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REVIEW

The use of databases, data mining and immunoinformatics in vaccinology: where are we?

Nagendra R. Hegde [a], S. Gauthami[b], H. M. Sampath Kumar[c] and Jagadeesh Bayry [d]

[a]National Institute of Animal Biotechnology, Miyapur, India; [b]Ella Foundation, Turkapally, Hyderabad, India; [c]Council of Scientific and Industrial Research – Indian Institute of Chemical Technology, Hyderabad, India; [d]Institut National de la Santé et de la Recherche Médicale (INSERM) Unité 1138, Centre de Recherche des Cordeliers, Paris, France

ABSTRACT

Introduction: Vaccinology has evolved from a sub-discipline focussed on simplistic vaccine development based on antibody-mediated protection to a separate discipline involving epidemiology, host and pathogen biology, immunology, genomics, proteomics, structure biology, protein engineering, chemical biology, and delivery systems. Data mining in combination with bioinformatics has provided a scaffold linking all these disciplines to the design of vaccines and vaccine adjuvants.

Areas covered: This review provides background knowledge on immunological aspects which have been exploited with informatics for the *in silico* analysis of immune responses and the design of vaccine antigens. Furthermore, the article presents various databases and bioinformatics tools, and discusses B and T cell epitope predictions, antigen design, adjuvant research and systems immunology, highlighting some important examples, and challenges for the future.

Expert opinion: Informatics and data mining have not only reduced the time required for experimental immunology, but also contributed to the identification and design of novel vaccine candidates and the determination of biomarkers and pathways of vaccine response. However, more experimental data is required for benchmarking immunoinformatic tools. Nevertheless, developments in immunoinformatics and reverse vaccinology, which are nascent fields, are likely to hasten vaccine discovery, although the path to regulatory approval is likely to remain a necessary impediment.

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Adjuvant; antigen; data mining; immunoinformatics; structural biology; systems immunology; vaccine design

1. Introduction

Vaccines have been one of the best interventional strategies to control infectious diseases [1]. Since the first demonstration of protection against small pox, numerous vaccines have been developed, and a huge number of other candidates have been experimentally tested in animal models as well as in target species. Classical ways to develop vaccines have been to mass produce the pathogens, inactivate them by physical or chemical means, and purify and formulate the antigen [2]. In many instances, this has proven to be a very useful strategy, especially given the safety of such vaccines. On the other hand, live attenuated vaccines have played a major role in the eradication or elimination of some infectious diseases at regional level. However, live attenuated vaccines are typically not preferred due to live nature of the antigen and the potential risks for their use in subjects where the immune system is underdeveloped (pediatric subjects), in decline (geriatric subjects), or suppressed (e.g. transplant patients, or due to infectious agents such as human immunodeficiency virus (HIV)), and in pregnant individuals.

It is well known that those who survive any infection are typically protected against homologous infection or disease, although the duration of protection varies vastly between pathogens and/or hosts. The breadth of protective immune responses following infection is generally a combination of antibody- and cell-mediated functions. On the other hand, typical evaluation of vaccine efficacy involves antibody titers and/or functionalities. This is possibly due not only to historical reasons (antibodies being studied for more than a century compared to less than half a century of study of cellular responses), but also to the fact that standardization of assays to measure cell-mediated responses, and more importantly their protective correlates, are difficult. Thus, levels of antibodies are still the only correlates of protection defined for several vaccines [3–5].

Several new developments in the last two decades have opened new vistas in vaccine development. The design of vaccines has moved from empirical methods to rational approaches based on the understanding of host–pathogen interactions, structure–function analyses, mining of various databases, omics technologies, and bioinformatics [6]. Systems immunology approaches are attempting to understand the similarities and differences between immune responses to natural infection and vaccination to better define correlates of protection. Immunoinformatics (i.e. application of bioinformatics tools to answer questions related to immune responses) has revolutionized rational approaches to designing vaccines. Computational methods developed and refined based on experimental immunology and large data sets save

CONTACT Nagendra R. Hegde hegde@niab.org.in National Institute of Animal Biotechnology, 1-121/1, 4th & 5th Floors, Axis Clinicals Building, Miyapur, Hyderabad, Telangana 500049, India

Article Highlights

- Data mining has contributed greatly to the identification of vaccine targets
- Databases have considerable utility in determining biomarkers and pathways of immune responses to antigens
- Informatics combined with structural biology has significant potential for future vaccine design
- Prediction of T cell targets (mostly sequential epitopes) has been easier than prediction of B cell targets (mostly conformational epitopes)
- Adjuvant discovery is another area where immunoinformatics could influence vaccine development

This box summarizes key points contained in the article.

time and cost. Generic methodologies to this 'genome-to-vaccine' approach have been described by others [7–9]. Here, we present our views on the status and the future immunoinformatics holds for vaccine design and development.

2. A prelude to immunoinformatics

Immune responses can be innate or adaptive. Innate responses are triggered by pathogen-associated molecular patterns, which signal through pattern recognition receptors (PRRs), on responder cells to initiate a cascade of events to stimulate antibody- and cell-mediated responses, and eliminate the pathogen [10,11].

Antibodies are heterodimeric molecules, which fold to form a three-dimensional antigen-binding cavity. The binding groove is formed by the combination of hypervariable, complementarity-determining regions (CDRs) contributed by light and heavy chains of the antibody molecule. Side chains of amino acids in the CDR contact charged moieties on the antigen to form a complementarily fitting structure of the antigen with the antibody. The sequences of amino acids partaking in the interaction form epitope on the antigen and paratope on the antibody. This interaction between epitope and paratope is the quintessential target of antibody-based vaccine design using immunoinformatics.

Cell-mediated immune responses, on the other hand, are initiated by contact between cognate receptors and ligands on cells, and the effector molecules are different from these recognition molecules. Typically, the T cell receptors (TCRs) recognize major histocompatibility complex (MHC) molecules on antigen presenting cells, to be activated. A dichotomy in the way antigen is processed results in the presentation of antigens in the context of MHC Class I and Class II molecules to $CD8^+$ and $CD4^+$ T lymphocytes, respectively [12–14]. Between antigen recognition and effector function, a plethora of soluble mediators initiate, maintain, and regulate cellular and humoral responses [15]. Compartmentalization into subpopulations of cells adds another layer of complexity since a balance between these subpopulations and polarization toward one or another may define protection against certain pathogens. Since TCRs typically recognize short linear peptides (i.e. T cell epitope) on an antigen [12], peptide binding by MHC molecules is the only aspect of cellular immunity that can be interrogated by immunoinformatics.

The classical method of identification of B or T cell epitopes involves the identification of whole antigen first, followed by the assessment of its fragments for reactivity with antibodies or T cells. Despite advancement in high-throughput methodologies in qualitative and functional assays, these methods are still laborious, time-consuming, and expensive. The advent of omics approaches, availability of various databases based on individual parameters, structural analyses, computational capabilities, and automation to perform high-throughput assays to experimentally validate theoretical predictions have paved the way for faster development of vaccines [9,16]. In this context, protein microarrays have been employed for the identification of reactivity to antibodies as well as T cells [17–23], and these data can be applied to build bioinformatics tools for antigen discovery. Thus, applying bioinformatics to predict vaccine targets provides a focused approach where limited number of potential candidates can then be investigated for their involvement in immune response through directed experimental exploration.

3. Antibody-based vaccine design: where are we?

Despite a century of research on antigen-antibody interactions, *in silico* prediction of B cell epitopes remains complicated. Initial prediction algorithms depended on individual or a combination of propensities for amino acids to fit certain characteristics such as hydrophilicity, surface probability, helicity, flexibility, solvent accessibility and turns. The later machine-learning methods, which combined a variety of propensity scores with neighborhood matrices, conformational geometry, and statistics-supported likelihood criteria, have seen vast improvements [24,25]. However, predictive performance of these approaches has been demonstrated to be close to random [26], not to mention the fact that their use has been mostly limited to simple pathogens such as viruses. The availability of crystal structures of many proteins as well as some antigen-antibody complexes, and our ability to carry out docking and modeling studies have further contributed to improvements in the prediction of B cell epitopes [27,28]. Indeed, structure-based predictions outperform sequence-based predictions [29].

Recent data mining and omics approaches have been a boon to our ability to tackle pathogens, which are complex (e.g. bacteria, protozoa, viruses with large genomes), present hypervariable antigenic regions (e.g. HIV, hepatitis C virus, *Plasmodium*, etc.), or cause chronic infections or mimic host proteins (e.g. human cytomegalovirus, hepatitis C virus, staphylococci, streptococci). The first example of using immunoinformatics and reverse vaccinology was that of group B meningococcal vaccine, which was developed following the whole genome sequencing of the pathogen and subsequent series of immunomic and structure–function analyses [30–35]. An approach using genome screening, proteomics, and/or antigenomics also led to the identification of protective antigens of group A and group B streptococci [8,36–43]. Genomics and proteomics followed by secretome analyses have also led

to the discovery of conserved protective antigens against *Chlamydia pneumoniae* [44–46].

Findings from structure biology have also been instrumental in designing novel vaccine candidates. From a metastable, difficult to purify, weak, recombinant antigen, structure–function analyses yielded a highly stable respiratory syncytial virus glycoprotein capable of eliciting strong functional antibody responses [36,47–51]. Similar structure-guided engineering has been employed to produce potent, conformationally stable, and homogenous spike protein of Middle East respiratory syndrome coronavirus [52]. Broadly cross-reacting neutralizing antibodies (bNAb) have also recently been described against dengue virus in both infected and vaccinated individuals, and these have been shown to bind to regions which are irrelevant to serotypes [53–56]. Importantly, recent studies have shown the importance and utility of structure-guided studies to engineer high affinity bNAbs [57]. In another example, structure-based studies have not only revealed the determinants of thermolability of foot-and-mouth disease virus but also have helped in the development of more stable variants, which are not typically represented in natural infections, as vaccine candidates [58–63].

Immunoinformatics can be used to design strategies to induce bNAbs against hypervariable pathogens. High throughput *in vitro* assessment combined with structure-based functional analyses of antibody responses to influenza virus hemagglutinin have revealed two regions within the protein to be targets of bNAbs [36, 64–69]. Hemagglutinin stalk-based vaccine constructs have been shown to protect mice against challenge with a broad range of influenza A viruses [70–73]. In the case of HIV-1, studies have identified the V2- and V3-glycans of HIV-1 to be targets of bNAbs, and Env expressing V3-high mannose glycans could induce bNAb precursors in macaques [74,75]. The ultimate hope with these studies is the development of vaccines that (a) do not depend on annual prediction of influenza virus strains to be included in the vaccine formulation and the associated delay in production of seasonal influenza vaccines, and (b) target all variants and quasi-species of HIV-1. Whether these will be successful in humans is far from clear due the influence of environment, microbiota, genetic background, and history of past infections. However, it is expected that databases of various parameters from infected and vaccinated individuals and immunoinformatics can provide valuable details on the selection of immunogens, their properties, and parameters for vaccination strategies. One could also look forward to the design of computationally guided scaffolds onto which antibody target regions can be grafted, as has been shown recently [76,77].

