Computational vaccinology and epitope vaccine design by immunoinformatics

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COMPUTATIONAL VACCINOLOGY AND EPITOPE VACCINE DESIGN BY IMMUNOINFORMATICS

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Human immune system includes variety of different cells and molecules correlating with other body systems. These instances complicate the analysis of the system; particularly in postgenomic era by introducing more amount of data, the complexity is increased and necessity of using computational approaches to process and interpret them is more tangible.

Immunoinformatics as a subset of bioinformatics is a new approach with variety of tools and databases that facilitate analysis of enormous amount of immunologic data obtained from experimental researches. In addition to directing the insight regarding experiment selections, it helps new thesis design which was not feasible with conventional methods due to the complexity of data. Considering this features immunoinformatics appears to be one of the fields that accelerate the immunological research progression.

In this study we discuss advances in genomics and vaccine design and their relevance to the development of effective vaccines furthermore several division of this field and available tools in each item are introduced.

Keywords: immunoinformatics, new vaccine design, immunology

Introduction

The advantages of computational strategies to expedite biological researches and immunology in particular have been become conspicuous in today's

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biology. However recent advancements in genomics and proteomics technologies fundamentally altered the circumstances and bioinformatics insinuates itself into every corner of biological researches. Sequencing of human and other species genomes along with extensive progress in various scientific areas not only have provided vast amounts of data for immunology researches, but also created novel field of "Immunomics" which refers to genes and proteins involved merely in immune response [1]. In other words, the study of all immunological responses and regulations of a given host interacting with a pathogen or antigens known as immunomics [2]. Immunomics like genomics and proteomics is an interdisciplinary field using high throughput methods to understand immune system [3, 4]. The knowledge provided by immunomics reflects the current status of human immunology and disease. This is a unique source for those researchers looking for a deep cognition of mechanisms involved in immune system and disease pathogenesis. In fact the need for management of growing immunological resources necessitates establishment of a new field capable of organizing this amount of data. Computational methods as rising research trends marked to be amenable tools to speed up biological investigations including the field of immunology using their ability to data management, making predictions, and finding rational connections. Immunoinformatics emerged as a novel computational field capable of coping with challenges lie ahead of immunomics and its subdomains. Nowadays immunoinformatics or in other words immunology using computational sciences became an inevitable part of modern immunological researches. This field links computer sciences with exprimental immunology and actually is a branch of bioinformatics focusing on immunology [5]. Immunoinformatics is scientific setting in which computational methods and sources are exploited to comprehend, produce, process, and develop immunological data [6]. Historically 90 years ago immunoinformatics attempts initiated with theoretical modeling of malaria epidemic while employing mathematical models was a common method to conduct studies of disease transmission [7]. Thereafter, this field has been expanded aiming every aspect of immunology and diseases [8]. The augmentation occurred regarding the number of novelsoftwares and databases developed based on immunological data is a fair indication of immunoinformatics far-reaching impact on immunological studies. Immunogenomics, immunoproteomics, epitope prediction and *in silico* vaccination are different sub branches forming computational immunology studies. Moreover systems biology approaches is used to investigate dynamic nature of immune system network [9] providing novel set of data applicable as computational immunology resources.

Developing B and T cell epitope prediction algorithms is one of the main applications of immunoinformatics. Accurate prediction of B and T cell epitopes in turn would result in decreased time and costs required for experimental analysis. Employing such methods provides immunologists with profound concepts of immune system and antigen interactions which lead to development of new vaccines. Reverse vaccinology is a technology based on the idea of whole genome analysis to identify genes which could be used as appropriate antigens for vaccine development. It employs the same algorithms designed by immunoinformatics to increase the number of potential vaccine targets and revealing their T and B cell epitopes [10]. Since conventional methods require costly and time consuming procedures such as pathogen culture and antigenic protein extraction to find novel vaccine targets, they could be superseded by more advantageous *in silico* methods offered by immunoinformatics. Immunoinformatics procedures seem to provide an alternative path through which pathogenic genes and surface proteins could be invstigated [9]. Figure 1 briefly represents various branches of current immunoinformatics. Although immune system is interconnected with other body systems, bioinformatics applications are most expanded in areas such as databases [11], genomic applications [12], epitope prediction [13], and modeling of immune responses [14]. Other fields of immunology like allergenicity analysis of proteins [15] and proteomics [16], in contrast to above-mentioned fields, are in their primary stages of bioinformatics applications (Fig. 1).

