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REVIEW

Immunoinformatics and Vaccine Development: An Overview

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Abstract: The use of vaccines have resulted in a remarkable improvement in global health. It has saved several lives, reduced treatment costs and raised the quality of animal and human lives. Current traditional vaccines came empirically with either vague or completely no knowledge of how they modulate our immune system. Even at the face of potential vaccine design advance, immune-related concerns (as seen with specific vulnerable populations, cases of emerging/re-emerging infectious disease, pathogens with complex lifecycle and antigenic variability, need for personalized vaccinations, and concerns for vaccines' immunological safety -specifically vaccine likelihood to trigger non-antigen-specific responses that may cause autoimmunity and vaccine allergy) are being raised. And these concerns have driven immunologists toward research for a better approach to vaccine design that will consider these challenges. Currently, immunoinformatics has paved the way for a better understanding of some infectious disease pathogenesis, diagnosis, immune system response and computational vaccinology. The importance of this immunoinformatics in the study of infectious diseases is diverse in terms of computational approaches used, but is united by common qualities related to host–pathogen relationship. Bioinformatics methods are also used to assign functions to uncharacterized genes which can be targeted as a candidate in vaccine design and can be a better approach toward the inclusion of women that are pregnant into vaccine trials and programs. The essence of this review is to give insight into the need to focus on novel computational, experimental and computation-driven experimental approaches for studying of host–pathogen interactions and thus making a case for its use in vaccine development.

Keywords: immunoinformatics, computational vaccinology, vaccine design, emerging infections, immune system; vaccinology

Introduction

Vaccination has been undeniably very helpful in promoting a healthy global population. It has severally saved lives, reduced healthcare costs and raised man's quality of life.[1] It greatly reduces disease burden, disability and death. However, newly emerging and re-emerging infectious diseases (ERID), infectious agents with complex lifecycle and antigenic variability and the need for personalized vaccination present additional challenges in vaccine development.[2,3]

For many pathogens (especially the emerging and those with antigenic variability), their genomes are known but their immune correlates of protection remain unclear.[1,4] Some of these reasons are why vaccine development for ERID and multi-lifecycle pathogenic diseases is a tall order.

Serendipitous discoveries in immunology coupled with knowledge of bioinformatics tools for epitope predictions have resulted in the emergence of new pattern of

vaccine design.[5,6] The art and science of efficient and comprehensive information extraction and analysis of data deposited in relevant databases is now increasingly essential in researches related to immunology.[7] Even with this capacity (efficient information extraction), some challenges in the application of bioinformatics in immunology include structure and/or function analysis and immune process analyses as concern the immune interaction specificity. Fortunately, although researches in immunology are experimentally costly and very intensive, colossal amounts of data are usually generated. Such data can only be analyzed with high precision and speed using bioinformatics tools. For instance, genome sequencing as well as in vitro T-cell confirmation is done in few months as opposed to years using the conventional vaccine design.[8] Also, computational immunological methods drastically reduce both time and labor needs in epitopes screening.[5,9] With computational immunology techniques, it is possible to discover vaccine candidate epitopes simply by scanning the protein sequences in a pathogen of interest.[5] Many of these proteins are yet to be isolated or at least cloned. Being pathogens specific and unique, they present ready candidates in vaccine construct.

This review describes the need to use immunoinformatics-based techniques to unveil vital determinants of immunity made available in the genome sequence database and design vaccines. Also, this review gives insight into the need to focus on novel computational, experimental and computation-driven experimental approaches for studying of host–pathogen interactions and thus making a case for its use in vaccine development. This review will further show the need for new approaches for effective drugs or vaccine design so as to combat the antigenic variability of some pathogens.

Vaccine Immunology and Design

The process of generating vaccine-induced immunity is somewhat challenging in immunology. Current conventional vaccines came empirically when there were vague or no knowledge of vaccine immune system activation. A lot of research[10–12] has been geared toward the understanding of this challenge, but the complexity of it requires a different dimension of approach.[13] An approach that must accommodate many factors affecting vaccine development like pathogen antigenic variability, the emergence of infectious disease, human genetic variation is the goal of immunoinformatics [Figure 1].

Activation of the immune system involves, among many processes, induction of the immune memory. The strength of this induction determines the efficacy of a vaccine. Hence, vaccine efficacy in the long run is influenced by the determinants of immunological memory stimulation, persisting antibodies and kind and type of immune memory cells induced.[14]

The primary vaccine-mediated immunological effectors (Table 1) are mainly the antibodies (from B lymphocytes/cell)[15,16] and sometimes CD8+ and CD4+ T cells. These antibodies bind specifically to a particular kind of toxin or

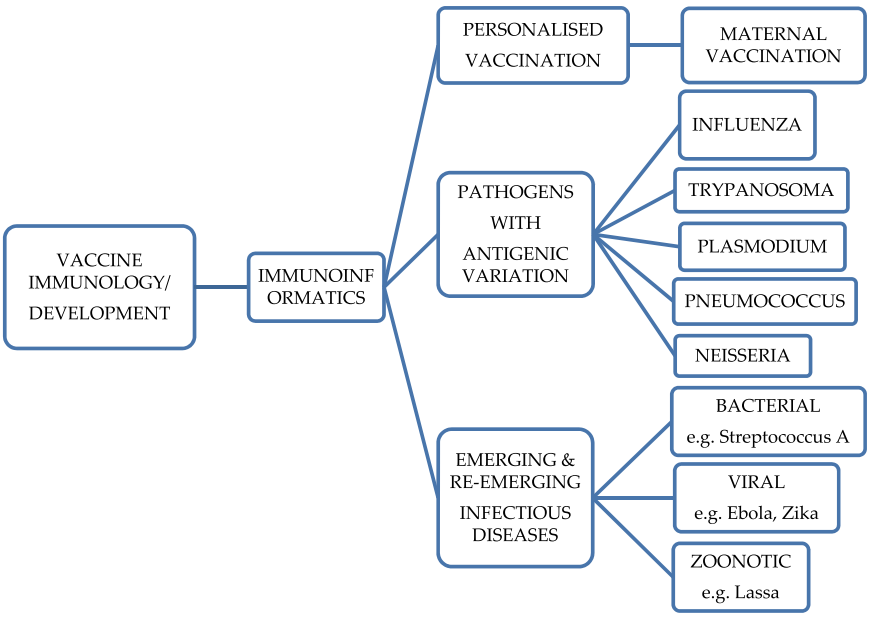

Figure 1 Schematic illustration of the cases stemming the need for immunoinformatics vaccine development approach.

