost for mass use in developing countries, the possibility of interference with the treatment, and the long interval needed for developing protection makes the OCVs a less feasible strategy to protect against cholera.[152,153]

In the last 1990s the idea of using recombinant vaccines for *Vibrio cholerae* has been proposed. One of the earliest attempts on a chimeric vaccine for preventing cholera was on 1996. With the fact that protective immunity to cholera is specific to serogroups and being infected or vaccinated with *V. cholerae* O1 provides no protection against O139 and vice versa, serogroup specific vaccines are of great interest. Dukoral® for instance contains 10^{11} killed *V. cholerae* O1 of both classical and El Tor strains with 1 mg of recombinant nontoxic B subunit of cholera toxin which cannot protect against other serogroups of *V. cholerae.* In an attempt to solve the problem OSP-core (OSPc) antigen derived from LPS was fused to recombinant heavy chain fragment of tetanus toxoid (TThc) and administered to mice. Anti-OSP responses evoked following administration of this conjugate vaccine in mice which is the effective and protective immunity against different serogroups of *V. cholerae*.[154] In 2014 a subunit chimeric vaccine was designed to confer mucosal resistance to both cholera toxin (CT) and toxin coregulated pilus (TCP)-the two most important virulence determinants of *V. cholerae*- in a mouse model.[153,155] Another approach is using genetically engineered strains that express *V. cholerae* antigens to act as live attenuated vaccines. The engineered vaccine strain *Salmonella* Typhimurium strain Z234-pMS101 which is capable of secreting CtxB can confer protection against both *V.cholerae* and also against lethal challenges of *Salmonella* Typhimurium in the murine model.[16] In silico studies are opening a new window to design, predict the spatial structure and efficiency of the designed chimeric protein. Chimeric proteins are now being developed that can act as multiple weapons capable of fighting an array of microorganisms. CII is such a protein constructed from entire cfaB protein and parts of intimin and ipaC. CII could be a candidate subunit vaccine against EHEC, ETEC and *Shigella*. Finding solutions for travelers to developing countries where diarrhoeagenic infections are not uncommon is of great concern. A chimeric construct is designed to being developed as a cocktail vaccine against the binding sites of AB5 toxins secreted by three most common diarrhoeagenic bacteria including cholera toxin of *Vibrio cholerae*, heat-labile enterotoxin (LT) of enterotoxigenic *Escherichia coli* and shiga-like cytotoxin (STX) of Enterohemorrhagic *Escherichia coli*.[156-158] Plant based edible vaccines also have come to assist solving the dilemma. With their long shelf-life, relatively high protein yield, stability at room temperature, reduced production costs, correct protein folding and post-translational modifications that are eukaryotic they introduce promising options to use.[159,160] There are transgenic plants available that express a chimeric protein comprising CTB and some epitopes of TCPA.[160]

Chimeric Recombinant Vaccines against Helicobacter pylori

Substantial effort has been devoted to introduce a vaccine for *Helicobacter pylori* (*H. pylori*) yet none of them gained great success to completely eliminating the bacterium in the tested population. Trials in human with different antigens and adjuvants lead in unsatisfactory outcomes.[161] A recombinant strain of *Lactococcus lactis* (NZ9000) was managed to produce the *H. pylori* antigen UreB fused with IL-2 as adjuvant to use as an edible vaccine. It couldn't completely remove *H. pylori* from infected mice but may play some role in controlling H. pylori infection when used as an edible vaccine.[162] In another study Yang and et al., designed a multiepitope vaccine (HUepi-LTB) against *H. pylori* that through oral prophylactic immunization could protect against *H. pylori* infection in BABL/c mice. Protection is probably mediated by specific IgA and secretory IgA antibodies and a mixed cells response of Th1/Th2/Th17. According to the results of this study the designed multi-epitope vaccine is a promising candidate for protection against *H. pylori* infection.[163] In another attempt to find a vaccine against *H. pylori* a dual-antigen epitope and dual-adjuvant vaccine called CTB-UE-CF (CCF) was designed which is constructed from cholera toxin B (CTB) subunit as well as tandem copies of the Th and B cell epitopes from *H. pylori* urease. In order to construct the CF moiety, the central variable region of *Salmonella typhimurium* phase I flagellin was replaced with the central variable region of FlaA. It was shown that administration of CCF

with adjuvant induces a gastric mucosal response and also a prominent humoral and proinflammatory cytokine production compared with CTB-UE. Determining *ure*C copy number using Real-time quantitative PCR assay showed that the designed construct can effectively abolishes *H. pylori* infection in the stomach and provides a new approach for more promising anti-*H. pylori* vaccines.[164]