Despite the advancements in the computational methods for prediction of antibody binding sites on antigens, the identification of conformational epitopes are best accomplished experimentally by e.g. studying escape mutants [78–80] or by using other techniques such as isotope exchange mass spectrometry or nuclear magnetic resonance spectroscopy, in combination with proteolytic fragmentation, epitope excision, and peptide panning [81–84]. Indeed, such methodologies provide fundamental data for building, verifying, and validating computational methods. And yet, prediction of B cell epitopes is far from ideal [85–87]. First, the prediction depends on three-dimensional structure of the antigen, and how the epitope is displayed on the surface of this structure. Although there has been tremendous advancement in our ability to predict protein structures, predicting the structure of bound antigen-antibody complexes is a relatively unchartered area. Added to this is the fact that co-crystals may not completely reflect binding in solution, since (a) the phenomena of intrinsic protein disorder and induced fit may be difficult to visualize, although it can be modeled based on minimal energy requirements, and (b) the influence of other physiochemical characteristics of the antigen, such as multimerization, posttranslational modification (e.g. glycosylation), folding (e.g. disulfide bridge), and charge (e.g. protonation of histidine), on function will need to be considered as well. However, biological consequence of antigen binding can be a continuum, which is not reflected by a dichotomous binding/nonbinding prediction [88]. In addition, it is difficult to predict affinity and avidity of an antibody to its cognate antigen, and further, complicated to equate this to the biological activity.

A second area of concern is the vaccine design itself. The best vaccines have been live or inactivated whole organisms or complex assembled structures. And yet, the simplicity of peptides makes them an attractive target for vaccine design, although not a single peptide vaccine has been commercialized despite half a century of research in this area [89]. The inability to form ordered structures could be a major reason for this. On the other hand, elicitation of antibodies reacting to peptides does not necessarily translate to reactivity with native antigen, and further, reflect on biological activity [88]. One way to address this is to rationally design scaffolds to present peptides which mimic spatial organization of epitopes, but production of reliable scaffolds is not a trivial issue. In addition, a fundamental problem with assessing correlates of protection is the fact that *in vitro* assays may not completely mirror biological activity *in vivo*, although this is an issue with the development of any vaccine. Furthermore, a multitude of conformation-dependent antibodies are more likely to be of *in vivo* functional relevance than a handful of antibodies specific to one or a mixture of a few peptides. Therefore, there is a need to redirect efforts to not only better predict conformational epitopes but also expand on structural and experimental data, both to support the predictions as well as to provide platforms to strengthen computation.

4. T-cell-based vaccine design: are we there yet?

Since T cells recognize linear peptides, the only information that is required for data mining to identify T cell epitopes is the way peptides, specifically the side chains of the amino acids of the epitope, fit into grooves of MHC proteins [90]. The size and location of the pockets represent the polymorphism of MHC molecules among a population. Although most of the MHC molecules can bind thousands of different peptides, the size and charge environment of the pockets dictate the kind of amino acid side chains that can fit, resulting in restrictions in the amino acids that can be present at specific positions of an epitope presented by each MHC allomorph [91]. Thus, availability of the structure of an MHC allomorph allows us to

predict not only the epitope but also the binding characteristics of the epitope to the specific allomorph. Several programs are available for the prediction of peptides binding to Class I or Class II MHC molecules, and these have been useful in the identification of $CD8^+$ or $CD4^+$ T cells, respectively [92]. In the case of MHC Class II, combining multiple prediction methods appear to be better than individual methods [93–95].

T cell antigens can be defined by using protein fragments or polypeptide pools. For practical considerations, these antigenic regions are sufficient, and may be better than minimal peptide epitopes, to elicit T cell responses. Nevertheless, delineation of T cell epitopes can reveal other information such as fine specificity and immunological dominance. Since the first identification of a minimal T cell epitope [96], a huge number of $CD4^+$ and $CD8^+$ T cell epitopes have been defined. A large proportion of these, including cross-clade epitopes of HIV, were identified through reverse immunogenetic or epitope-driven approaches [97,98]. It was later discovered that MHC polymorphism can be circumvented somewhat by combining several allotypes into a handful of supertypes [99] or by selecting promiscuous epitopes [100–102] to overcome MHC polymorphism. Epitopes delivered through various vehicles (plasmid DNA, virus vectors, etc.) were explored and shown to protect against challenge with the pertinent pathogen in experimental models. In addition, epitopes encoded in tandem (string-of-beads) [103] were envisaged and engineered to immunize against multiple antigens or pathogens simultaneously [104]. Bioinformatics has also been employed to identify regions within vaccine candidates that enhance regulatory responses, as evidenced by the enhancement in protective immunity when such epitopes were removed or modified [105].

In the field of oncology, besides contributing to accurate and early cancer detection, biomarker-based predictions, grading of tumors, identification of metastases, and predicting prognosis and survival of patients, data mining based on genomic, transcriptomic, proteomic, and imaging data sets have helped in the identification of new targets for cancer vaccines and therapy [106–110]. As tumor antigens are self-antigens, breaking immunological tolerance to these antigens is a difficult task, and hence, exploiting data mining for anti-tumor vaccine development requires close collaboration with adjuvant and other vaccinology areas. Also, several factors contribute to tumor escape; thus, gene expression profiles in particular cancer types, tumor microenvironment, frequency of specific effector T cells to tumor antigens, *in silico* prediction of antigen presentation by MHC and peptide selection, adjuvant selection, and monitoring of immune responses can benefit from data mining. Since the last few years, more than 60–70 clinical trials have been conducted every year in the field of oncology (ClinicalTrials.gov), and data from those trials are valuable in designing future vaccines. Thus, data mining can play a crucial role in discovery and development of cancer vaccines.

However, T-cell-based vaccine design has mainly remained experimental, possibly because: (a) cellular response to multiple epitopes and a combination of cellular and antibody responses are required in most cases, and (b) it is still not clear what exactly defines correlates of cell-mediated protection. In fact, evaluation of cellular immunity is much more complex than evaluating antibody responses, and assays that constitute evaluation of vaccine efficacy based on cellular immunity are a major focus of systems immunology research. In addition, a report has recently cautioned that extrapolating data obtained from self-antigen-derived peptides to prediction of antigenic peptides has its own limitations, requiring further fine-tuning [111].

5. Contribution of data mining and immunoinformatics to other areas of vaccine research

Data mining and immunoinformatics have also greatly facilitated adjuvant discovery, systems immunology, and pathogen engineering. Designing ligands to stimulate specific PRRs is an area of immunoinformatics, which is being employed to tweak adaptive responses as well as to potentiate or channelize responses through adjuvants.

Adjuvants are an integral part of vaccine formulation, especially given that several of the current vaccines use inactivated pathogen or subunits as antigen [2]. Application of informatics to vaccine adjuvant research spans receptor-ligand docking studies to analysis of gene expression, safety, mechanism of action, and correlates of effective vaccine adjuvant response [112]. The most investigated is the systematic discovery of small molecule adjuvants through targeting receptors implicated in initiating and/or regulating innate and/or adaptive immune responses. For example, *in silico* designing and/or homology modeling have been employed to develop toll-like receptor agonists as potential adjuvants [113–117]. A combination of homology modeling, molecular docking, and through-put screening *in vitro* and *in vivo* has also identified CCR4 receptor antagonists to enhance T cell and antibody responses [118,119], and CD1d agonists to activate invariant natural killer T cells [120]. Other computationally predicted adjuvant candidates include citrus-derived molecules [121] and microbe-derived macromolecules [122]. Vaccine adjuvant informatics, which encompasses collating diverse information ranging from structure to immune response to toxicity to function, and applying bioinformatics tools, and vaccine ontology, which attempts to integrate all data (both basic and clinical) related to vaccines in order to standardize annotation, and guide informatics-based reasoning [122,123], are nascent areas of research but have the potential to lead to the development of new and better adjuvants. Since it is highly challenging to predict optimal adjuvants for developing disease-specific vaccines, advanced computational and statistical algorithms provide researchers more capabilities to conduct adjuvant research.

Systems immunology, which includes transcriptomic, proteomic, and immunomic analysis of responses to infectious agents, vaccine antigens, and adjuvants, has recently revolutionized vaccine development. Importantly, attempts have been made to understand signature immunological markers during responses to natural infection versus following vaccination in order to better define correlates of protection, especially that of cell-mediated responses, and to identify early

Table 1. Online (web interphase) databases, tools, and resources for informatics and related aspects of basic and applied immunology.