Table 1 Functions of the Immunological Memories

Immune Cells	Immunological Memories (Effectors of the Immune Response)	Mechanistic Functions
B cells produced (humoral immunity)	Antibodies play vital roles in the control (including prevention) and complete removal or destruction of both extracellular and intracellular pathogens as well as in response to vaccination.	- activate the complement cascade - neutralize the replicating power of viruses (viral infectivity) - prevent the diffusion of toxins and/or bind to their enzymatic active sites (comprising of the binding site and the catalytic site) - induction of the macrophages and neutrophils for the purpose of clearing extracellular bacteria
T-cell produced (cellular immunity)	T cells of the CD4+ class. Clears the pathogens residing within and outside the cells	- produces several interleukins and supports B-cell stimulation and differentiation (Th2 cells response) - produces Th1 cell responses (produces interferon-γ, tumor necrotic factor-α/-β and Interleukins-2 and 3 and supports the proliferation and differentiation of CD8+T cells, B cells, and macrophages - activate the B cells, cytotoxic T cells and other cells not involved in the immune system
	T cells of the CD8+ T class. Clear the pathogens residing in the cells	- induces the release of antimicrobial cytokine for the purpose of killing microbial infected cells - kills infected cells directly by releasing proteolytic enzymes

pathogen. Vaccines and most antigens evoke humoral as well as cell-mediated immune responses.[17] Vaccines that mediate immune responses through these systems (B and T cell responses) are said to be more effective. Although B cells are regarded as the primary vaccine immune effectors, T cells induce immune memory cells and high-affinity antibodies. Studies in reverse vaccinology and immunomics had also proved T cells as prime immune effectors following the discovery of novel vaccine targets with EpiMatrix.[18–20] This change of immune target has led to successful advances in vaccine design.

Even at the face of potential vaccine design advances, immune-related questions are now focused on specific vulnerable populations such as the young, elderly and immunocompromised.[21,22] These concerns have propelled a better understanding of the efficacy of current vaccines on this vulnerable population and have also paved way for the application of new approaches that can put into consideration the differences of population and better targets that can generate optimum immune induction[23–25] with the exception of type II T-cell-independent (TI-2) antigens (i.e., polysaccharide antigens).

Antigens that could provoke the B lymphocytes as well as the T lymphocyte responses stimulate the germinal centers causing antigen-specific highly efficient B-cell multiplication and eventual differentiation into antibody-forming plasma cells and memory B cells. All existing protein and DNA antigens induce immunological memory B cells unlike type II T-cell-independent (TI-2) antigens (i.e., polysaccharide antigens). These polysaccharide antigens do not generate memory B cells but can induce long-lasting humoral immunity even when recall responses are lacking.[26] Vaccine efficacy may be short term[27] if only the B cells are activated.

The traditional approach for developing vaccines for infectious disease threats has shifted to include other vaccine design techniques like cloning and expressing major surface antigens[28] although this frequently resulted in the formulation of vaccines with poor immunogenicity, requiring strong adjuvantation.[29] This approach is particularly likely to be less specific for pathogens with complex life-cycles (e.g., parasites) or very high mutability (e.g., RNA viruses). These pathogens do not depend on one route for their virulence of pathogenesis in human and thus to alter this process, increasing the specificity of the vaccine should be the aim and not just the effectiveness as seen in the current conventional vaccines.[28,29]

Vaccines for several neglected tropical diseases are in various stages of development,[30] thanks to mega drug companies that have continued to demonstrate a willingness to invest money in the research and development as regards to diseases plaguing the developing nations.[16,30-32] It is very pertinent to invest in researches that have an interest in vaccine specificity on the pathogen antigens than totally

on the efficacy. Fortunately, with global research efforts, the genomes for several pathogens causing neglected tropical diseases and several emerging pathogens are becoming available.[33] Computational vaccinology may now be applied to screen these genomes for possible vaccine target. With these tools, many proteins of virulence interest can be sequenced and the most essential gene of interest modeled for a potential vaccine candidate specific for that pathogen. Immunoinformatics is the way forward in the identification of vaccine candidates for these tropical ERID, for pathogens with varying antigens and for individualized therapy.

Immunoinformatics and Infectious Disease

Immunology studies produce data in colossal quantities. Also, with proteomics and genomics projects, extensive screening of pathogens and/or pathogen–host interaction, it has become increasingly necessary to store, manage and analyze these data, hence the birth of immunoinformatics. Immunoinformatics deals with computational techniques and resources used to study the immune functions. Statistical, computational, mathematical and biological knowledge and tools are applied in immunoinformatics in order to accurately and specifically store, and analyze data concerning the immune system and its functions.