Chimeric Recombinant Vaccines against Borrelia

Lyme disease is a tick born disease in North America and Europe. This infection caused by *Borrelia burgdorferi, B. garinii* and *B. Afzelii* that can be treated with antibiotics. If the patient is not diagnosed until sever stage of disease, it may interference in different parts of the body such as heart, nervous system and joints.[165,166]

LYMErix monovalent vaccine containing OspA (outer membrane protein A) was available for several years (from 1998 to 2002). Production of the vaccine was discontinued due to vaccine‐associated autoimmune arthritis side effects. Currently there is no human vaccine available for it. There is an essential requirement for vaccine production with high safety, better efficacy, low cost with minimal side effects.[167] Several studies have been performed for design a new vaccine. Studies show that Outer surface protein C (OspC) is an immunodominant antigen with high antigenicity that can be used as second generation vaccine candidate. However, due to heterogeneity, they have not been vaccinated until now. Using sequence analysis data were detected about 21 OspC phyletic clusters or types that are differentiated by letter marked (A–U). Recently other types have been added and identified .Although OspC exhibits significant diversity, it is genetically stable during infection. In previous study done in USA they designed a recombinant, tetravalent, chimeric construct contain OspC types A, B, K, and D. This construct was found to be highly immunogenic in mice and the induced antibodies against each of four OspC type.[167]
Another construct vaccine was a chimeric immunogen containing epitopes from OspA serotypes 1 and 2. Mice was immunized with this chimeric vaccine candidate. Then mics was infected by *B.burgdorferi* s.s. (OspA-1) and B. afzelii (OspA-2). Immunization with chimeric vaccine candidate provided dose-dependent protection against infection with *B. burgdorferi* s.s. and B. afzelii.[168] Another recombinant, Octavalent, chimeric construct contain type E, N, I, C, A, B, K and D OspC r‐proteins had high immunogenicity and was presented as a chimeric vaccine candidate.[169]

Chimeric Recombinant Vaccines against Bacillus anthraces

Anthrax disease is a zoonosis severe illness caused by *bacillus anthraces*. Two factors capsule and exotoxins contribute to the pathogenicity of this pathogen. The capsule that made up Poly-D-γ-glutamic acid, protects bacteria against macrophage phagocytosis during infection. Endotoxin consists of three proteins including protective antigen (PA), lethal factor (LF), and edema factor (EF), encoded by a 181-pair plasmid. This toxin belongs to the A-B toxin superfamily. Subunit B moiety (PA) is attached to the cell surface and assists in the translocation of the enzymatic A moiety (LF and EF) inside the cell. Vaccination is known as the best way to fight this disease. Currently, Anthrax Vaccine Adsorbed (AVA) is the only commercially vaccine available for human use. AVA is known to be the crude preparation of *B. anthracis* culture supernatant which mainly consists of PA and trace amounts of LF and EF. The vaccine has a series of limitations that require a new alternative vaccine. The limitations of this vaccine include crude preparation, allergic side reactions, ineffective in neutralizing the LF component, require multiple boosters, and so on.[170]

Studies show that N-terminal domain of LF has high immunogenicity with good protection against anthrax infection in animal model. Several chimeric vaccine candidates have been suggested in the past by using N-terminal domain LF (LFn) linked to PA. In this way, construct were designed can be used as a pre-exposure and post exposure application.[171] Chimeric protein of domain 4 of protective antigen (PA4) and c-terminal region of antigen 1 (EA1C) have better protection than PA or EA1 against toxin and bacilli. Another chimeric DNA vaccine candidate was composed of calreticulin (CRT) fused to domain 4 of protective antigen (PA4) which was significantly leads to the production of lymphocyte TCD4 dependant antibodies.[172]