Database	For mining and/or predicting	URL
Category: MHC Class I or Class II binding, MHC-related proteins, T-helper or cytotoxic T lymphocyte epitopes, proteasome cleavage, TAP binding, endogenous or exogenous antigens, adaptive immunity, transplantation and transfusion		
AFND	• Population frequency for human MHC, MIC-A, KIRs, cytokines • Adverse drug reactions associated with HLA alleles	http://www.allelefrequencies.net/
ATLAS	Repository of peptides interacting with MHC and TCR, and their binding affinities	https://zlab.umassmed.edu/atlas/web/
BIMAS	Human MHC peptide-binding prediction	https://www-bimas.cit.nih.gov/molbio/hla_bind/
BMDW	Compilation of HLA phenotypes and other relevant data of volunteer stem cell donors and cryopreserved cord blood products for bone marrow and related transfusions	https://www.bmdw.org/bmdw/about-bmdw
CTLPred	CTL epitopes	http://www.imtech.res.in/raghava/ctlpred/
dbMHC	Human MHC sequences, allele and haplotype frequencies, HLA testing; clinical data sets for certain diseases, and stem cell transplantation	https://www.ncbi.nlm.nih.gov/Web/Newsltr/Summer03/dbMHC.html
EpiDOCK	Molecular docking-based MHC Class II-binding prediction	http://epidock.ddg-pharmfac.net/EpiDockPage.aspx
EpiJen	Prediction based on proteasome cleavage, TAP restriction and binding to some HLA Class I	http://www.ddg-pharmfac.net/epijen/EpiJen/EpiJen.htm
EpiMatrix	Binding to MHC Class I and II, and T cell epitope prediction	http://www.epivax.com/immunogenicity-screening/epimatrix/
EPIMHC	Binding ligands for human, NHP, and other vertebrate MHC Class I and II, and non-classical MHC	http://imed.med.ucm.es/epimhc/
EpiPox	Poxvirus bioinformatics resource	http://bio.med.ucm.es/epipox/
EpiToolKit	MHC genotyping, epitope and neo-epitope prediction, epitope selection for vaccine design, and epitope assembly	http://www.epitoolkit.de/
EpiTOP	Prediction of peptide binding to 12 HLA-DRB alleles	http://www.pharmfac.net/EpiTOP/
Epitope prediction	Peptides binding to supertypes as well as individual alleles	http://boson.research.microsoft.com/bio/epipred.aspx
Expitope	Prediction of cross-reactive peptides to avoid in T cell therapy	http://webclu.bio.wzw.tum.de/expitope/
FDR4	Core and affinity of HLA-DRB*0401-binding peptides	http://crdd.osdd.net/raghava/fdr4/
FRED	T cell epitope prediction	http://fred-2.github.io/(desktop tool)
HLA-DR4Pred	Peptides binding HLA-DR1*0401	http://www.imtech.res.in/raghava/hladr4pred/
HLaffy	Structure-based HLA (Class I) peptide epitope affinity prediction	http://proline.biochem.iisc.ernet.in/HLaffy/
HLA assignment	Identification of HLA-restricted epitopes form ELISpot data	http://boson.research.microsoft.com/bio/hlaassign.aspx
HLAPRED	MHC Class I or Class II-binding peptides	http://crdd.osdd.net/raghava/hlapred/
HLA restrictor	Patient-specific predictions of HLA restriction and optimal peptide epitopes	http://www.cbs.dtu.dk/services/HLArestrictor/
HPVdb	Database and analytical tools for T cell epitope discovery for HPV	http://cvc.dfci.harvard.edu/hpv/
IDAG	Database of human MHC Class I and KIR	http://www.igdawg.org/
IEDB	Human and mouse MHC Class II binding	http://tools.immuneepitope.org/mhcii/
IFNepitope	IFN-g inducing epitopes	http://crdd.osdd.net/raghava/ifnepitope/
IHWG	Genomic reference lines, purified genomic DNA, DNA panels or individual HLA genes (in cDNA or transfectant cell line) for human MHC	https://www.fredhutch.org/en/labs/clinical/projects/ihwg.html
IL4pred	IL-4 inducing MHC Class II-binding peptides	http://crdd.osdd.net/raghava/il4pred/
KIR	Killer inhibitory receptors	https://www.ebi.ac.uk/ipd/kir/
MAPPP	Peptide processing (proteasome cleavage) for MHC Class I	http://www.mpiib-berlin.mpg.de/MAPPP/
MHC	MHC sequences from a number of different species	https://www.ebi.ac.uk/ipd/mhc/
MHCBENCH	Benchmarking of MHC-binding prediction algorithms through comparisons	http://www.imtech.res.in/raghava/mhcbench/
MHCBN	Experimentally determined MHC- or TAP-binding peptides and non-binders, T cell epitopes	http://www.imtech.res.in/raghava/mhcbn/
MHCcluster	Functional clustering of MHC Class I molecules based on binding specificity	http://www.cbs.dtu.dk/services/MHCcluster-2.0/
MHCMIR	Binding affinity of 13 HLA-DR and 3 mouse H2-IA alleles	
MHC2Pred	Promiscuous peptides binding to human, mouse, and rat MHC Class II	http://www.imtech.res.in/raghava/mhc2pred/
MMBPred	Mutated high affinity and promiscuous peptides binding to MHC Class I	http://www.imtech.res.in/raghava/mmbpred/
MPID-T2	Sequence-structure-function information for MHC-peptide interactions	http://biolinfo.org/mpid-t2/
NetChop	Cleavage specificities of human proteasome	http://www.cbs.dtu.dk/services/NetChop/
NetCTLpan	CTL epitopes in protein sequences	http://www.cbs.dtu.dk/services/NetCTLpan/
NetMHC	Peptides binding to human MHC Class I (both classical and non-classical)	http://www.cbs.dtu.dk/services/NetMHC/
NetMHCIIpan	Peptides binding to human and mouse MHC Class II	http://www.cbs.dtu.dk/services/NetMHCIIpan/
NetTepi	Prediction of T cell epitopes for 13 human MHC Class I supertypes	http://www.pharmfac.net/EpiTOP/
nHLAPred	MHC Class I-binding peptides	http://www.imtech.res.in/raghava/nhlapred/

(*Continued*)

Table 1. (Continued).

Database	For mining and/or predicting	URL
MULTIPRED2	Large-scale screening of allele-, genotype-, and supertype-specific HLA associated peptides	http://cvc.dfci.harvard.edu/multipred2/index.php
NMDP	HLA resources for marrow donor program of the US	https://bethematch.org/
NNAlign	Simultaneous identification of MHC Class II binding core and affinity	http://www.cbs.dtu.dk/services/NNAlign/
PAProC	Proteasomal cleavage	http://www.paproc.de/expl2.html
PolyCTL	Design of CTL-based polyepitope immunogens	http://tepredict.sourceforge.net/PolyCTLDesigner.html
POPISK	T cell reactivity of HLA-A2-binding peptides	http://140.113.239.45/POPISK/
PREDEP	MHC Class I-binding peptides	http://margalit.huji.ac.il/Teppred/mhc-bind/index.html
PREDIVAC	Prediction of peptide binding to MHC Class II, population coverage; database of validated peptides	http://predivac.biosci.uq.edu.au/#
Propred	Promiscuous MHC Class II-binding regions	http://www.imtech.res.in/raghava/propred/
RANKPEP	Peptides binding to mouse or human MHC Class I or II	http://imed.med.ucm.es/Tools/rankpep.html
Riken BRC DNA Bank	Sequences of HLA Class I genes and cDNA clones	http://dna.brc.riken.jp/en/GENESETBANK/HLA2en.html
SMM	High affinity HLA-A2-binding peptides	https://zlab.bu.edu/SMM/
SVMHC	Peptides binding to MHC Class I and II	http://www.sbc.su.se/~pierre/svmhc/
SVMTAP	TAP-binding peptides	https://abi.inf.uni-tuebingen.de/Services/SVMTAP
SVRMHC	Prediction of peptides binding to some human, mouse, monkey, and chimpanzees	http://c1.accurascience.com/SVRMHCdb/
SYFPEITHI	Ligands and peptide motifs (from eluted peptides) for humans, mouse, and rat MHC	http://www.syfpeithi.de/bin/MHCServer.dll/EpitopePrediction.htm
TABTIGEN	Human tumor antigen database	http://cvc.dfci.harvard.edu/tadb/
TAPpred	TAP-binding peptides	http://www.imtech.res.in/raghava/tappred/
TepiTool	Prediction of peptide binding to MHC Class I and II	http://tools.iedb.org/tepitool/
TEPITOPEpan	Peptides binding to HLA Class II	http://datamining-iip.fudan.edu.cn/service/TEPITOPEpan/TEPITOPEpan.html
Immunoglobulin (antibody) binding/structure/modeling/engineering, B cell epitopes (linear or conformational), adaptive immunity		
AAAAA	Antibody sequences, structure, modeling, engineering	http://www.bioc.uzh.ch/plueckthun/antibody/
ABangle	Orientation and angle of VH-VL	http://www.stats.ox.ac.uk/~dunbar/abangle/
ABCPred	B cell epitope (using artificial neural network)	http://www.imtech.res.in/raghava/abcpred/
ABG	3D structures of antibodies	http://www.ibt.unam.mx/vir/index.html
ABodyBuilder	Models of Ab Fv region	http://opig.stats.ox.ac.uk/webapps/sabdab-sabpred/Modelling.php
AgAbDb	Interaction between antigen and antibody, and prediction of epitope	http://196.1.114.46:8080/agabdb2/home.jsp
AntigenDB	Experimentally validated antigens from pathogens, their sequence, structure, and other data	http://www.imtech.res.in/raghava/antigendb/
BASIC	Assembly and determination of full-length sequence of B cell receptor in single B cells from scRNA sequence data	http://ttic.uchicago.edu/~aakhan/BASIC/
Bcepred	B cell epitope (using physicochemical properties)	http://www.imtech.res.in/raghava/bcepred/
BCPreds	B cell epitope prediction	http://ailab.ist.psu.edu/bcpred/
Bcipep	Experimentally determined B cell epitopes of antigenic proteins	http://www.imtech.res.in/raghava/bcipep/
bcRep	Analysis of B cell receptor data such as gene usage, mutations, clones, diversity	https://cran.r-project.org/web/packages/bcRep/
BepiPred	Linear B cell epitope prediction	http://www.cbs.dtu.dk/services/BepiPred/
BEpro	Conformational epitope prediction	http://pepito.proteomics.ics.uci.edu/index.html
bNAber	Database of broadly neutralizing anti-HIV antibodies, their potency, sequences, and 3-D profile	http://bnaber.org/
CBTOPE	Conformational B cell epitopes	http://www.imtech.res.in/raghava/cbtope/
CED	Conformation epitopes	http://immunet.cn/ced/
CEKEG	Conformational epitope prediction	http://cekeg.cs.ntou.edu.tw/
CIDB (SEREX)	Identification of Ab-binding sites through recombinant expression cloning	http://www2.licr.org/CancerImmunomeDB/
COBEPro	Continuous B cell epitope	http://scratch.proteomics.ics.uci.edu/
CombiNAber	Prediction and analysis of HIV neutralization by antibody combinations	https://www.hiv.lanl.gov/content/sequence/COMBINABER/combinaber.html
DIG IT	Ig variable domain sequences annotated with the type of antigen, the germline sequences, and pairing information between light and heavy chains	http://circe.med.uniroma1.it/digit/
DiscoTope	Prediction of discontinuous B cell epitopes from 3-D protein structures	http://www.cbs.dtu.dk/services/DiscoTope-2.0/
ElliPro	B cell epitope prediction	http://tools.immuneepitope.org/ellipro/(IEDB)
EPCES	Epitopes on protein surfaces	http://sysbio.unl.edu/EPCES/
EpIC	B cell epitope prediction	http://saphire.usask.ca/saphire/epic/
Epitome	Ag-Ab complex structure-inferred antigenic residues	https://www.rostlab.org/services/epitome/

(*Continued*)

Table 1. (Continued).