In this study, the field of immunoinformatics was outlined and its various applications and are explained. Various databases, prediction tools, and challenges in each sub-branch is inspected.

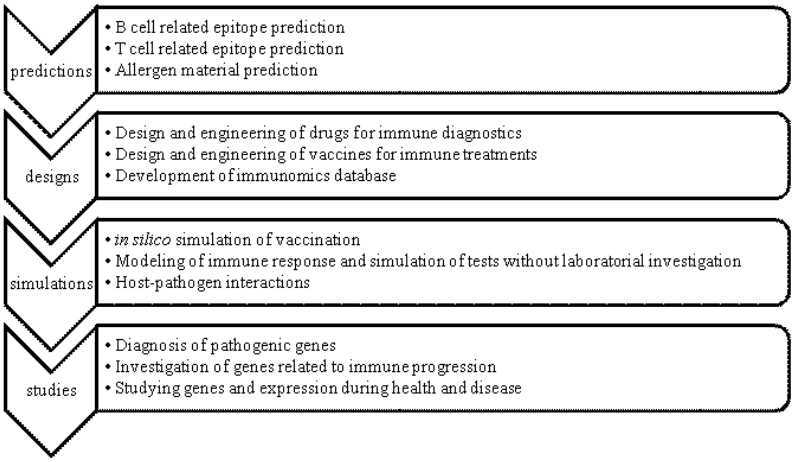

Figure 1. Immunoinformatics and different sections of immunology

Immunoinformatics and epitope prediction

Immune system is the defensive barrier of vertebrates against foreign infectious organisms. Innate immune responses are the primary mechanism activated to protect the host from infection. This mechanism includes rapid and non-specific responses against some conserved non-self-molecules which are molecules associated with groups of pathogens. These molecules are referred as pathogen-associated molecular patterns, or PAMPs, such as lipopolysaccharide of bacterial cell walls or flagella proteins [17]. Secondary barrier of immune system is adaptive immunity that responses specifically against each infectious agent. Memory cells differentiated and lingered from first encounter with infectious agent can elicit a rapid and strong response in the case of another encounter. Adaptive immunity has two arms: cellular and humoral immune systems that T and B lymphocytes are responsible cells for, respectively. Immune response is triggered in both cases with recognition of a fragment of antigen called epitope. Antibodies usually recognize intact proteins. B cell epitopes could be continuous (linear) or non-continuous (conformational). Conformational epitopesare spatially separated segments brought into proximity during folding of protein. More than 90 percent of B cell epitopes are postulated to be conformational. Even linear epitopes are usually structure dependent and proper antigen–antibody interaction would be achieved in effect of correct folding of epitopes. Predicting conformational B cell epitopes is more complex due to need for contemplating antigens 3-dimensional structure. The relative number of linear and conformational B cell epitope predictors reflects this fact. T cell epitopes are linear short peptides processed out of antigenic proteins [18, 19]. These epitopes are presented on Major Histocompatibility Complex (MHC) and Human Leukocyte Antigen (HLA) classes 1 and 2, in mice and human, respectively. Epitope presentation depends on MHC and peptide along with T Cell Receptor (TCR) interactions [20, 21]. MHC proteins are polymorphic and each of them interacts with specific groups of peptides restricted to that MHC. Exposed set of MHC complexes of host cells determines potential eptitopes would be exposed to immune system during infections. Structures of T cell epitopes on MHC proteins are critical for recognition by TCR [22, 23]. T cells can be categorized based on expression of CD4 and CD8 proteins each of which could recognize displayed epitopes on MHC I and II proteins. $CD8^+$ T cells can induce virus infected cells to undergo apoptosis [24]. This function of $CD8^+$ cells relays on previous encounter with particular antigen [25]. $CD4^+$ cells produce cytokines that regulate other parts of immune system. However, $CD4^+$ cells can induce apoptosis [24] and $CD8^+$ cells can produce regulatory molecules alternatively. To be recog-

nized by $CD4^+$ T cells antigens should be engulfed into antigen presenting cells and proteolyzed into peptide fragments capable of interacting with MHC class 2. Thereupon MHC-Epitope complexes could be transferred onto cell surface to be recognized with $CD4^+$ TCRs [10]. Viral and endogenous antigens (proteins inside cytoplasm that are fragmented from C-terminal [26] with immunoproteasomes [27] are usually recognized with $CD8^+$ T cells [28]. Epitopes eventually displayed in association with MHC II could be possible targets for $CD8^+$ T cells [27, 29, 30]. Both B and T cells are antigen specific and any mutation within the epitopes could have noticeable impact on MHC-TCR or Antigen-Antibody interactions.