Chimeric Recombinant Vaccines against Leptospira spp

Leptospirosis is a disease has been reported in developed and developing countries .It is a serious public health Problem in manty of countries especially after flood. The main route for transmission of this disease is through direct contact of the wound or mucous membranes with soil and water contaminated with this pathogen. The disease has variable symptoms from a middle fever to renal failure. Despite the advancement in antibiotic therapy for this disease, vaccination is the most appropriate way to prevent disease. Inactivated or attenuated vaccine has been used for human and animal but this vaccine has several side effects, such as aches and anaphylaxis, and they confer short-term immunity and immunity only against serovars used in vaccination. There are more than 270 serovar of *leptospira* spp. Antigen diversity that is among species is due to variation structure and lipopolysaccharide (LPS) composition of the outer membrane.[39]

Many studies have been done on the design of a chimeric vaccine. Chimeric protein including amino acid sequences of the LigA, Mce, Lsa45, OmpL1, and LipL41 proteins was survey in the hamster infection model. However only 50% of animal were protected against leptospirosis.[39]

Another chimeric vaccine candidate containing four repeats

Table 1. Vaccines that have been approved for Use in human

Proper Name	Tradename	Manufacturer	Indication
Hepatitis A Inactivated & Hepatitis B (Recombinant) Vaccine	Twinrix	GlaxoSmithKline Biologicals	Active immunization of persons 18 years of age or older against disease caused by hepatitis A virus and infection by all known subtypes of hepatitis B virus
Hepatitis B Vaccine (Recombinant)	RECOMBIVAX HB	Merck & Co, Inc	For prevention of infection caused by all known subtypes of hepatitis B virus
Hepatitis B Vaccine (Recombinant)	ENGERIX-B	GlaxoSmithKline Biologicals	ENGERIX-B is a vaccine indicated for immunization against infection caused by all known subtypes of hepatitis B virus
Hepatitis B Vaccine (Recombinant), Adjuvanted	HEPLISAV-B	Dynavax Technologies Corporation	Indicated for prevention of infection caused by all known subtypes of hepatitis B virus in adults 18 years of age and older
Human Papillomavirus Quadrivalent (Types 6, 11, 16, 18) Vaccine, Recombinant	Gardasil	Merck & Co., Inc(US)	Prevention of vulvar and vaginal cancer
Borrelia burgdorferi (Recombinant)	Lymerix	GlaxoSmithKline Biologicals	Prevention of Lyme disease in the US
Neisseria meningitides (Recombinant)	Bexsero	Novartis	Causative agent of meningococcal meningitis and septicemia
Human papilloma virus	Cervarix	GlaxoSmithKline Biologicals(EU)	Prevention of Human papillomavirus
Influenza virus	Flublok	Protein Sciences Corporation	Prevention of Influenza

of six T- and B-cell combined epitopes from the *leptospiral* outer membrane proteins, OmpL1, LipL32 and LipL21. This chimeric vaccine can be developed for vaccine against leptospirosis.[173]

Commercial Recombinant Vaccines

According to studies on recombinant vaccines, good progress has been done in recent years. Also, this type of vaccine has benefits such as high production, low costs and ability to produce target proteins with desired structures and biological functions. Therefore, some of these products have commercial produced and approved for use in human (Table1).[174]

Conclusion

Millions of people die annually because of the lack of vaccines against from infectious diseases in the world. On the other hand, with the emergence of emerging diseases, it is more necessary to deal with infectious agents in order to continue life. With the advancement of the biology sciences, the world of vaccines and vaccinations has also undergone an evolution. The first generation of vaccines is live-weakened and inactivated or vaccines killed. These type vaccines are so similar to the natural pathogen with a strong and long-lasting immune response but they have some limitations. With the advancement of vaccine sciences other types of vaccines including subunit, recombinant, polysaccharide, toxoid and conjugate vaccines also created. One of these types is recombinant vaccines which were developed with the advancement of recombinant technology. After that chimeric proteins and nucleic acids encoding selected antigens were appeared as a vaccine. The Recombinant protein-based vaccine is producing using heterologous expression systems in bacteria, yeast, mammalian cells and insect cells for vaccination. In these systems genes can be chimeric with expression of several genes from different agents. In recent years, special attention has been paid to highly purified recombinant proteins or subunits of pathogens as a source of recombinant vaccines. Advantages of these vaccines include high production, low costs and ability to produce target proteins with desired structures and biological functions.

Authors' Contributions

All authors contributed equally to this study.

Conflict of Interest Disclosures

The authors declare they have no conflicts of interest.

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