To handle evidence diversity, immunoinformatics uses tools that cut across several aspects of bioinformatics such as creation and management of databases,[34,35] use and definition of both structural and functional signatures and the formation and application of predictive tools.[35–37] These strategies can synergize toward a better understanding of the immune system of both man and animals and fight against some less predictable pathogenesis. The complex nature of vertebrates' immune system, the variable nature of pathogens and environmental antigens coupled with the multi-regulatory pathways show that colossal quantities of data will be needed to unveil how the human immune systems work. Conventionally, much cannot be achieved based on the complexity of the immune system and the virulent antigen but with the application of computational vaccinology, researches on vaccine design have been made easier, accurate and specific. Applying immunoinformatics in disease study (Table 2) requires the knowledge of disease pathogenesis, the immune system dynamics, and computational vaccinology, painstaking searches of the database, sequence comparison, structural modeling as well as motif analysis.[35,38] These methods can go a long way in analyzing the pathogenesis of a disease and identification of vaccine candidates.

In order to help understand complex pathogen-related processes, computational models were developed for viral[46,47] bacterial,[48] parasitic[49] and fungal pathogens.[50]

The bioinformatics tools (Table 3) are used to identify possible epitopes for vaccine formulation. Each tool can screen protein sequences and identify aggregates of MHC binding and supertype motifs for possible use in epitope-based vaccine development and for use among human populations with genetic variability.

There are several databases (Table 4) that can provide a wide range of information for all forms of immunological studies. Generated data from the studies are further organized and stored in the databases (Table 4) to provide a means for the development and advance in

Table 2 The Importance of Bioinformatics in the Research on Infectious Diseases

Importance	Applications	Refs
Surveillance of infectious disease	Microbial genotyping is used to either confirm or refute epidemiological links with potential environmental sources.	39
Determining the various strains of pathogens in circulation	The proteins used by variants of pathogens can be predicted and mutated for better analysis. Even the genes that code for the proteins can be manipulated in silico in order to predictive a better targeting	40,41
Diagnostic microbiology	Bio-surveillance focused text-mining tools and microbial profiling are used to detect infectious disease outbreak	42
Databases for Pathogens	Array of data on pathogens can help in their genome study and their virulence toward development of vaccine candidate	43
Vaccinology	Bioinformatics have helped in the advance of DNA and Epitope-based vaccines both in silico and as a preliminary study for the in vivo validation study	44,45

Table 3 Bioinformatics Tools for MHC Cluster Binding and Super-Type Motifs and Protein Sequences

Bioinformatics Tools	Applications/Description	Refs
EpiMatrix	This is an in-silico product of EpiVax developed for predicting and identifying the immunogenicity of therapeutic proteins and epitopes. It is also used to re-design proteins and in designing T-cell vaccine	51
Conservatrix	Has been applied in comparing strings from different strains of same pathogens and for pathogens identification. Configuration of Conservatrix allows for amino acid replacement at unusual positions. Highly conserved T-cell epitopes in variable genomes such as some viruses are amenable to the algorithm	51,52
ClustiMer	Potential T-cell epitopes usually aggregate in specific immunogenic consensus sequence (ICS) regions as clusters of 9–25 amino acids with 4–40 binding motifs instead of randomly distribute throughout protein sequences. In combination with EpiMatrix, the ClustiMer algorithm may be used to identify those peptides with EpiMatrix immunogenicity cluster scores ≥ +10. Such peptides are usually immunogenic and tend to make a promising vaccine candidate.	1,53,54
BlastiMer	Using BlastiMer program, one may also choose to automatically BLAST "putative epitopes against the human sequence database at GenBank". BLASTing screens off those epitopes with possible autoimmunity and cross reactivity questions and locates the epitopes that can safely be used in developing human or animal vaccine. BlastiMer can also BLASTs sequences against PDB, SwissProt, PIR, PRF and non-redundant GenBank CDS translations.	55–57
Vaccine CAD	This algorithm evaluates junctional epitopes for possible immunogenicity and inserts "spacers and breakers into the design of any string-of-beads construct".	1,58,59
NERVE	Predicts the best vaccine candidates starting from the flat file proteome of a prokaryotic pathogen. It's a fully automated reverse vaccinology system, developed to predict best VCs from bacteria proteomes and to manage and show data by user-friendly output.	60
Jenner-Predict	Predicts PVCs from proteomes of bacterial pathogens. The web server targets host-pathogen interactions and pathogenesis by considering known functional domains of protein classes such as adhesin, virulence, invasion, porin, flagellin etc	61
Vaxign	Is a vaccine target prediction and analysis system based on the principle of reverse vaccinology? Two programs exist in Vaxign: 1) Vaxign Query and 2)Dynamic Vaxign Analysis	62
VaxiJen	Is the first server for alignment-independent prediction of protective antigens. It was developed to allow antigen classification solely based on the physicochemical properties of proteins without recourse to sequence alignment.	63
VacSol	A high throughput in silico pipeline to predict potential therapeutic targets in prokaryotic pathogens using subtractive RV.	64
PanRV	Pangenome-reverse vaccinology approach for identifications of potential vaccine candidates in microbial pangenome. It comprises of four functional modules including i) Pangenome Estimation Module(PEM) ii) Reverse Vaccinology Module(RVM) iii) Functional Annotation Module(FAM) and iv) Antibiotic Resistance Association Module(ARM).	65

immunological research. A tour on these databases will actually stimulate some interest in the vaccinology of emerging and re-surging disorders attributable to pathogen including cancer.