On one hand knowledge about the mechanisms of B and T cell responses is rapidly growing on the other hand immune responses against cancerous cells or pathogens are being elucidated employing various methods. While pieces of immunological puzzle are found one after another in scientific literatures, collection and classification of this knowledge and its storage as databases is of great worth. Moreover developing computational tools to interpret them would help to have a better concept of what happens in an immune response. Databases of B and T cell epitopes, bioinformatics tools, and prediction algorithms would be helpful understanding structure and sequence of epitopes. Such knowledge would be essential in basic immunology studies, diagnostics, treatment of various diseases and vaccinology researches [31]. A list of databases and prediction tools of B and T cell epitopes are represented Tables I–IV.

Allergens

Allergy is an immunological hypersensitivity reaction to substances normally considered as harmless [80]. Recent studies reveals that allergy have become a serious problem in industrial countries where involves a considerable portion of population [81–83]. In fact, allergy is the most important chronic disease in industrial countries [84]. This fact makes the allergy as one of highest priority research fields and growing data sources. Coping with such an active research field necessitates developing immunoinformatics tools which could shed light on this field. Creating new databases and allergen prediction tools would help recognition of new or potential protein and non-protein allergens [80].

Qualitative data management, B and T cell epitope prediction, allergenecity, and allergic interactions are primary focus of allergy informatics. Currently allergenicity analyses are still in their initial stages. World Health Organization and Farmery and Agriculture Organization suggested guidelines regarding

Table I

Databases of B cell epitopes

Database name	Brief introduction	URL	References
ABcheck	Evaluates DNA sequence of existing antibodies in Kabat database, so sequencing and cloning errors could be recognized.	http://www.bioinf.org.uk/abs/seqtest.html	[32]
AntiJen	This database contains qualitative data related to epitopes of different antigens.	http://www.ddg-pharmfac.net/antijen/AntiJen/antijenhomepage.htm	[33]
BCIPEP	It provides experimentally validated data of B cell epitopes along with a tool for mapping of epitopes on antigens.	http://www.imtech.res.in/raghava/bcipep	[34]
CED	It has 293 reports of well-described structural B cell epitopes based on literatures.	http://immunet.cn/ced	[35]
Epitome	It has all known interactions of antigen-antibody complexes and is a recognition and storage tool for any antigenic interaction.	http://www.rostlab.org/services/epitome/	[36]
IEDB	Contains 79230 peptide epitopes till Feb 2011. Data includes epitope sequence, reference antigen and the original organism sequences retrieved from.	http://www.iedb.org/	[37]
IMGT/IG	It provides immunoglobulin structures and their annotated sequences.	http://www.imgt.org/IMGTrepertoire/	[38]
HaptenDB	It contains information about haptens, methods to induce immune response against them, specificity and cross-reactivity of developed antibodies, and utilization of antibodies in diagnostic kits.	http://www.imtech.res.in/raghava/haptendb	[39]
HIV Immunology	It presents list of monoclonal and polyclonal responses against HIV proteome. Also contains information about changes and locuses of epitopes, mutations, structures, and biological outcomes of immune responses.	http://www.hiv.lanl.gov/content/immunology/	[40]
HCV Immunology	It presents a list of monoclonal and polyclonal responses against HCV proteome. It also contains information about changes and locuses of epitopes, mutations, structures, and biological outcomes of immune responses.	http://hcv.lanl.gov/content/immuno/immuno-main.html	[41]
MMDB	Largest source of antibody, HLA, and TCR crystallography structures.	http://www.ncbi.nlm.nih.gov/Structure/MMDB/mmdb. shtml	[42]
SACS	It has a summary of antibody crystallography structures.	http://www.bioinf.org.uk/abs/sacs	[43]