Database	For mining and/or predicting	URL
ePitope informatics	Antibody epitope discovery	http://www.epitope-informatics.com/
Epitopia	Immunogenic region in protein sequences or structures	http://epitopia.tau.ac.il/
EPSVR	B cell epitope prediction	http://sysbio.unl.edu/EPSVR/
Glycoepitope	Encyclopedia of carbohydrate epitopes and their antibodies	http://www.glycoepitope.jp/
Glydin	Ab epitopes against glycans, and visualization of relationships between epitopes based on monosaccharide composition	http://glycoproteome.expasy.org/epitopes/
Humanization bY design	Humanized murine antibodies	http://people.cryst.bbk.ac.uk/~ubcg07s/
IgBLAST	Sequences analysis for human, mouse, rat, rabbit, rhesus monkey Igs	https://www.ncbi.nlm.nih.gov/igblast/
IgDiscover	Analysis of antibody repertoires and identification of germline V genes	http://docs.igdiscover.se/en/latest/
IGPred	B cell epitopes for specific class of antibodies	http://www.imtech.res.in/raghava/igpred/
iHMMune-align	Alignment, identification of germline genes for human Ig H chain	http://cgi.cse.unsw.edu.au/~ihmmune/iHMMune/
JOINSOLVER	Analysis of V-D-J recombination (especially CDR3) of human Ig genes	https://joinsolver.niaid.nih.gov/
Kotai Antibody Builder	Ab Fv modeling	http://kotaiab.org/
LBEEP	Linear B cell epitope prediction	https://github.com/brsaran/LBEEP
LBTOPE	Linear B cell epitope prediction	http://www.imtech.res.in/raghava/lbtope/
MAP	Design of antibody variable domains	http://maranas.che.psu.edu/submission/maps.htm
NEP	Neutralization-based epitope prediction	https://exon.niaid.nih.gov/nep/#home
OptCDR	Design of Ab-binding pockets	http://maranas.che.psu.edu/submission/OptCDR_2.htm
PyIgClassify	Database of Ab CDR structure	http://dunbrack2.fccc.edu/pyigclassify/
PEASE	Epitope prediction on proteins based on antibody sequences	http://www.ofranlab.org/PEASE
Pep-3D-Search	B cell epitope prediction	http://kyc.nenu.edu.cn/Pep3DSearch/
PIGS	Modeling of variable domains	http://circe.med.uniroma1.it/pigs/
ROSIE	Prediction of Ab variable region (CDR loops)	http://antibody.graylab.jhu.edu/antibody
SAbPred	Structural analysis of antibodies, epitope prediction	http://opig.stats.ox.ac.uk/webapps/sabdab-sabpred/WelcomeSAbPred.php
SEPIa	Conformational epitope prediction	https://github.com/SEPIaTool/SEPIa
SEPPA	Conformational epitope prediction	http://lifecenter.sgst.cn/seppa/index.php
SVMTrip	Linear epitope prediction	http://sysbio.unl.edu/SVMTriP/
TabHu	Design of humanized antibody and building 3D structure	http://circe.med.uniroma1.it/tabhu/
VBASE2	Germline variable gene sequences from human Igs	http://www.vbase2.org/
Innate immunity, adjuvants, vaccine design		
ALGPred	Pattern recognition receptors and their ligands	http://www.imtech.res.in/raghava/algpred/
CancerTope	Genome-based personalized immunotherapy or vaccine against cancer	http://crdd.osdd.net/raghava/cancertope/
ESTDAB	Tumor cell lines, HLA type, antigens (predominantly melanoma)	https://www.ebi.ac.uk/ipd/estdab/
FLAVIdB	Data and analysis of flavivirus antigens	http://cvc.dfci.harvard.edu/flavi/
ImmunoBase	Genetics and genomics of immunologically related human diseases; metazoan immunity genes and orthologs	https://www.immunobase.org/
ImRNA	Immunomodulatory RNAs – adjuvants	http://crdd.osdd.net/raghava/imrna/
InnateDB	Platform to facilitate systems-level analyses of mammalian innate immunity networks, pathways, and genes	http://www.innatedb.ca/
Interferome	IFN-regulated genes	http://interferome.its.monash.edu.au/interferome/home.jspx
LRRsearch	Prediction of conformational NOD-like receptor family	http://www.lrrsearch.com/
Mosaic Vaccine Tool Suite	T cell epitope-based prediction for viral proteins	https://www.hiv.lanl.gov/content/sequence/MOSAIC/
MtbVeb	Vaccine against tuberculosis	http://www.imtech.res.in/raghava/mtbveb/
PolysacDB	Polysaccharide antigens and their antibodies	http://crdd.osdd.net/raghava/polysacdb/
PRRDB	Pattern recognition receptors and their ligands	http://www.imtech.res.in/raghava/prrdb/
VaccineDA	Oligo-deoxy-nucleotide-based adjuvants	http://crdd.osdd.net/raghava/vaccineda/
VaxiJen	Prediction of protective antigens of bacterial, viral, and tumor origin	http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html
VaxinPAD	Peptide-based vaccine adjuvants	http://crdd.osdd.net/raghava/vaxinpad/
	Multi-category databases	
ANARCI	Numbering amino acids of Ab and TCR variable domains	http://opig.stats.ox.ac.uk/webapps/sabdab-sabpred/ANARCI.php
AntiJen (JenPep)	Peptides binding to MHC Class I, MHC Class II, TAP, TCR (T cell epitope), Ig (B cell epitope); Immunological protein–protein interactions; peptide libraries	http://www.ddg-pharmfac.net/antijen/AntiJen/antijenhomepage.htm

(*Continued*)

Table 1. (Continued).

Database	For mining and/or predicting	URL
AntigenDB	Database of sequence, structure, origin, etc. of antigens with additional information such as B- and T cell epitopes, MHC binding, function, gene expression, post-translational modification, etc.	http://www.imtech.res.in/raghava/antigendb/
HCV Immunology	B-, T- helper and CTL-epitopes, epitope maps for HCV, HIV	https://hcv.lanl.gov/content/immuno/immuno-main.html
HIV Molecular Immunology	B-, T-helper, and CTL-epitopes for HIV; epitope variants; epitope maps; antibody neutralization indices	https://www.hiv.lanl.gov/content/immunology/
IEDB	B and T cell epitopes, MHC restriction of humans, nonhuman primates, other animal species for infectious diseases, allergy, cancer, autoimmunity, and transplantation	http://www.iedb.org/
ImmPort	Database and tools for the advancement of research in basic and clinical immunology	https://immport.niaid.nih.gov/home
Immunesapce	Database of various parameters of the human immune system in diverse populations	https://www.immunespace.org/
Kabat database	Proteins of immunological interest	ftp://ftp.ebi.ac.uk/pub/databases/kabat/
IMGT	• Sequences of human, nonhuman primate, canine and feline MHC; MHC structure, grooves and superfamilies • Sequence information, 3D structure, naming, analysis, and phylogeny of germline and rearranged Ig and TCR genes from human and other vertebrates • Antibody engineering • Monoclonal antibodies with clinical indications • Leucocyte receptor complex • Allergens, their cross-reactivities • Integrins	http://www.imgt.org/
ImmGen	Expression profiles of >250 mouse immune cell types, and data browsers	https://www.immgen.org/
ImmuSort	Variability and plasticity of human and mouse immune genes	http://immu.tipsci.com/Account/
EpiCombFlu	Database and exploration of conserved Ab and T cell epitopes, and their combination in various hosts against influenza viruses	http://14.139.240.55/influenza/home.html
IPD	Polymorphism in genes of the immune system • MHC of human, mouse, other vertebrates • KIR • Tumor cells	http://www.ebi.ac.uk/ipd/
dbLRC	Leucocyte receptor complex (NK receptors, KIR, Ig superfamily genes such as LILR, LAIR)	https://www.ncbi.nlm.nih.gov/gv/lrc/main.fcgi?cmd=init
LYRA	B cell and T cell receptor structure modeling	http://www.cbs.dtu.dk/services/LYRA/
MUGEN	Murine models of immune processes and immunological diseases	http://www.mugen-noe.org/
Protegen	Protective antigen database and analysis	http://www.violinet.org/protegen/
RefDIC	Quantitative transcript and protein expression profiles for immune cells and tissues	http://refdic.rcai.riken.jp/welcome.cgi
RSS	Recombination signal sequence in human and mouse chromosomes	http://www.itb.cnr.it/rss/index.html
VDJsolver	Analysis of V-J and V-D-J junctions for Ig and TCRs	http://www.cbs.dtu.dk/services/VDJsolver/
ViPR	Genomes, genes and proteins, epitopes, host factors, therapeutic molecules for viruses	https://www.viprbrc.org/brc/home.spg?decorator=vipr
	Systems Immunology	
BloodExpress	Database of gene expression in mouse hematopoiesis	http://hscl.cimr.cam.ac.uk/bloodexpress/
BloodSpot	Gene expression of human and mouse hematopoietic cells at different stages	http://servers.binf.ku.dk/bloodspot/
GPX-MEA	Transcriptome profile of a range of macrophage cell types following treatment with pathogens and immune modulators	http://gpxmea.gti.ed.ac.uk/
ImmGen	Gene networks of mouse hematopoiesis	http://www.immgen.org/
ImmQuant	Prediction of immune cell types in a tissue based on transcriptomic data	http://csgi.tau.ac.il/ImmQuant/
ImmuCo	Co-expression of genes in different human and mouse hematopoietic cells	http://immuco.bjmu.edu.cn/search.jsp
ImmunomeBase	Database of immunity genes and orthologs for metazoans	http://structure.bmc.lu.se/idbase/ImmunomeBase/index.php
Immunome Database	Database for genes and proteins of the human immune system	http://structure.bmc.lu.se/idbase/Immunome/index.php
ImmTree	Database of evolutionary relationships of proteins and genes of the human immune system	http://structure.bmc.lu.se/idbase/ImmTree/index.php
macrophages.com	Database of macrophage images, transcriptional analyses, protein expression, pathways, etc.	http://www.macrophages.com/

predictors of vaccine efficacy [65,124–126]. In addition, stimulation and polarization of immune responses by using specific adjuvants or delivery systems [16,127,128] have also accelerated approaches to induce directed immune functions. Furthermore, it has been suggested that biomarker screening could be used to rapidly identify adverse reactions to adjuvants [129–131].