Case of Emerging and Re-Surging Disorders/Diseases Attributable to Pathogens

Emerging infections (EIs) include infections that are entirely new in a population or that may have existed before in the population but are now gaining rapid and continued spread and/or wide geographical range. Re-emerging or re-surging infections represent the infections that were previously of historical relevance but are now quickly becoming relevant because of either increasing incidence or increasing geographical and/or human host range while emerging infections represent the infections that were not originally observed in man.[66] Several factors such as human behavioral changes, environmental changes, and host/intermediate factors, animal-human switching and microbial genetic changes all affect infectious disease emergence and spread.[67] These factors interact to promote the evolution of pathogens into new ecosystems, infect, spread and thrive in their new hosts.[68] The overall consequences of these are continued

Table 4 List of Some Immunological Databases

Immunological databases		
IMGT	It contains well over 32, 000 entries of Immunoglobulins and T cells Receptor sequences from both human and non-human vertebrate species. A lot of information on the human leukocyte antigen is also provided.	http://www.imgt.org/IMGTindex/databases.php
HIV Molecular Immunology	It contains an annotated pull of HIV-1 $CD4^+$ and $CD8^+$ epitopes and antibody binding sites.	https://www.hiv.lanl.gov/content/immunology/
ExactAntigen	Employed for the search of monoclonal antibodies utilised for therapeutic, commercial and academic purposes.	http://www.labome.com/index.html
EPIMHC	This the major curated database for MH ligands and Epitopes for tailor-made Computational Vaccinology	http://imed.med.ucm.es/epimhc/
JenPen (AntiJen)	This database contains quantitative binding data for peptides that bind to MH1, MH2 and TAP molecules. Also provides quantitative binding data for TAP, TCR-MHC complexes and MHC-ligand interactions	http://www.ddg-pharmfac.net/antijen/AntiJen/antijenhomepage.htm
SNPBinder	A known database of predicted antigenic peptides and minor histocompatibility antigens (mHAgs)	http://www.imgt.org/about/immunoinformatics.php
SYFPEITHI	This is a collection of MH ligands and peptide motifs	http://www.syfpeithi.de/bin/MHCServer.dll/EpitopePrediction.htm
Bcipep	This a repertoire containing immunant dominant B cell epitopes	http://crdd.osdd.net/raghava/bcipep/
kabat database immunoglobulin	It contains sequences of important immunological proteins of including Ig, TcR, MHC molecules, etc	http://immuno.bme.nwu.edu OR http://www.ftp.ebi.ac.uk/pub/database/kabat/
EpiVaxb	Contains information on promiscuous and conserved epitopes of class I and II	http://www.epivax.comb
IEDB Binding, MHC Class I	Uses 3 different methods to predict class I-peptide binding	http://www.immuneepitope.org/analyze/html/mhc_binding.html
IEDB Binding, MHC Class II	Prediction of class II-peptide binding	http://www.immuneepitope.org/tools/matrix/iedb_input
MotifScan	Summary and location of anchor motifs	http://www.hiv.lanl.gov/content/immunology/motif_scan
MHC Haplotype Project	The haplotype of MHC-linked-diseases, showing full genomic sequences, ancestral relationships and vital variations (SNPs and DIPs).	http://www.sanger.ac.uk/HGP/Chr6/MHC
HCV Immunology	CD8þ and CD4þ T cell proteome and HCV epitopes maps	http://hcv.lanl.gov/immuno/

infectious disease emergence and re-emergence, epidemics and public health challenges. Emerging infections and multi-antibiotic-resistant strains of pathogenic bacteria usually surge from one geographic location from where it spreads to other places due to immigration of people.[67,69] Most emerging infections originate from a specific population and can spread to a new population or become selectively advantaged that it can lead to the emergence of new strains of the pathogen.[67,70,71] Also, there could be microbial traffic, in which case, an infectious agent transfers from animals to humans or spreads from isolated groups to new populations.[67,71,72]

Several factors, including ecology, are known to be associated with infectious disease outbreaks. These factors bring man into close contact with a natural disease reservoir/host.[70] With an increasing world population and

poor infection control, the emergence of infection and increased microbial populations are sure. The human growth population will only increase the spread of the infection across populations. The information provided in Table 5 is the list of remerging infections and current emerging diseases put forward during the WHO 2018 annual review.[73] The review noted that these infections, if not well controlled, can cause disease outbreaks, bioterrorism and similar occurrences requiring urgent public health attention and that with the dearth of efficacious medicines or vaccines, there is a compelling demand for continuous as well as accelerated research and development in those areas.

Advances in Genomics, proteomics, immunomics, vaccinomics and nanotechnology are being continually exploited in diagnostic, therapeutic and in rational drug and vaccine development. These advances have also served in the control of the afore-mentioned emergences.[74,75] The knowledge of the emerging pathogen's genome, protein make-up, pathogen-immune system interactions and researching the possible therapeutics will go a long way in directing the optimum path to containing the infection spread and controlling potential re-emergence or emergence in a different population. Approaches in direct and computer-based structural determinations,[76] protein–protein interactions predicting, and bioinformatics tools now exist and are used in modern-day development of drug and biologics.[77]

Vaccine development has been sped up through the advance in the knowledge of the immune system of man. Researches in the traditional targets of vaccines (adaptive immune response) and the less specific and fast-acting innate immune responses have been clear evidences for this advance.[78–80] As our understanding of the intercourse between innate and adaptive immunity increases, reasons and opportunities for more effective vaccine adjuvants will open up. This can be a step forward in solving a critical world's health challenge per population. Following the conventional approach of vaccine design, much cannot be achieved but when the knowledge of immunoinformatics is applied, population safety and disease control can be achieved through pathogen's genome sequencing leading to optimum new vaccine design or development of a novel vaccine for the infection.