Table II

B cell epitope prediction servers

Database name	Brief introduction	URL	References
ABCpred	It is a tool for B cell epitope prediction based on neural network algorithms. Predicted data are evaluated in BCIPEP database.	http://www.imtech.res.in/raghava/abcpred	[44]
AgAbDb	Contains molecular interactions of co-crystallized structures (that are crystallized and crystallographied together) of antigen-antibody that makes B cell epitopes recognizable.	http://115.111.37.206:8080/agabdb2/home.jsp	[45]
Bcepred	This server predicts linear B cell epitopes with 58.7% accuracy, based on availability, hydrophobicity, flexibility, polarity, surface accessible area and turns.	http://www.imtech.res.in/raghava/bcepred	[46]
Bepipred	Uses HMM model for prediction of B cell epitopes.	http://www.cbs.dtu.dk/services/BepiPred	[47]
BEPITOPE	Predicts continuous epitopes based on protein turns.	jlpellequer@cea.fr	[48]
COBEpro	A two-stage system for prediction of B cell epitopes that is linked with SCRATCH prediction database. This software cannot recognize antigen from non-antigen.	http://scratch.proteomics.ics.uci.edu	[49]
3DEX	Recognizes sequences of linear epitope on 3D structures of antigens.	http://www.schreiber-abc.com/3dex	[50]
Discotope	Recognizes amino acids of conformational epitopes with 95% accuracy, based on statistical data of amino acids, spatial data and surface accessible area.	http://www.cbs.dtu.dk/services/DiscoTope	[51]
IMGT	A comprehensive web site having 6 databases and 15 different tools to analyze gene sequence and 3D structure of proteins.	http://www.imgt.org/	[38]
MIMOP	Recognizes linear and conformational epitopes using MIMALIGN and MIMCONS algorithms.	franck.molina@cpbs.univ-montp1.fr	[52]
MIMOX	Like MIMOP uses sequence similarity search for recognition of epitopes on antigens.	http://immunet.cn/mimox/	[53]
Pepitope	An advanced server for prediction of epitopes based on similarity search and epitope mapping.	http://pepitope.tau.ac.il/	[54]

Table III

Databases of T cell epitopes

Database name	Brief introduction	URL	Reference
Allele frequencies	Summarizes HLA frequencies and polymorphism of cytokines.	http://www.allelefrequencies.net	[55]
AntiJen	Provides experimental and quantitative data of interacted peptides with MHC, TAP, B and T cell epitopes, etc.	http://www.ddg-pharmfac.net/antijen/AntiJen/antijenhomepage.htm	[33]
dbMHC	Contains summary of HLA genetic regions, alignments of genetic sequences and tools for recognition of HLA types.	http://www.ncbi.nlm.nih.gov/projects/mhc	[56]
dbMHC Anthropology	Allele frequencies and individual haplotypes of populations, nations and geographical regions are presented.	http://www.ncbi.nlm.nih.gov/proects/gv/mhc/ihwg.cgi?cmd=page&page=AnthroMain	[56]
FRED	Functions based on data processing methods and could evaluates prediction methods using experimental data.	http://www-bs.informatik.uni-tuebingen.de/Software/FRED	[57]
HIV Immunology	Contains CD8+ and CD4+ T cell epitopes and eiptope mapping of HIV proteome.	http://www.hiv.lanl.gov/content/immunology	[40]
IEDB	Contains 79230 peptide epitopes till Feb 2011. Data includes epitope sequence, reference antigen and the original organism sequences retrieved from.	http://www.iedb.org	[31]
IMGT/HLA	Contains aligned and annotated HLA sequences.	http://www.ebi.ac.uk/imgt/hla/allele.html	[38]
IMGT/TR	Contains aligned and annotated TCR sequences.	http://www.imgt.org/IMGTrepertoire	[38]
JenPep	Contains B and T cell epitopes, peptide-MHC-TCR complexes, etc.	http://www.jenner.ac.uk/JenPep.	[58]
MHCBN	Includes 20717 MHC binding and 4022 MHC non-binding peptides, 1053 TAP binding and non-binding peptides, and 1600 antigen.	http://www.imtech.res.in/raghava/mhcbn	[59]
MHC Haplotype Project	Includes haplotypes data of MHC related diseases, complete genomic sequences, polymorphisms, and phylogenetic relations.	http://www.sanger.ac.uk/HGP/Chr6/MHC	[60]
MHCPEP	Contains collection of data of MHC binding epitopes.	http://www.hiv.lanl.gov/contentimmunology/motif_scan /	[61]

Table III (cont.)