Finally, omics investigations have been carried out to identify markers of attenuation in classically attenuated vaccines such as those for Japanese encephalitis, measles, mumps, polio, tuberculosis, varicella-zoster, yellow fever, etc. but definitive answers have been difficult to get. On the other hand, subtractive and deductive approaches to attenuate pathogens have involved systematic identification of target regions on pathogens based on experimental evidence on essential genes or virulence determinants (e.g. influenza, bluetongue, West Nile, rabies, dengue, herpes, etc.). Studies on pathogen biology and host–pathogen interactions based on omics approaches are likely to lead to the derivation of genetically defined attenuated pathogens for vaccine development. For example, expression profiling of pre-erythrocytic stages of *Plasmodium* parasite have identified genes which could be deleted to generate gene-deficient, attenuated parasites [132]. Another approach has involved antigenome analysis (mining of expressed antigens) of pathogenic versus nonpathogenic strains [133,134].

6. Summary

In silico analytical and predictive methods have greatly facilitated all aspects of biological research and the field of vaccinology is no exception. While early immunoinformatics work focused on prediction of antibody or T cell recognition regions within antigens, explosion in research in both experimental immunology and informatics and structural biology have in the last two decades contributed immensely to the application of bioinformatics to the understanding immune responses at a higher order, and to harness the outcome for the prediction of vaccine design and development. Although shortcomings remain in immunoinformatics approaches, recent developments support a view of bright future for immunoinformatics.

7. Expert opinion

Vaccine development has come a long way from variolation to reverse vaccinology and systems immunology. Application of bioinformatics to data mining and analysis, structure prediction, and intermolecular and pathway interactions have tremendously advanced, and have great potential to further contribute to, vaccine development. Several tools have been developed as well as employed by researchers to identify antigenic regions targeted by both antibodies and T cells, as well as for systems immunology (see Table 1). There have been efforts to integrate informatics with biological phenomena; however, the vertebrate immune system is a very complex homeostatic system. The application of immunoinformatics to vaccinology, therefore, requires knowledge of a broader biological perspective which includes host genetic background and population diversity, physiological status of the host, pathogen variation, antigenic drift and shift, host–pathogen interaction, environmental influences, microbiota, the complexity and adaptability of the immune system, comorbidities, delivery methods, the route and mode of immunization, and others.

One area that needs progress and implementation is benchmarking of the bioinformatic tools. For T cell epitopes, this has been easier because of the continuous nature of the epitopes; however, surrogate *in vitro* assays of *in vivo* T cell function will need to be identified. On the other hand, benchmarking bioinformatics tools for the prediction of antibody epitopes, most of which are discontinuous [135–139], needs improvement. Elucidation of more structures of bound antigen-antibody complexes might be the only answer to this, but could still fall short since we may not be able to define structural commonalities, owing to potential uniqueness of each antigen-antibody complex.

Computational biology depends on elucidation of biological phenomena through experimental observations. Since interrogation of biological phenomena is time-consuming, development of computational methods could face lag periods. On the other hand, computational methods could complement experimental biology by reducing investigations to arrive at definitive answers on biological phenomena, in turn reducing the time required for the cycle of discovery. Immunoinformatics is, therefore, a tremendous boost to experimental immunology, and vaccine design and evaluation, although the ultimate test of *in vivo* consequence in the target species is sometimes difficult to gauge.

Classical vaccine development approaches have been to either derive attenuated pathogens or to inactivate them. There have also been several subunit vaccines to combat bacterial diseases, and some viral vaccines which have been derived through recombinant approaches. However, time lines of vaccine development using these approaches have been prolonged, albeit variable depending on the type of vaccine, due both to (a) the time required for basic understanding of attenuation, or balancing inactivation versus immunogenicity, or technological hurdles in developing systems to mass produce antigens, and (b) the acceptance by regulatory bodies. Data mining and immunoinformatics have the potential to hasten the process of vaccine discovery, thereby enabling faster time to vaccine availability for deployment in society.

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Declaration of Interest

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ORCID

Nagendra R. Hegde http://orcid.org/0000-0001-5260-2159
Jagadeesh Bayry http://orcid.org/0000-0003-0498-9808

References

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Plotkin SLP. S.A. A short history of vaccination. In: Plotkin SAO, Orenstein W, Offit P, editor. Vaccines. 6th ed. Philadelphia (PA): Saunders; 2013. p. 1–13.
2. Rappuoli R. Bridging the knowledge gaps in vaccine design. Nat Biotechnol. 2007 Dec;25(12):1361–1366. PubMed PMID: 18066025.
3. Plotkin SA. Correlates of protection induced by vaccination. Clin Vaccine Immunol. 2010 Jul;17(7):1055–1065. PubMed PMID: 20463105; PubMed Central PMCID: PMC2897268.
4. Plotkin SA. Complex correlates of protection after vaccination. Clin Infect Dis. 2013 May;56(10):1458–1465. PubMed PMID: 23386629.
• **The article captures the multiplicity and redundancy of the mechanisms of immune functions involved in protection against pathogens, and drives home the point that one or more of these functions may be involved in protection.**
5. Plotkin SA, Gilbert PB. Nomenclature for immune correlates of protection after vaccination. Clin Infect Dis. 2012 Jun;54(11):1615–1617. PubMed PMID: 22437237; PubMed Central PMCID: PMC3348952.
• **The article attempts to standardize the terminology for use in defining correlates of protection following vaccination.**
6. Rappuoli R. Reverse vaccinology. Curr Opin Microbiol. 2000 Oct;3 (5):445–450. PubMed PMID: 11050440.
• **This and reference 8 succinctly describe vaccine design based on genomics data.**
7. Moise L, Moss SF, De Groot AS. Moving Helicobacter pylori vaccine development forward with bioinformatics and immunomics. Expert Rev Vaccines. 2012 Sep;11(9):1031–1033. PubMed PMID: 23151160.
8. Sette A, Rappuoli R. Reverse vaccinology: developing vaccines in the era of genomics. Immunity. 2010 Oct 29;33(4):530–541. PubMed PMID: 21029963; PubMed Central PMCID: PMC3320742.
• **This and reference 6 succinctly describe vaccine design based on genomics data.**
9. Vaishnav N, Gupta A, Paul S, et al. Overview of computational vaccinology: vaccine development through information technology. J Appl Genet. 2015 Aug;56(3):381–391. PubMed PMID: 25534541.
•• **Has information on reverse vaccinology (including a list of vaccine candidates developed and predicted), immunoinformatics databases and tools.**
10. Akira S, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. Cell. 2006 Feb 24;124(4):783–801. PubMed PMID: 16497588.
11. Turvey SE, Broide DH. Innate immunity. J Allergy Clin Immunol. 2010 Feb;125(2Suppl 2):S24–32. PubMed PMID: 19932920; PubMed Central PMCID: PMC2832725.
12. Blum JS, Wearsch PA, Cresswell P. Pathways of antigen processing. Annu Rev Immunol. 2013;31: 443–473. PubMed PMID: 23298205; PubMed Central PMCID: PMC4026165.
13. Neefjes J, Jongsma ML, Paul P, et al. Towards a systems understanding of MHC class I and MHC class II antigen presentation. Nat Reviews Immunol. 2011 Nov 11;11(12):823–836. PubMed PMID: 22076556.
14. Rossjohn J, Gras S, Miles JJ, et al. T cell antigen receptor recognition of antigen-presenting molecules. Annu Rev Immunol. 2015;33:169–200, PubMed PMID: 25493333
15. Zuniga EI, Macal M, Lewis GMet al. Innate and adaptive immune regulation during chronic viral infections. Annu Rev Virol. 2015 Nov;21:573–597. PubMed PMID: 26958929; PubMed Central PMCID: PMC4785831.
16. Bagnoli F, Baudner B, Mishra RP, et al. Designing the next generation of vaccines for global public health. Omics. 2011 Sep;15 (9):545–566. PubMed PMID: 21682594.
•• **An excellent review on the application of omics technologies and integrative biology on the development of next generation of vaccines for tackling complex diseases.**
17. Davies DH, Liang X, Hernandez JE, et al. Profiling the humoral immune response to infection by using proteome microarrays: high-throughput vaccine and diagnostic antigen discovery. Proc Natl Acad Sci USA. 2005 Jan 18;102(3):547–552. PubMed PMID: 15647345; PubMed Central PMCID: PMC545576.
• **This, along with references 20, 21, 22, and 23 describe the application of high throughput protein microarray analysis in the discovery of vaccine antigens to complex infectious diseases of humans.**
18. Gaseitsiwe S, Valentini D, Mahdavifar S, et al. Peptide microarray-based identification of Mycobacterium tuberculosis epitope binding to HLA-DRB1*0101, DRB1*1501, and DRB1*0401. Clin Vaccine Immunol. 2010 Jan;17(1):168–175. PubMed PMID: 19864486; PubMed Central PMCID: PMC2812096.
19. Jing L, Davies DH, Chong TM, et al. An extremely diverse CD4 response to vaccinia virus in humans is revealed by proteome-wide T-cell profiling. J Virol. 2008 Jul;82(14):7120–7134. PubMed PMID: 18480455; PubMed Central PMCID: PMC2446988.
20. Jing L, McCaughey SM, Davies DH, et al. ORFeome approach to the clonal, HLA allele-specific CD4 T-cell response to a complex pathogen in humans. J Immunol Methods. 2009 Aug 15;347(1–2):36–45. PubMed PMID: 19520082; PubMed Central PMCID: PMC2715169.
21. Liang L, Felgner PL. A systems biology approach for diagnostic and vaccine antigen discovery in tropical infectious diseases. Curr Opin Infect Dis. 2015 Oct;28(5):438–445. PubMed PMID: 26237545; PubMed Central PMCID: PMC4666702.
22. Magnan CN, Zeller M, Kayala MA, et al. High-throughput prediction of protein antigenicity using protein microarray data. Bioinformatics. 2010 Dec 01;26(23):2936–2943. PubMed PMID: 20934990; PubMed Central PMCID: PMC2982151.
23. Vigil A, Davies DH, Felgner PL. Defining the humoral immune response to infectious agents using high-density protein microarrays. Future Microbiol. 2010 Feb;5(2):241–251. PubMed PMID: 20143947; PubMed Central PMCID: PMC2841399.
24. El-Manzalawy Y, Honavar V. Recent advances in B-cell epitope prediction methods. Immunome Res. 2010 Nov 03;6(Suppl 2):S2. PubMed PMID: 21067544; PubMed Central PMCID: PMC2981878.
25. Sun P, Ju H, Liu Z, et al. Bioinformatics resources and tools for conformational B-cell epitope prediction. Comput Math Methods Med. 2013;2013:943636 PubMed PMID: 23970944; PubMed Central PMCID: PMC3736542;2013:943636.
• **Covers several topics such as prediction methods, databases, tools and their applications and the problems relevant to the prediction of B cell epitopes.**
26. Blythe MJ, Flower DR. Benchmarking B cell epitope prediction: underperformance of existing methods. Protein Science. 2005 Jan;14(1):246–248. PubMed PMID: 15576553; PubMed Central PMCID: PMC2253337.
• **This article examines the correlation between B cell epitope prediction methods and the wet lab experimental data, and shows that the methods of predicting B cell epitopes, which are mostly conformational, fall short of expectations.**
27. He L, Zhu J, Computational tools for epitope vaccine design and evaluation. PubMed PMID: 25837467; PubMed Central PMCID: PMC4456225. Curr Opin Virol. 2015 Apr;11:103–112.
• **Along with reference 28, this article presents how integration of experimental data and structure-based engineering can help antibody epitope design for human viral vaccines.**
28. Sela-Culang I, Ofran Y, Peters B. Antibody specific epitope prediction-emergence of a new paradigm. PubMed PMID: 25837466; PubMed Central PMCID: PMC4456244. Curr Opin Virol. 2015 Apr;11:98–102
29. Kringelum JV, Lundegaard C, Lund O, et al. Reliable B cell epitope predictions: impacts of method development and improved