Table 5 Current Emerging and Re-Surging Infectious Disease

Infectious Disease	Endemic Population	Contributing Factors	Current Treatment
Lassa fever	West Africa	Urbanization favoring rodent host, increasing exposure	Ribavirin (no specific vaccine)
Streptococcus A (Invasive necrotizing)	Global	Uncertain	Antibiotic
Ebola	Central & West Africa	Unknown (In Europe, importation of monkeys)	No proven therapy (Experimental vaccine)
Variant Creutzfeldt-Jakob disease (cattle)	UK, France, Spain	Changes in rendering processes	No specific therapy
Shigellae sonnei Infection		Consumption of iceberg lettuce	
SARS	Southern China, Canada	Animal-to-animal transmission	No specific therapy
2009 H1N1 Influenza	Global attack	Droplets of unprotected cough or sneeze	Antiviral therapy (No specific vaccine)
Hantavirus pulmonary syndrome	USA	Rodent infestation	No specific therapy
MERS-CoV	Saudi Arabia,	Human-to-human, dromedaries	No specific therapy
Human T lymphocyte Virus I (HTLV-I)	Japan, Central & South Africa, USA	Human-to-human, Sexual contact	Experimental vaccines
Human immunodeficiency virus-2 (HIV-2)	Africa	Sooty mangabey monkey	No specific therapy
Human herpes virus-6 (HHV-6)	USA, UK, Japan, Taiwan	Shedding of viral particle into saliva	No specific therapy

Pathogens with Variable Antigens

Antigenic variability is an important mechanism pathogens use to evade their host immunity. The surface proteins of pathogens are normally variable. This assists them to escape recognition by the immune system. A successful infectious agent presents to the host immune system information that differs from that of its virulence. Pathogenic organisms have organized systems of escaping destruction by the immune system of their hosts. For instance, Toxoplasma invades and appropriates the host cells thereby circumventing phagocytosis and then spread within their host to establish infection.[81] Vertebrates on their own are endowed with immune system robust enough to efficiently and effectively surmount the non-self-attacks. Yet the more the host's immune system elaborates, the better the organisms in their evasion of immune effector cells.

Antigenic variation refers to a pathogen's ability to modify its surface proteins such that it can circumvent the host's immunological attacks. It involves several mechanisms including the varying of surface protein's phase, shifting and drifting of surface protein antigens and/or any other form of alteration of antigenic protein.[82] Antigenic variation plays significant roles in the pathogenicity of microorganisms by evasion of the host immune responses and establishment of re-infection. When a pathogen alters its surface antigens, it can evade the host's adaptive immunity and so re-establishes infection. The immune system may battle to generate new immunoglobulins against the new antigen. Certain bacteria like *Neisseria gonorrhea, Neisseria meningitides, Mycoplasma* and species of the genus *Streptococcus* show antigenic diversity.[83] In eukaryotic pathogens, antigenic variation is shown by *Trypanosoma brucei* and *Plasmodium falciparum*.[81,84]

Another vital cause of antigenic variation in bacteria is horizontal gene transfer (more important than point mutation) through plasmid acquisition and transduction via bacteriophages. Virulence genes are normally acquired by non-virulent organisms via these routes. Once this occurs, the new bacteria may quickly get established and cause fresh epidemic outbreak.

Neisseria

Species of the genus *Neisseria* are champions in the rapid change of surface antigens amongst bacterial pathogens. Pathogenic forms exhibit an amount of phenotypic variability not found in the commensal species. The pathogenic forms are implicated in STD and meningitis. They employ amazing varieties of antigenic variability mechanisms.

- They can recombine their pilin genes in a similar manner that eukaryotes recombine their own genes, such that they can express variable surface protein.[85]
- Some cell-surface proteins and enzymes synthesizing bacterial cell-surface carbohydrates are expressed in a variety of ways. This is as a result of replication slipping or slippage errors and repairs of simple tandem nucleotide repeats involving either the di-, or tri- or tetra-nucleotides.[86]
- Neisseria is able to take up and incorporate environmental DNA into its genomes.[83,87]
- Again, the chances of Neisseria acquiring new mutations by replication errors are high compared to other bacteria like *E. coli*.[88]

These are why an effective vaccine against *Neisseria* infections is not yet developed. *Neisseria* may be considered as an extreme example. However, many other bacterial pathogens like *Streptococcus* and *Mycoplasma* in promoting their antigenic variation tend to utilize one or more of these techniques. Additionally, there are reports that DNA-related defects have a much greater association with bacterial pathogen from symptomatic patients than samples of the same bacterial species isolated from environmental sources.[87–89]

Pneumococcus

Streptococcus pneumoniae, Gram-positive cocci bacteria that cause otitis media, bacteremia and pneumonia, are a public health concern, causing morbidity and mortality in adults and children.[90] Two forms of vaccines (polysaccharide and conjugate vaccines) are currently marketed for the prevention of pneumococcal infections. While the polysaccharide vaccines are for vaccination in the adult population, conjugate vaccines have an added immunogenic non-pneumococcal protein conjugated to the pneumococcal polysaccharides for enhanced immunogenicity in children. It is not yet known that these vaccines can evoke complete immunity against the infection.

A polysaccharide capsule is a major virulence factor in the bacteria. Several of these capsule types have been identified, and these form the basis of pathogen's antigenic serotyping.[91,92] Current pneumococcal vaccines are

combinations of various capsular (polysaccharide) antigens from the serotypes most common in a particular population. Currently, over 100 different serotypes are known but are not all covered in the available vaccines.[92] The discovery of a common antigen(s) will produce an effective vaccine. Knowledge of the genome of the organism and the different strains has led to a possible advance in driving the pneumococcal potential vaccine search through a different approach. And this consideration will help solve a lot of concerns about the current vaccines. With this knowledge, many methods are been tried to determine whether they can be a source of effective vaccine design that can accommodate all the serotypes of the organism. Search for antigen that is common to all the serotypes can be achieved with the knowledge of Genomics and immunoinformatics. The introduction of genomic and computational technologies has given new directions in the study of bacterial pathogenesis and vaccine design.[93,94]