Database name	Brief introduction	URL	Reference
PDB	A database of protein 3D structures. It provides structure visualization tools and contains MHC/peptide/TCR complexes.	http://www.syfpeithi.de	[62]
SYFPEITHI	Provides accurate MHC interacted epitopes and anchor and supporting specific parts of MHC.	http://thr.cit.nih.gov/molbio/hla_bind	[63]
ELF	Provides maps of HLA anchor motifs on proteins and peptides in association with known epitopes provided by HIV and HCV epitope databases.	http://tools.immuneepitope.org/tools/ElliPro	[64]
iMAPPP	Provides all predicted epitopes of SYFPEITHI and FRAGPREDICT along with molecular weights, and MHC-peptide binding affinity.	http://www.ddg-pharmfac.net/mhcpred/MHCPred/	[65]

allergenicity evaluation of genetically manipulated organisms. Based on Codex Alimentarius standards, a potentially allergenic protein should contain ≥6 consecutive amino acids similar to a known allergen and/or have a ≥35% of amino acidic similarity to a known allergen in an 80 amino acidic region. Despite their overall usefulness, intrinsic limitations of such guidelines along with existing exceptions are among hurdles of their implementation [85]. However, novel algorithms developed to evaluate allergenicity potential of genetically engineered foods, medical biotechnology drugs, and other products [86]. Comparing allergen prediction algorithms is defiantly hard due to lack of a reliable definition for allergenicity and experimentally approved non-allergenic sequences. Most of novel allergen prediction algorithms employ theoretical non-allergens to examine and train their algorithms. Currently there are limited evaluation regarding the importance of their application and possibly they need serious investment to be optimized and managed. Table V demonstrates a list of allergen databases and allergenicity prediction servers.

Table IV

T cell epitope prediction servers

Database name	Brief introduction	URL	Reference
ElliPro	Employs upgraded modeling software and visualizing platform to predict and display epitopes.	http://www.epitoolkit.org	[66]
EpiToolKit	Provides different methods to predict MHC I and II ligands and can analyze mutations on T cell epitopes.	http://www.epivax.com	[67]
EpiVax	Predicts types I and II epitopes with binding specificity for a wide range of MHC and conserved epitopes.	http://www.imtech.res.in/raghava/ctlpred	[68]
IEDB Binding, MHC	Predicts peptide binding to MHC I and II, proteasome cleavage and TAP binding.	http://www.mpiib-berlin.mpg.de/MAPPP	[69]
MHCPred	Predicts peptide-MHC or TAP binding possibility based on energetic calculations of ligand-protein interaction.	http://www.imtech.res.in/raghava/mhc2pred	[70]
MHC2Pred	Predicts a broad range of MHC II binding epitopes.	http://www.imtech.res.in/raghava/mmbpred	[71]
MMBPred	Predicts a broad range of MHC I binding epitopes and mutations causing more powerful interactions.	http://www.cbs.dtu.dk/services/NetChop	[72]
NetChop	Predicts proteasomal and immunoproteasomal cleavage sites based on non-linear artificial neural networks.	http://www.cbs.dtu.dk/services/NetCTL	[73]
NetMHC	Predicts peptide-HLA interactions based on artificial neural networks.	http://www.uni-tuebingen.de/uni/kxi	[74]
PAProC	Provides human proteasomal cleavage sites.	http://www.imtech.res.in/raghava/pcleavage	[75]
Pcleavage	Predicts proteasomal cleavage sites in antigens.	http://www.imtech.res.in/raghava/propred	[76]
ProPred	Predicts peptide-MHC II binding.	http://www.imtech.res.in/raghava/propred1	[70]
ProPred-I	Provides predictions of peptide-MHC I binding along with optional proteasomal cleavage filters.	http://www.syfpeithi.de	[62, 77]
SYFPEITHI	Predicts T cell epitopes based on ranking of amino acids according to their frequencies.	http://www.imtech.res.in/raghava/tappred	[78]
TAPPred	Predicts binding affinities of TAP proteins.	http://www.allelefrequencies.net	[55]
BIMAS	Ranks potential 8-mer, 9-mer, or 10-mer peptides based on literature tables and predicted half-time of dissociation to HLA class I molecules.	http://www.bimas.cit.nih.gov/molbio/hla_bind/	[79]