benchmarking. PLoS Comput Biol. 2012;8(12):e1002829. PubMed PMID: 23300419; PubMed Central PMCID: PMC3531324.
•• **The authors show that prediction methods can be improved by applying proper benchmark definitions, with the potential for better B cell epitope discovery.**
30. Delany I, Rappuoli R, Seib KL. Vaccines, reverse vaccinology, and bacterial pathogenesis. Cold Spring Harb Perspect Med. 2013 May 01;3(5):a012476. PubMed PMID: 23637311; PubMed Central PMCID: PMC3633180.
•• **This, along with reference 32, provides excellent reviews on specific examples and successes of candidate vaccine development from genome information.**
31. Giuliani MM, Adu-Bobie J, Comanducci M, et al. A universal vaccine for serogroup B meningococcus. Proc Natl Acad Sci USA. 2006 Jul 18;103(29):10834–10839. PubMed PMID: 16825336; PubMed Central PMCID: PMC2047628.
32. Masignani V, Rappuoli R, Pizza M. Reverse vaccinology: a genome-based approach for vaccine development. Expert Opin Biol Ther. 2002 Dec 2;(8):895–905. PubMed PMID: 12517268.
•• **This, along with reference 30, provides excellent reviews on specific examples and successes of candidate vaccine development from genome information.**
33. Pizza M, Scarlato V, Masignani V, et al. Identification of vaccine candidates against serogroup B meningococcus by whole-genome sequencing. Science. 2000 Mar 10;287(5459):1816–1820. PubMed PMID: 10710308.
34. Scarselli M, Arico B, Brunelli B, et al. Rational design of a meningococcal antigen inducing broad protective immunity. Sci Transl Med. 2011 Jul 13;3(91):91ra62. PubMed PMID: 21753121.
•• **This is an excellent example of reverse vaccinology resulting in a vaccine candidate. Work described in references 31, 33, and 35 led to the work described in this reference.**
35. Tettelin H, Saunders NJ, Heidelberg J, et al. Complete genome sequence of Neisseria meningitidis serogroup B strain MC58. Science. 2000 Mar 10;287(5459):1809–1815. PubMed PMID: 10710307.
36. Dormitzer PR, Grandi G, Rappuoli R. Structural vaccinology starts to deliver. Nat Reviews Microbiol. 2012 Dec;10(12):807–813.
37. Giefing C, Meinke AL, Hanner M, et al. Discovery of a novel class of highly conserved vaccine antigens using genomic scale antigenic fingerprinting of pneumococcus with human antibodies. J Exp Med. 2008 Jan 21;205(1):117–131. PubMed PMID: 18166586; PubMed Central PMCID: PMC2234372.
38. Lauer P, Rinaudo CD, Soriani M, et al. Genome analysis reveals pili in Group B Streptococcus. Science. 2005 Jul 01;309(5731):105. PubMed PMID: 15994549.
39. Maione D, Margarit I, Rinaudo CD, et al. Identification of a universal Group B streptococcus vaccine by multiple genome screen. Science. 2005 Jul 01;309(5731):148–150. PubMed PMID: 15994562; PubMed Central PMCID: PMC1351092.
40. Margarit I, Rinaudo CD, Galeotti CL, et al. Preventing bacterial infections with pilus-based vaccines: the group B streptococcus paradigm. J Infect Dis. 2009 Jan 01;199(1):108–115. PubMed PMID: 19086816.
•• **This paper, the data for which was built from those in references 37, 38, and 39, describes reverse vaccinology approach to the identification of candidate vaccines for group B streptococcus.**
41. Mora M, Bensi G, Capo S, et al. Group A Streptococcus produce pilus-like structures containing protective antigens and Lancefield T antigens. Proc Natl Acad Sci USA. 2005 Oct 25;102(43):15641–15646. PubMed PMID: 16223875; PubMed Central PMCID: PMC1253647.
•• **This paper, the data for which was built from those in references 37, 38, and 39, describes reverse vaccinology approach to the identification of candidate vaccines for group B streptococcus.**
42. Nuccitelli A, Cozzi R, Gourlay LJ, et al. Structure-based approach to rationally design a chimeric protein for an effective vaccine against Group B Streptococcus infections. Proc Natl Acad Sci USA. 2011 Jun 21;108(25):10278–10283. PubMed PMID: 21593422; PubMed Central PMCID: PMC3121859.
43. Rodriguez-Ortega MJ, Norais N, Bensi G, et al. Characterization and identification of vaccine candidate proteins through analysis of the group A Streptococcus surface proteome. Nat Biotechnol. 2006 Feb;24(2):191–197. PubMed PMID: 16415855.
44. Kalman S, Mitchell W, Marathe R, et al. Comparative genomes of *Chlamydia pneumoniae* and *C. trachomatis*. Nat Genet. 1999 Apr;21(4):385–389. PubMed PMID: 10192388.
45. Stephens RS. Chlamydial genomics and vaccine antigen discovery. J Infect Dis. 2000 Jun;181(Suppl 3):S521–523. PubMed PMID: 10839752.
46. Thorpe C, Edwards L, Snelgrove R, et al. Discovery of a vaccine antigen that protects mice from *Chlamydia pneumoniae* infection. Vaccine. 2007 Mar 08;25(12):2252–2260. PubMed PMID: 17275142.
• **Describes the application of reverse vaccinology for the identification of vaccine candidates for chlamydial pneumonia.**
47. McLellan JS, Chen M, Joyce MG, et al. Structure-based design of a fusion glycoprotein vaccine for respiratory syncytial virus. Science. 2013 Nov 01;342(6158):592–598. PubMed PMID: 24179220; PubMed Central PMCID: PMC4461862.
48. McLellan JS, Yang Y, Graham BS, et al. Structure of respiratory syncytial virus fusion glycoprotein in the postfusion conformation reveals preservation of neutralizing epitopes. J Virol. 2011 Aug;85(15):7788–7796. PubMed PMID: 21613394; PubMed Central PMCID: PMC3147929.
49. Swanson KA, Settembre EC, Shaw CA, et al. Structural basis for immunization with postfusion respiratory syncytial virus fusion F glycoprotein (RSV F) to elicit high neutralizing antibody titers. Proc Natl Acad Sci USA. 2011 Jun 07;108(23):9619–9624. PubMed PMID: 21586636; PubMed Central PMCID: PMC3111287.
50. Graham BS. Vaccines against respiratory syncytial virus: the time has finally come. Vaccine. 2016 Jun 24;34(30):3535–3541. PubMed PMID: 27182820; PubMed Central PMCID: PMC4912855.
•• **Summarizes years of research on vaccines against childhood pneumonia caused by respiratory syncytial virus. Importantly, the paper reviews recent advances in the identification of structure-guided vaccine candidates, as described in references 47, 48, 49, and 50.**
51. Krarup A, Truan D, Furmanova-Hollenstein P, et al. A highly stable prefusion RSV F vaccine derived from structural analysis of the fusion mechanism. Nat Commun. 2015 Sep 03;6:8143. PubMed PMID: 26333350; PubMed Central PMCID: PMC4569726.
52. Pallesen J, Wang N, Corbett KS, et al. Immunogenicity and structures of a rationally designed prefusion MERS-CoV spike antigen. Proc Natl Acad Sci USA. 2017 Aug 29;114(35):E7348–E7357. PubMed PMID: 28807998.
53. Dejnirattisai W, Wongwiwat W, Supasa S, et al. A new class of highly potent, broadly neutralizing antibodies isolated from viremic patients infected with dengue virus. Nat Immunol. 2015 Feb;16(2):170–177. PubMed PMID: 25501631; PubMed Central PMCID: PMC4445969.
54. Rouvinski A, Guardado-Calvo P, Barba-Spaeth G, et al. Recognition determinants of broadly neutralizing human antibodies against dengue viruses. Nature. 2015 Apr 02;520(7545):109–113. PubMed PMID: 25581790.
•• **This paper, along with reference 53, describes the potential for universal dengue fever vaccine candidates through the identification of common determinants among serotypes.**
55. Smith SA, de Alwis AR, Kose N, et al. The potent and broadly neutralizing human dengue virus-specific monoclonal antibody 1C19 reveals a unique cross-reactive epitope on the bc loop of domain II of the envelope protein. mBio. 2013 Nov 19;4(6):e00873–13. PubMed PMID: 24255124; PubMed Central PMCID: PMC3870244.
56. Smith SA, de Alwis R, Kose N, et al. Human monoclonal antibodies derived from memory B cells following live attenuated dengue virus vaccination or natural infection exhibit similar characteristics. J Infect Dis. 2013 Jun 15;207(12):1898–1908. PubMed PMID: 23526830; PubMed Central PMCID: PMC3654755.
57. Robinson LN, Tharakaraman K, Rowley KJ, et al. Structure-guided design of an anti-dengue antibody directed to a non-