Plasmodium

Plasmodium falciparum undergoes two life cycles: one in humans and the other in mosquitoes. The human host's erythrocytes and hepatocytes usually display modified parasite proteins called *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1) and *Plasmodium falciparum* hepatocyte membrane protein 1 (PfHMP1), respectively. These proteins function to assist the parasite to evade destruction by the host immune systems.[95,96] The PfEMP1 proteins were identified as the prime ligands responsible for cytoadherence and resetting.[97] They cause the infected RBCs of host tissues to sequester thus helping the parasite to circumvent clearance by the host's spleen.[98] The membrane proteins also shield infected host cells from destruction by the spleen by adhering to the endothelium. Luckily, if the PfEMP1 protein is expressed for a long while; it comes under attack by the naturally acquired immunity.[98,99] In defence to this, the parasite has expanded the var genes coding for PfEMP1 such that the genes can exist as a polymorphic family of as much as over 50 members in every genomic haploid. Antigenic switches work well here in that members of the polymorphic family (also called antigenic-variant-protein family) can be interchanged and cannot express their proteins at the same time. In this way, only one particular protein at the surface of the infected RBC is expressed at any given time.[97,100]

When studying antigenic-variant-protein families, it is pertinent to understand if grouping them into single-family results in any meaningful antigenic activity. Studies have tried to understand the "languages" of the antigenic variant of PfEMP1 proteins.[97,101,102] They sought to know the PfEMP1 proteins binding properties or search to understand the correlation between motifs and infection severity.

The varDB database is a repository for protein sequences involved in antigenic variation and their associated functionalities.[103] Antigenic variant data obtained from several pathogens may be regrouped into a unified database. This will enable researches from several multicopy gene families to be accessed and compared swiftly in a single moment. Updated varDB database contains close to 10,000 DNA sequences, several protein translations, tens of infectious diseases and pathogens with their gene families. With a novel sequencing-based approach, PacBio, the different PfEMP1 proteins can be sequenced and the related sequences used as potential vaccine targets.[104,105]

Trypanosoma

For many pathogens, antigenic variability occurs during the infection pathogenesis and is to enable them to escape destruction by the host antibodies. For instance, some eukaryotic parasites take to genetic assortment and rearrangement thereby changing their surface antigens. A ready example is seen in *Trypanosoma brucei*, the causative organism for sleeping sickness. *Trypanosoma brucei* replicates in the bloodstream (outside the cells) of their host, but at maturity, it crosses the blood–brain barrier to cause several fatal complications. During replication in the bloodstream, the parasites are subjected to humoral as well as cellular immune responses. It evades the host defenses by encasing itself in homogeneous coat of glycoprotein called the variant surface glycoprotein (VSG).[106,107] Though at initial invasion, the protein coat tends to protect the microbe from the immune system but on constant exposure, the coat will be identified as a foreign matter, and at this point the immune effectors can launch an attack against it. In a particular *Trypanosoma brucei*, there are diversities of the VSG protein being coded by more than a thousand genes in the parasite's genome. Unfortunately for the host, the expression of these genes is mutually exclusive. Expressed VSG gene is normally due to genetic reassortments causing new alleles to be copied into the sites of expression. Some trypanosomes with these abnormal VSG genes evade humoral immunity and multiply thereby causing re-infection and chronic recurring infections.[107]

Influenza Virus

Influenza is a viral infectious disease due to infection by any of the three types of RNA viruses, namely influenza Types A, B and C. Current vaccines contain double Type A and single Type B strains and induce strong antibody responses to neuraminidase and the surface glycoprotein hemagglutinin. These vaccines, however, cannot effectively protect against newly emerging viruses with antigenic shift and drift.[108,109]

Antigenic drift results in changes in the antigenic site (a minor change) while antigenic shift results in a new virus subtype. Hemagglutinin and neuraminidase are the two enzymes dictating the antigenic properties of the viruses. While inside its host, defined host proteases break the peptide bonds in the hemagglutinin molecule to form hemagglutinin 1 and 2 subunits. Virulence tendencies are decreased when the amino acids at the cleavage sites are lipophobic, the virus exhibits high virulence tendencies.[110] The surface glycoprotein can be regarded as antigen and hence can serve as a target for the immune system which if sequenced, using the new immunoinformatics approach and a common site for the varying proteins identified, a potent vaccine can be developed which can accommodate the antigenic drift/shifts of the virus.

Influenza viruses are able to thrive for a long while in a given human population.[111,112] The virus has a high mutation rate such that a once effective vaccine can easily lose efficacy. Antigenic variability is only one of the evidences of phenotypic variation in the biology of the Influenza virus.

Multiepitope Vaccines

The use of immunoinformatics in vaccine development has been accelerated toward the design of a multiepitope vaccine construct which has and will fully address the challenges faced with pathogens with mutagenic antigens. Previous vaccines developed by conventional approaches consist of several proteins or a whole pathogen. This constitutes unwarranted antigenic load and increases the chances of inducing allergy. The use of peptide-based vaccines surmounts these challenges. The vaccines are made from short peptide fragments capable of eliciting highly specific immune responses, precision targeting and multiepitope constructs, in the case of varying antigenic peptides, which has been made feasible with the advancements in the field of computational biology.[113–116]

Vaccines for pathogens with immune escape potentials can basically be constructed by using most, if not all, of their immunogenic peptides[116,117] because such vaccines prove to be better than single-epitope and classical vaccines. Multiepitope vaccines enjoy the following advantages over single-epitope and classical vaccines: a) they are an assemblage of several epitopes obtained from distinct protein targets/antigens of an intended infection; b) the multiple T-cell receptors (TCRs) in the vaccine recipient can easily recognize vaccines with multiple HLA epitopes; c); they can be easily adjuvanted to improve their immunogenicity; d) they can activate antibody-mediated and cell-mediated immunological responses because of their overlapping helper T lymphocytes (HTL), CD8+ T-cell and B-cell epitopes; and e) unwanted protein antigens are excluded in such construct thereby reducing the chances of untoward effects and/or immune responses likely to cause disease(s).[118–123] Thus producing a vaccine with these qualities can provide chances of combating most infections such as *Streptococcus pneumoniae* and HIV infections.