Table V

Allergy related databases

Database name	Brief introduction	URL	Reference
AlgPred	Provides different choices for allergen prediction.	http://www.imtech.res.in/raghava/algpre	[87]
AllergenPro	Contains 2434 microbial, animal, and plant allergens and includes three major functional parts: data lists, allergen search, and allergy prediction.	http://www.niab.go.kr/nabic/	[88]
Allergome	Insists on allergens with IgE related diseases and contains data obtained from 5800 scientific literatures.	http://www.allergome.org/	[89]
Allermatch	Predicts potential allergenic proteins based on FAO/WHO guidelines.	http://www.allermatch.org	[90]
APPEL	Predicts new allergenic proteins based on statistical methods.	http://jing.cz3.nus.edu.sg/cgi-bin/APPEL	[91]
BIFS	Contains 500 alimentary, 600 non-alimentary and 80-wheat gluten proteins.	http://www.iit.edu/sgendel/fa.htm	[92]
Database of IUIS	Contains allergens and iso-allergens named and hold by International Union of Immunological Societies (IUIS).	http://www.allergen.org	[93]
EVALLER	Differentiate between allergen and non-allergen molecules base on advanced algorithms.	http://bioinformatics.bmc.uu.se/evaller.html	[94]
FARRP	Contains 1491 sequences of known and potential allergens mined from scientific literatures and databases.	http://www.farrp.org/	[95]

Immunoinformatics and evolution of immune genes and proteins

Reference genes and proteins should be defined, to elucidate evolutionary course of human immune system. Ortutay et al. developed IMMTREE database to provide a unique data set of evolutionary relationships of human immune related genes and proteins along with their phylogenetics and other related statistical information [97]. This database provides information about orthologs in 80 species collected from different databases including HomoloGene, OrthoMCL and EGO. IMMUNOME is another database constructed to characterize human immune system. This database includes information about 893 genes and immunity related proteins, domain structure of them and their related ontology term [98].

Immunome Knowledge Database (IKB) is one single service providing access to several databases and resources. This database contains ortholog groups of 1811 metazoan immune-related genes which are used to study immune system evolution. Moreover, it contains variation data on genomic, transcriptomic and proteomic levels (Table VI).

Table VI

The databases contain evolutionary data of immune genes

Database name	Brief introduction	URL	References
ImmTree	Provides evolution trees for human immune system proteins and contains human ortholog genes in 80 other species.	http://bioinf.uta.fi/ImmTree/	[97]
Immunome database	Provides information about 893 immune proteins, structures, gene locuses based on literatures until 2008.	http://bioinf.uta.fi/Immunome/	[98]
Immunome Knowledge Base	Combination of Immunome, ImmTree, and ImmunomeBase databases along with other comprehensive informations about immune proteins and genes.	http://bioinf.uta.fi/IKB/	[1]

Immunoinformatics and applications

Immunoinformatics provides scientists with tools applicable in different fields of immunology [99–102]. Here *in silico* vaccine design and immune system modeling are discussed as common applications of immunoinformatics.

In silico *vaccine design*

Since 1995 genome of more than 180 species are completely sequenced, while new informatics tools have been developed to analyze huge amount of available data. Several *in silico* tools developed simultaneously capable of epitope prediction as the best components regarding vaccine design. Using these *in silico* tools along with *in vitro* methods simply lead to the field of immunoinformatics. Exploiting these tools scientist could increase the number of candidate antigens for vaccine design purposes and could limit the search range to specific regions of high immunogenicity [103, 104]. Gradual optimization and improvement of these

tools could lead to deletion of many experimental steps. Primary evaluation of such epitope vaccines designed against bestial bacteria, are approved in animal models [105, 106]. Apparently confirmation of these vaccines for human subjects would open a new era in this field [107]. Table VII presents some *in silico* vaccinology database.

Table VII

Some *in silico* vaccinology databases and internet links

Database name	Brief introduction	URL	References
VaxiJen	First alignment independent antigenicity prediction tool of protective antigens that only classifies antigens based on physico-chemical characteristics.	http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html	[108]
DyNAVacs	Provides services such as codon optimization, CpG motif engineering, insertion of Kozak sequences, and determination of host type for development of proper and practical DNA vaccines.	http://www.miracle.igib.res.in/dynavac/	[109]
NERVE	Provides tools to recognize best vaccine candidates among intact bacterial proteome.	http://www.bio.unipd.it/molbinfo	[110]
VIOLIN	Investigates, stores, and processes scientific literatures of immune system and vaccinology.	http://www.violinet.org	[111]
Vaxign	Uses reverse vaccinology rules along with Vaxign Query and Dynamic Vaxign Analysis softwares to predict proper vaccines.	http://www.violinet.org/vaxign/	[112]