immunodominant epitope. Cell. 2015 Jul 30;162(3):493–504. PubMed PMID: 26189681; PubMed Central PMCID: PMC4758460.
58. Hegde NR, Maddur MS, Rao PP, et al. Thermostable foot-and-mouth disease virus as a vaccine candidate for endemic countries: a perspective. Vaccine. 2009 Apr 06;27(16):2199–2201. PubMed PMID: 19189856.
59. Mateo R, Luna E, Mateu MG. Thermostable variants are not generally represented in foot-and-mouth disease virus quasispecies. J Gen Virol. 2007 Mar;88(Pt 3):859–864. PubMed PMID: 17325358.
60. Mateo R, Luna E, Rincon V,et al. Engineering viable foot-and-mouth disease viruses with increased thermostability as a step in the development of improved vaccines. J Virol. 2008 Dec;82 (24):12232–12240. PubMed PMID: 18829763; PubMed Central PMCID: PMC2593342.
61. Rincon V, Rodriguez-Huete A, Lopez-Arguello S, et al. Identification of the structural basis of thermal liability of a virus provides a rationale for improved vaccines. Structure. 2014 Nov 04;22 (11):1560–1570. PubMed PMID: 25308865.
62. Scott KA, Kotecha A, Seago J, et al. SAT2 foot-and-mouth disease virus structurally modified for increased thermostability. J Virol. 2017 May 15;91(10):e02312-02316. PubMed PMID: 28298597; PubMed Central PMCID: PMC5411616
63. Scott KA, Rathogwa NM, Capozzo AV, et al. Evaluation of immune responses of stabilised SAT2 antigens of foot-and-mouth disease in cattle. Vaccine. 2017 Apr 18;35(40):5426-5433. PubMed PMID: 28431813.
64. Corti D, Voss J, Gamblin SJ, et al. A neutralizing antibody selected from plasma cells that binds to group 1 and group 2 influenza A hemagglutinins. Science. 2011 Aug 12;333(6044):850–856. PubMed PMID: 21798894.
65. Ekiert DC, Bhabha G, Elsliger MA, et al. Antibody recognition of a highly conserved influenza virus epitope. Science. 2009 Apr 10;324 (5924):246–251. PubMed PMID: 19251591; PubMed Central PMCID: PMC2758658.
66. Shriver Z, Trevejo JM, Sasisekharan R. Antibody-based strategies to prevent and treat influenza. Front Immunol. 2015;6:315. PubMed PMID: 26217334; PubMed Central PMCID: PMC4500096.
•• **Reviews broadly neutralizing antibodies, their targets, and potential for therapy of influenza.**
67. Steel J, Lowen AC, Wang TT, et al. Influenza virus vaccine based on the conserved hemagglutinin stalk domain. mBio. 2010 May 18; 1 (1). PubMed PMID: 20689752; PubMed Central PMCID: PMC2912658
68. Sui J, Hwang WC, Perez S,et al. Structural and functional bases for broad-spectrum neutralization of avian and human influenza A viruses. Nat Struct Mol Biol. 2009 Mar;16(3):265–273. PubMed PMID: 19234466; PubMed Central PMCID: PMC2692245.
69. Whittle JR, Zhang R, Khurana S, et al. Broadly neutralizing human antibody that recognizes the receptor-binding pocket of influenza virus hemagglutinin. Proc Natl Acad Sci USA. 2011 Aug 23;108 (34):14216–14221. PubMed PMID: 21825125; PubMed Central PMCID: PMC3161572.
70. Krammer F, Hai R, Yondola M,et al. Assessment of influenza virus hemagglutinin stalk-based immunity in ferrets. J Virol. 2014 Mar;88 (6):3432–3442. PubMed PMID: 24403585; PubMed Central PMCID: PMC3957929.
71. Krammer F, Palese P. Influenza virus hemagglutinin stalk-based antibodies and vaccines. Curr Opin Virol. 2013 Oct 3;3(5):521–530. PubMed PMID: 23978327; PubMed Central PMCID: PMC3804342.
•• **Summarizes research on universal influenza vaccine based on antibodies binding to the conserved stalk region of the hemagglutinin protein.**
72. Krammer F, Pica N, Hai R,et al. Chimeric hemagglutinin influenza virus vaccine constructs elicit broadly protective stalk-specific antibodies. J Virol. 2013 Jun;87(12):6542–6550. PubMed PMID: 23576508; PubMed Central PMCID: PMC3676110.
73. Margine I, Krammer F, Hai R,et al. Hemagglutinin stalk-based universal vaccine constructs protect against group 2 influenza A viruses. J Virol. 2013 Oct;87(19):10435–10446. PubMed PMID: 23903831; PubMed Central PMCID: PMC3807421.
74. Rusert P, Kouyos RD, Kadelka C,et al. Determinants of HIV-1 broadly neutralizing antibody induction. Nat Med. 2016 Nov;22(11):1260–1267. PubMed PMID: 27668936.
75. Saunders KO, Nicely NI, Wiehe K, et al. Vaccine elicitation of high mannose-dependent neutralizing antibodies against the V3-glycan broadly neutralizing epitope in nonhuman primates. Cell Rep. 2017 Feb 28;18(9):2175–2188. PubMed PMID: 28249163; PubMed Central PMCID: PMC5408352.
76. Azoitei ML, Correia BE, Ban YE, et al. Computation-guided backbone grafting of a discontinuous motif onto a protein scaffold. Science. 2011 Oct 21;334(6054):373–376. PubMed PMID: 22021856.
77. McLellan JS, Correia BE, Chen M, et al. Design and characterization of epitope-scaffold immunogens that present the motavizumab epitope from respiratory syncytial virus. J Mol Biol. 2011 Jun 24;409(5):853–866. PubMed PMID: 21549714; PubMed Central PMCID: PMC3107930.
78. Crowther JR, Farias S, Carpenter WC,et al. Identification of a fifth neutralizable site on type O foot-and-mouth disease virus following characterization of single and quintuple monoclonal antibody escape mutants. J Gen Virol. 1993 Aug;74(Pt 8):1547–1553. PubMed PMID: 8393912.
79. Erdmann N, Du VY, Carlson J,et al. HLA class-II associated HIV polymorphisms predict escape from CD4+ T cell responses. PLoS Pathog. 2015 Aug;11(8):e1005111. PubMed PMID: 26302050; PubMed Central PMCID: PMC4547780.
80. Ferreira HL, Lambrecht B, van Borm S,et al. Identification of a dominant epitope in the hemagglutinin of an Asian highly pathogenic avian influenza H5N1 clade 1 virus by selection of escape mutants. Avian Dis. 2010 Mar;54(1 Suppl):565–571. PubMed PMID: 20521695.
81. Clementi N, Mancini N, Criscuolo E, et al. Epitope mapping by epitope excision, hydrogen/deuterium exchange, and peptide-panning techniques combined with in silico analysis. Methods Mol Biol. 2014;1131:427–446. PubMed PMID: 24515481.
• **This, along with reference 82, describes methodologies used for epitope mapping.**
82. Gallagher ES, Hudgens JW. Mapping protein-ligand interactions with proteolytic fragmentation, hydrogen/deuterium exchange-mass spectrometry. Methods Enzymol. 2016;566:357–404. PubMed PMID: 26791987.
• **This, along with reference 81, describes methodologies used for epitope mapping.**
83. Saito NG, Paterson Y. Nuclear magnetic resonance spectroscopy for the study of B-cell epitopes. Methods. 1996 Jun;9(3):516–524. PubMed PMID: 8812707.
84. Wei H, Mo J, Tao L,et al. Hydrogen/deuterium exchange mass spectrometry for probing higher order structure of protein therapeutics: methodology and applications. Drug Discov Today. 2014 Jan;19(1):95–102. PubMed PMID: 23928097; PubMed Central PMCID: PMC4081482.
85. Bergmann-Leitner ES, Chaudhury S, Steers NJ, et al. Computational and experimental validation of B and T-cell epitopes of the *in vivo* immune response to a novel malarial antigen. PloS One. 2013;8(8): e71610. PubMed PMID: 23977087; PubMed Central PMCID: PMC3745447.
86. Greenbaum JA, Andersen PH, Blythe M,et al. Towards a consensus on datasets and evaluation metrics for developing B-cell epitope prediction tools. J Mol Recognit. 2007 Mar-Apr;20(2):75–82. PubMed PMID: 17205610.
87. Ponomarenko JV, Bourne PE. Antibody-protein interactions: benchmark datasets and prediction tools evaluation. BMC Struct Biol. 2007 Oct 02;7:64.
88. Caoili SE. Benchmarking B-cell epitope prediction for the design of peptide-based vaccines: problems and prospects. J Biomed Biotechnol. 2010;2010:910524. PubMed PMID: 20368996; PubMed Central PMCID: PMC2847767.
• **Describes parameters and their implications for benchmarking B cell epitopes.**
89. Sirskyj D, Diaz-Mitoma F, Golshani A,et al. Innovative bioinformatic approaches for developing peptide-based vaccines against