Immunoinformatics can be employed in the docking of single and multiepitope vaccines and subsequently to predict their properties (physicochemical, allergenic and antigenic). This approach has seen the use of diverse tools and database in the analysis of ligands with their targets and has greatly helped to predict the binding score of antigenic peptides with the immune proteins like HLA. Peptides and HLA allele modeling can be done by the 3D structural designing of the epitopes using PEPFOLD3 (an online server),[124] retrieving from Protein Data Bank (PDB) the x-ray crystallographic structure of human population most occurring HLA alleles (HLA-DRB1 01:01, 15:01 and HLA-A 02:01) followed by filtration of previously bound ligands.

The following is a step-wise detail on how to construct a single or multiepitope-based vaccine and its property prediction;

- Molecular Docking Analysis: to determine the interaction pattern of the screened out epitopes with the HLA alleles by employing ClusPro v.2 (a protein-protein docking web server). This server performs this task by energy minimization, calculation of both the binding energy scores of the docked complex and electrostatic/shape complementarity.
- Target-Protein Comparative Modeling and associated Structure Validation: the sequence of the amino acid in the target protein (e.g., TLR-9) can be retrieved from UniProt and the tertiary structure with Raptor-X and I tasser (online comparative modeling tools). The server constructs and creates a 3D model

(mathematical representation) of the target protein using hierarchical algorithms.[118,125]

- Designing of Final Single/Multiepitope Vaccine Construct: in the case of multiepitope-based vaccine; the different epitopes can be linked to each other using AAY and GPGPG linkers and to the adjuvant using EAAAK linker.
- Predicting the antigenicity and allergenicity together with other physicochemical information: allergenicity of the final vaccine construct can be analyzed by Vaxijen v.2 tool, antigenicity of the vaccine by the use of AlgPred server and the physicochemical properties (isoelectric point, half-life, solubility, molecular weight, aliphatic index, and average hydropathicity) with the ProtParam server.
- Structure modeling, Refinement and Validation of the final Vaccine-these can be investigated using the SOPMA server/Raptor-x, Galaxy Refine Server and ProSA-web, respectively. This process is being followed by molecular docking and molecular dynamics of receptor and the epitope vaccine using ClusPro and Molecular Dynamic Simulation (Desmond)[126] respectively.

Case of Personalized Vaccination

Personalized vaccine refers to vaccines "targeted" toward an optimized outcome. Immunogenicity is maximized while either the risk of vaccine failure or reactogenicity and side effect is minimized. Personalized are developed in the following cases;

The Individual Level

Vaccines are developed to take care of haplotype and polymorphism knowing that they can retard the formation of a protective immune response or become pointers to the risk of an adverse vaccine reaction.

The Gender Level

This is needed when it is clear that females produce a higher antibody titre against a particular vaccine antigen than do their male counterparts.

The Racial/Ethnic Level

Where it is clear that a particular human race or ethnic group has a higher or lower immune response to a particular vaccine antigen.

The Subpopulation Level

Personalized vaccines arise due to known complex interactions between host environmental, genetic and some other factors that may be influencing the vaccine immune responses. The associations between the immune response gene polymorphisms and variations in immune responses to a particular gene must be pine-pointed when it is clear that a particular drug either suppresses or augments the transcription of an immune response gene.[127,128] This could help in designing vaccines or vaccine adjuvants that can circumvent restrictions due to immunological differences arising from varying genetic compositions.[129,130]

Personalized vaccines stem from our understanding of how, within the human leukocyte antigen (HLA) system – also referred to as the major histocompatibility complex (MHC), the T cells are able to recognize peptides of pathogenic origin.[131,132] HLA molecules enjoy the double advantages of having stable polymorphisms and being fully characterized.[133] These advantages make good candidates for personalized vaccine design. HLA polymorphism, although stable, is complex. For instance, more than 12,000 alleles of HLA class I molecules and greater than 4000 class II molecules have been identified among human populations.[133,134] HLA class I and II molecules have heterodimeric character comprising of α and β chains, three highly variable extracellular domains (α1, α2, and α3) and then transmembrane and intracytoplasmic domains that are less variable.[133,135] HLA genes contain eight exons. Exon 1 is responsible for producing the leader peptide; exons 2,3,4 produce α1, α2, and α3 extracellular domains, respectively, for MHC class 1 or α1, β1and α3, respectively, for MHC class II; exon 5 encodes transmembrane anchor; exons 6 and 7 encodes the cytoplasmic tail while exon 8 encodes the 3′-untranslated region.[135] Most of the several forms associated with the class I and II genes are seen in α-1 and α-2 (as known as class I) and in α-1 and β-1 (as known as class II) domains.[133] MHC I and II bind and present the peptide to T cells.

T cell responses to viral pathogens differ from one patient to the other, basically because of the expression of differing HLA (MHC) alleles which determine the several viral amino acid sequence brought to the T cells to read.[136,137] It is most likely that during an infection, diverse epitopes are usually presented to the T cells to read owing to the several forms of HLA alleles and also because each human person expresses six HLA Class I and six HLA Class II alleles.[138] Now, antibody-binding sites in a given

HLA (MHC) molecule are mostly prediction-servers predetermined on the basis of particular binding motifs and the anchor residues.[139,140] These residues refer to known amino acids located at defined locations in the peptide chain and which are peculiar to each MHC molecule.[141,142] Prediction-server database of peptide motifs and/or of MHC ligands may be obtained from web-based and/or from prediction-servers dedicated to NetMHC family.[143,144]