CTL epitope predictors

All nucleated cells present fragments of their nuclear and cytoplasmic proteins in association with major MHC I moleculesto discriminate between healthy and infected cells. However, all possible fragments or epitopes originated from cell proteins are not presented by MHCI molecules. It is postulated that just one out of 2000 potential epitopes are immunodominant. MHC binding is the most restricting step [113] in the process of epitope presentation but it is not the only step for MHC associated epitope presentation. Actually natural course of epitope presentation starts with proteosomal cleavage [73, 114–119]. Next step is primary trimming by cytosolic exopeptidases [120–124] which is followed by transporter

associated with antigen processing (TAP) mediated transport of epitopes to endoplasmic reticulum (ER). Final step of further N-terminal trimming of the epitopes occurs in ER where resulted epitope could associate with MHCI. CTL epitopes are MHCI associated epitopes presented by CTLs. Successful prediction and inclusion of immunodominant CTL epitopes is the key to have an effective vaccine capable of eliciting strong CTL responses [113]. Therefore in the case of T cell epitope based vaccine design using a CTL predictor seems more rational rather than MHC predictor since we know not all MHC binders necessarily act as CTL epitopes (Table VIII).

Table VIII

CTL epitope predictors

Database name	Brief introduction	URL	References
NetCTLpan	Predicts 8–11mer CTL epitopes in protein sequences. This method integrates prediction of peptide MHC class I binding, proteasomal C terminal cleavage and TAP transport efficiency.	http://www.cbs.dtu.dk/services/NetCTLpan/	[125]
NetCTL	Predicts 9mer CTL epitopes in protein sequences. This method integrates prediction of peptide MHC class I binding, proteasomal C terminal cleavage and TAP transport efficiency.	http://www.cbs.dtu.dk/services/NetCTL/	[126]
IEDB	Provides a proteasomal cleavage/TAP transport/MHC class I combined predictor.	http://tools.immuneepitope.org/processing/	[31]
CTLpred	A CTL predictor based on elegant machine learning techniques like an Artificial Neural network and support vector machine.	http://www.imtech.res.in/raghava/ctlpred/	[127]
WAPP	It is a prediction server for the Whole Antigen Processing Pathway of MHC class I molecules.	http://abi.inf.unituebingen.de/Services/WAPP/index_html	[128]
EpiJen	This server tries to model several important stage of the MHC degradation pathway: proteasome cleavage, TAP binding and MHC binding.	http://www.ddg-pharmfac.net/epijen/EpiJen/EpiJen.htm	[129]

Modeling of immune system

Modeling of immune system represents a qualitative and quantitative perspective for immune system. These models could clarify antigen-antibody interaction and immune responses against specific antigen in the context of a vaccine test and/or prescription of a specific drug. A reliable model could result in a significant cost and expanses reduction. Several online immune system simulation and modeling projects are undergoing such as VIROLAB (http://www.virolab.org) and IMMUNOGRID (http://wwwimmunogrid.org). IMMUNOGRID project is supposed to simulate immune processes based on immunological studies and computations. VIROLAB project seeks to develop a virtual laboratory for infectious diseases based on human genetic diseases tests [130]. SIMISYS 0.3 is a software [131], models and simulates components of innate and adaptive immune systems based on cells automatic machinery. This software simulates healthy and diseased conditions according to intracellular interactions of macrophages, dendritic cells, B cells, T helper cells, and pathogen bacteria. There must be a balance between virtual and experimental data. This is evident that computational data need to be proved experimentally to become actual knowledge. In fact, post-genomic area needs dynamic transfer of data between simulation and laboratorial experiments [132].

Conclusion

Immunoinformatics is immensely expanding and progressing field, almost inevitably employed in every aspect of immunological studies. Despite great success in developing novel softwares and databases, there is a long way ahead to achieve a virtual immune system like Immunogrid. Further progress in this field would be possible providing the formation of concordant cooperation between immunologists and bioinformatists. Thereafter given the accurate cognition of both fields and specifying common standards designing novel and optimized tools would be achieved. *In silico* calculations should be evaluated experimentally to upgrade algorithms. Considering the great deal of existing immunological data and rising amounts of them in future, the necessity of immunoinformatics approaches become more evident. Particularly, immunoinformatics could have more tangible role regarding research time and costs reduction. Eventually immunoinformatics would accelerate the pace of immune system understanding.

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