hypervariable viruses. Immunol Cell Biol. 2011 Jan;89(1):81–89. PubMed PMID: 20458336.
90. Madden DR. The three-dimensional structure of peptide-MHC complexes. Annu Rev Immunol. 1995;13:587–622. PubMed PMID: 7612235.
91. Harris PE. Self-peptides bound to HLA molecules. Crit Rev Immunol. 1994;14(1):61–87. PubMed PMID: 7741977.
92. Singh SP, Mishra BN. Major histocompatibility complex linked databases and prediction tools for designing vaccines. Hum Immunol. 2016 Mar;77(3):295–306. PubMed PMID: 26585361.
93. Karpenko O, Huang L, Dai Y. A probabilistic meta-predictor for the MHC class II binding peptides. Immunogenetics. 2008 Jan;60(1):25–36. PubMed PMID: 18092156.
94. Mallios RR. A consensus strategy for combining HLA-DR binding algorithms. Hum Immunol. 2003 Sep;64(9):852–856. PubMed PMID: 12941539.
95. Wang P, Sidney J, Dow C, et al. A systematic assessment of MHC class II peptide binding predictions and evaluation of a consensus approach. PLoS Comput Biol. 2008 Apr 04;4(4):e1000048. PubMed PMID: 18389056; PubMed Central PMCID: PMC2267221.
96. Townsend AR, Rothbard J, Gotch FM, et al. The epitopes of influenza nucleoprotein recognized by cytotoxic T lymphocytes can be defined with short synthetic peptides. Cell. 1986 Mar 28;44(6):959–968. PubMed PMID: 2420472.
• **This paper was the first to identify a minimal T cell epitope through the classical approach.**
97. Davenport MP, Hill AV. Reverse immunogenetics: from HLA-disease associations to vaccine candidates. Mol Med Today. 1996 Jan;2(1):38–45. PubMed PMID: 8796850.
98. De Groot AS, Bosma A, Chinai N, et al. From genome to vaccine: *in silico* predictions, *ex vivo* verification. Vaccine. 2001 Aug 14;19(31):4385–4395. PubMed PMID: 11483263.
99. Sette A, Sidney J. HLA supertypes and supermotifs: a functional perspective on HLA polymorphism. Curr Opin Immunol. 1998 Aug;10(4):478–482. PubMed PMID: 9722926.
100. Panina-Bordignon P, Demotz S, Corradin G, et al. Study on the immunogenicity of human class-II-restricted T-cell epitopes: processing constraints, degenerate binding, and promiscuous recognition. Cold Spring Harb Symp Quant Biol. 1989;54(Pt 1):445–451. PubMed PMID: 2484166.
101. Panina-Bordignon P, Tan A, Termijtelen A,et al. Universally immunogenic T cell epitopes: promiscuous binding to human MHC class II and promiscuous recognition by T cells. Eur J Immunol. 1989 Dec;19(12):2237–2242. PubMed PMID: 2481588.
102. Van Den Berg HA, Molina-Paris C, Sewell AK. Specific T-cell activation in an unspecific T-cell repertoire. Sci Prog. 2011;94(Pt3):245–264. PubMed PMID: 22026148.
103. Whitton JL, Sheng N, Oldstone MB, et al. A "string-of-beads" vaccine, comprising linked minigenes, confers protection from lethal-dose virus challenge. J Virol. 1993 Jan;67(1):348–352. PubMed PMID: 7677954; PubMed Central PMCID: PMC237369.
104. An LL, Whitton JL. A multivalent minigene vaccine, containing B-cell, cytotoxic T-lymphocyte, and Th epitopes from several microbes, induces appropriate responses *in vivo* and confers protection against more than one pathogen. J Virol. 1997 Mar;71(3):2292–2302. PubMed PMID: 9032365; PubMed Central PMCID: PMC191338.
•• **This study, built upon that described in reference 103, demonstrated that multiple linear epitopes from a number of pathogens could be strung together in a single construct to induce relevant immune responses against respective pathogens in animal models.**
105. De Groot AS, Moise L, Liu R, et al. Immune camouflage: relevance to vaccines and human immunology. Hum Vaccin Immunother. 2014;10(12):3570–3575. PubMed PMID: 25483703; PubMed Central PMCID: PMC4514035.
106. Goldman M, Craft B, Swatloski T,et al. The UCSC Cancer Genomics Browser: update 2015. Nucleic Acids Res. 2015 Jan;43(Database issue):D812–7. PubMed PMID: 25392408; PubMed Central PMCID: PMC4383911.
107. Klonowska K, Czubak K, Wojciechowska M, et al. Oncogenomic portals for the visualization and analysis of genome-wide cancer data. Oncotarget. 2016 Jan 05;7(1):176–192. PubMed PMID: 26484415; PubMed Central PMCID: PMC4807991.
108. Lambin P, Leijenaar RTH, Deist TM, et al. Radiomics: the bridge between medical imaging and personalized medicine. Nat Reviews Clin Oncology. 2017 Oct 04;14:749–762. PubMed PMID: 28975929.
109. Li J, Lu Y, Akbani R,et al. TCPA: a resource for cancer functional proteomics data. Nat Methods. 2013 Nov;10(11):1046–1047. PubMed PMID: 24037243; PubMed Central PMCID: PMC4076789.
110. Xiong Y, Wei Y, Gu Y, et al. DiseaseMeth version 2.0: a major expansion and update of the human disease methylation database. Nucleic Acids Res. 2017 Jan 04;45(D1):D888–895. PubMed PMID: 27899673; PubMed Central PMCID: PMC5210584.
111. Vaughan K, Xu X, Caron E, et al. Deciphering the MHC-associated peptidome: a review of naturally processed ligand data. Expert Rev Proteomics. 2017 Aug;11:1–8. PubMed PMID: 28756714.
112. He Y. Vaccine adjuvant informatics: from data integration and analysis to rational vaccine adjuvant design. Front Immunol. 2014;5:32. PubMed PMID: 24550916; PubMed Central PMCID: PMC3909948.
113. Gentile F, Deriu MA, Licandro G, et al. Structure based modeling of small molecules binding to the TLR7 by atomistic level simulations. Molecules. 2015 May 08;20(5):8316–8340. PubMed PMID: 26007168.
114. Savar NS, Bouzari S. In silico study of ligand binding site of toll-like receptor 5. Adv Biomed Res. 2014;3:41. PubMed PMID: 24627849; PubMed Central PMCID: PMC3949343.
115. Wu TY, Singh M, Miller AT, et al. Rational design of small molecules as vaccine adjuvants. Sci Transl Med. 2014 Nov 19;6(263):263ra160. PubMed PMID: 25411473.
116. Yu H, Jin H, Sun L, et al. Toll-like receptor 7 agonists: chemical feature based pharmacophore identification and molecular docking studies. PloS One. 2013;8(3):e56514. PubMed PMID: 23526932; PubMed Central PMCID: PMC3603940.
117. Zatsepin M, Mattes A, Rupp S, et al. Computational discovery and experimental confirmation of TLR9 receptor antagonist leads. J Chem Inf Model. 2016 Sep 26;56(9):1835–1846. PubMed PMID: 27537371.
118. Bayry J, Tchilian EZ, Davies MN, et al. In silico identified CCR4 antagonists target regulatory T cells and exert adjuvant activity in vaccination. Proc Natl Acad Sci USA. 2008 Jul 22;105(29):10221–10226. PubMed PMID: 18621704; PubMed Central PMCID: PMC2481334.
119. Davies MN, Bayry J, Tchilian EZ, et al. Toward the discovery of vaccine adjuvants: coupling in silico screening and in vitro analysis of antagonist binding to human and mouse CCR4 receptors. PloS One. 2009 Nov 30;4(11):e8084. PubMed PMID: 20011659; PubMed Central PMCID: PMC2787246.
120. De Spiegeleer A, Wynendaele E, Vandekerckhove M, et al. An in silico approach for modelling T-helper polarizing iNKT cell agonists. PloS One. 2014;9(1):e87000. PubMed PMID: 24498010; PubMed Central PMCID: PMC3909045.
121. Pappalardo F, Fichera E, Paparone N, et al. A computational model to predict the immune system activation by citrus-derived vaccine adjuvants. Bioinformatics. 2016 Sep 01;32(17):2672–2680. PubMed PMID: 27162187.
122. Sayers S, Ulysse G, Xiang Z, et al. Vaxjo: a web-based vaccine adjuvant database and its application for analysis of vaccine adjuvants and their uses in vaccine development. J Biomed Biotechnol. 2012;2012:831486. PubMed PMID: 22505817; PubMed Central PMCID: PMC3312338.
123. He Y. Ontology-supported research on vaccine efficacy, safety and integrative biological networks. Expert Rev Vaccines. 2014 Jul;13(7):825–841. PubMed PMID: 24909153; PubMed Central PMCID: PMC4815432.
124. Maertzdorf J, Kaufmann SH, Weiner J 3rd. Molecular signatures for vaccine development. Vaccine. 2015 Sep 29;33(40):5256–5261. PubMed PMID: 25858856.

125. Nakaya HI, Li S, Pulendran B. Systems vaccinology: learning to compute the behavior of vaccine induced immunity. Wiley Interdiscip Rev Syst Biol Med. 2012 Mar-Apr;4(2):193–205. PubMed PMID: 22012654; PubMed Central PMCID: PMC3288517
126. Pulendran B, Li S, Nakaya HI. Systems vaccinology. Immunity. 2010 Oct 29;33(4):516–529. PubMed PMID: 21029962; PubMed Central PMCID: PMC3001343.
•• **References 124, 125, and 126 provide excellent reviews on systems immunology.**
127. Grimm SK, Ackerman ME. Vaccine design: emerging concepts and renewed optimism. Curr Opin Biotechnol. 2013 Dec;24(6):1078–1088. PubMed PMID: 23474232; PubMed Central PMCID: PMC3696422.
128. Olafsdottir T, Lindqvist M, Harandi AM. Molecular signatures of vaccine adjuvants. Vaccine. 2015 Sep 29;33(40):5302–5307. PubMed PMID: 25989447.
• **Briefly reviews the application of omics technologies to evaluate various aspects of immunological adjuvants.**
129. Poland GA, Ovsyannikova IG, Jacobson RM. Adversomics: the emerging field of vaccine adverse event immunogenetics. Pediatr Infect Dis J. 2009 May;28(5):431–432. PubMed PMID: 19395950; PubMed Central PMCID: PMC2843136.
130. Poland GA, Ovsyannikova IG, Kennedy RB. Personalized vaccinology: a review. Vaccine. 2017 Jul 31. PubMed PMID: 28774561.
131. Whitaker JA, Ovsyannikova IG, Poland GA. Adversomics: a new paradigm for vaccine safety and design. Expert Rev Vaccines. 2015 Jul;14(7):935–947. PubMed PMID: 25937189; PubMed Central PMCID: PMC4630804
•• **References 129, 130, and 131 present the importance of adverse events to vaccines.**
132. Mueller AK, Labaied M, Kappe SH, et al. Genetically modified Plasmodium parasites as a protective experimental malaria vaccine. Nature. 2005 Jan 13;433(7022):164–167. PubMed PMID: 15580261.
133. Moriel DG, Bertoldi I, Spagnuolo A, et al. Identification of protective and broadly conserved vaccine antigens from the genome of extraintestinal pathogenic Escherichia coli. Proc Natl Acad Sci USA. 2010 May 18;107(20):9072–9077. PubMed PMID: 20439758; PubMed Central PMCID: PMC2889118.
134. Rasko DA, Rosovitz MJ, Myers GS,et al. The pangenome structure of Escherichia coli: comparative genomic analysis of E. coli commensal and pathogenic isolates. J Bacteriol. 2008 Oct;190(20):6881–6893. PubMed PMID: 18676672; PubMed Central PMCID: PMC2566221.
135. Barlow DJ, Edwards MS, Thornton JM. Continuous and discontinuous protein antigenic determinants. Nature. 1986 Aug 21–27;322 (6081):747–748. PubMed PMID: 2427953.
136. Rubinstein ND, Mayrose I, Halperin D,et al. Computational characterization of B-cell epitopes. Mol Immunol. 2008 Jul;45(12):3477–3489. PubMed PMID: 18023478.
137. Rubinstein ND, Mayrose I, Pupko T. A machine-learning approach for predicting B-cell epitopes. Mol Immunol. 2009 Feb;46(5):840–847. PubMed PMID: 18947876.
138. Van Regenmortel MH. Antigenicity and immunogenicity of synthetic peptides. Biologicals. 2001 Sep-Dec;29(3–4):209–213. PubMed PMID: 11851317.
139. Walter G. Production and use of antibodies against synthetic peptides. J Immunol Methods. 1986 Apr 17;88(2):149–161. PubMed PMID: 2420900.