In another example, sequence analysis of Lassa fever virus (the LASV) and other viruses' immunoproteomic was used to identify the best immunogenic protein predicting T-cell as well as B-cell epitopes and also target sequence and binding sites.[145,146] The SSNLYKGVY peptide sequence at AA41-49 of glycoprotein 1 (produced by the L segment) was the best candidate epitope for the induction of humoral as well as the cell-mediated immunity for Lassa fever vaccine construct. 17 HLA-I and 16 HLA-II molecules have been proven in sizable African populations and their combination with the SSNLYKGVY peptide sequence may prove useful in such Lassa fever virus endemic areas.[145] This approach will strongly improve individualized vaccination and help combat emerging infections. The HLA region is suspected to contribute, to a large extent, to genetic propensity to infections and differences in vaccine expected immune responses.[132,147]

Studies show that females exhibit stronger immune responses to immunization compared to males.[148,149] There are differential antibody responses to rubella and measles viral protein between males and females and that both hormonal and genetic difference may be influencing the immune responses.[148,150,151]

Practical issues may stand in the way of achieving this new development (personalized vaccinology). Having to use different vaccines for different persons based because of personal genetic composition requires more time and labor during the vaccination process. Also, screening for individual factors for targeted vaccination can significantly increase vaccination cost. But with all these challenges, personalized vaccination is the new age approach in achieving an optimum immunization that takes into consideration the individual immune differences in a particular population and it is a new dawn for vaccine development.

Personalized vaccine development is strongly improved by vaccinomics. The field of vaccinomics looks at how immune response gene polymorphisms affect the cell-mediated, humoral and innate immune responses to vaccine antigens at population and specific individual levels. "Vaccinomics" encompasses both immunogenomics and immunogenetics as it concerns immune responses to vaccine antigen.[152] The fields of personalized vaccinology and vaccinomics were the products of Phase I of the international HapMap and that of the Human Genome Project. Also, modern molecular assay techniques permitting high-throughput detection of variations at gene level, in particular linkage disequilibrium maps and single nucleotide polymorphism (SNP), played significant roles in the development of personalized vaccinology and vaccinomics. It has also been shown that polymorphisms at vital immune response genes can bring about differing immune responses to biopharmaceutical products including vaccines.[152–154]

Newer, accurate, cheap and reproducible sequencing technologies; validated databases containing genotype-phenotype data; statistical and bioinformatics tools are needed in order to analyze and interpret data that will help and improve vaccine adverse and immune response quantifiability and predictability.[155] The information will enhance clinical practice and accelerate rational and directed vaccine development.

The Pregnant Women

Safe vaccines are a critical requirement for any immunization program.[156] Conventional vaccination has been an approach targeted at all groups and individuals but has failed toward the enrolment of pregnant women into vaccination programs because of presumed fetal and maternal harms.[157,158] Evidence on the safety of vaccination in pregnancy is very small because pregnant women were usually excluded from participating in vaccine trials.[159] Pregnancy can alter the maternal as well as fetal immunological responses.[160] It is pertinent to explore research opportunities presented in advanced vaccine designs such as immunoinformatics (multiepitope vaccine docking) by studying human immune system functions and responses specific to pregnant women and their unborn children.[157]

According to a report[161] from the Dominican Republic of Congo, during the 2016–2017 Zika virus outbreaks, over a thousand pregnant women were suspected of being infected with the virus and a sizable number were at their first trimester. The report further stated that fetal loss was approximately one-tenth of the pregnancies and that there were up to 3 cases of fetus with head circumferences smaller than normal. The widespread morbidity during the epidemic showed that Zika virus infection adversely affects pregnancy outcome.[160,161]

Currently, there is no proof that pregnancy predisposes to Ebola virus infections in comparison with the non-

pregnant population, but there is some evidence suggesting pregnancy to worsen the disease prognosis including fetal loss. Also, evidence showed that the virus can pass the placental barriers to establish infection in the unborn child.[162] Designing, implementing and enrolling pregnant women as well as perspective pregnant women into vaccine trials and programs is imperative in order to protect this group and ensure good vaccine uptake by them during infection outbreaks and epidemics.[157,163]

These recommendations will give an informed decision to be investigated using the immunoinformatic tools to determine the immunogenic responses worthy of safe vaccine development for the pregnant women and perspective pregnant women group.

Maternal immunization offers palpable benefits in several ways. Some vaccines are primarily administered to shield these pregnant women from morbid conditions and/or death including fetal death.[164,165] Pregnant women stand the risk of being exposed to virulent pathogens and may be at a higher risk of morbidity and/or mortality when compared to the general population.[166]

Conclusion and Future Research Directions

There has been an explosion of new immunological data (Table 4) due to an increase in research to understand the immune system pathway in infectious disease pathogenesis and the application of the knowledge of bioinformatics has led to a better exposition of the immune system importance through immunoinformatics. The knowledge of immune system and the cost-effective, specific and effective approach like immunoinformatics, the concerns for emerging and re-surging diseases caused by pathogenic organisms, antigenic variability/complex lifecycle of pathogens and the need of personalized vaccination can be combated on a molecular level.

The future of immunological research is sharpened by the ability to make discoveries in biologics (e.g., vaccines) more effectively and efficiently. This will mean reduction and better targeting of wet laboratory experiments and will only be possible if wet laboratory experimentation is combined with bioinformatics techniques.

Limitation of Immunoinformatics Predictions in Vaccine Design

- Immunoinformatics depends on experimental science (wet laboratory) to produce raw data for analysis. The predictions are not formal proofs of any concepts. They do not replace the traditional experimental research methods of actually testing hypotheses.
- The quality of immunoinformatics predictions depends on the quality of data and the sophistication of the algorithms being used. Sequence data from high-throughput analysis often contain errors. If the sequences are wrong, or annotations incorrect, the results from the downstream analysis are misleading as well.[167]

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Availability of Data

All the needed data are included in this manuscript.

Author Contributions

All authors contributed to data analysis, drafting or revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

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