

# Prediction of epitopes of viral antigens recognized by cytotoxic t lymphocytes as an immunoinformatics approach to anti-HIV/AIDS vaccine design

## Abstract

Vaccine design is a principal application of the recently emerged biomedical field of immunoinformatics, also known as computational immunology. Algorithms may be used to model immunological data in order to map potential B- and T-lymphocyte epitopes, or antigenic determinants, of foreign proteins which elicit specific responses from an immunized individual. Determination of antibody residues is a key factor in identifying antigen resistance mutations, designing epitopespecific probes for antibody isolation, and for developing epitopebased vaccines. Optimally, epitopes are determined at the atomic level structure of an antibodyantigen complex, although this may not in all instances be possible. Epitopes are of particular interest to both clinical and basic biomedical researchers since they hold huge potential for vaccine design, disease prevention, diagnosis and treatment. Since human immunodeficiency virus (HIV)1 was identified in 1983, it has attracted a research impetus unrivalled in breadth and complexity, the ultimate objectives of which - a preventive vaccine and a curative treatment - remain elusive. Development of an efficacious and cost-effective vaccine is part of a strategy that is required urgently to combat acquired immunodeficiency syndrome, AIDS, the spectrum of conditions which is caused by infection with HIV-1. The challenge of predicting a viral phenotype from sequence data has considerable potential in HIV-1 vaccine design. Here, the major approaches which may be used for epitope prediction in HIV/AIDS research are discussed.

**Keywords:** HIV, virus, immunity, vaccine, t-cell, epitope, bioinformatics

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**Abbreviations:** AIDS, acquired immunodeficiency syndrome; B-cell, B lymphocyte; CTL, cytotoxic t-lymphocyte; ELISA, enzyme-linked immunosorbent assay; Env, envelope; ER, endoplasmic reticulum; HIV, human immunodeficiency virus; MHC, major histocompatibility complex; NMR, nuclear magnetic resonance; TAP, transporter associated with antigen processing; T-cell, T lymphocyte

## Introduction

Since immunoinformatics is still in its infancy as a scientific discipline,<sup>1</sup> the primary focus of this article on current prediction methods available for determining T-cell epitopes of HIV-1 antigens is to provide a primer to clinical and basic biomedical researchers who may be less familiar with these computational biology-based approaches as a powerful tool to investigate peptide vaccines.<sup>2</sup>

Antigenicity or epitope prediction approaches can be classified into one of two categories:

1. Predictors of individual TAP-binding peptides, proteasomal cleavage sites, MHC-binding regions or B- and T-cell epitopes;
2. Predictors of several steps of this multi-stage process of epitope identification.

Epitope prediction complements laboratory bench experimentation and has the potential to enhance greatly research productivity by unravelling complexity, saving time and reducing financial considerations.

Determination of epitopes targeted by antibodies is valuable for understanding virus escape,<sup>3</sup> facilitating antibody optimization<sup>4,5</sup> and informing epitope-based design of vaccines.<sup>6</sup> Identifying the structure of antibody-antigen complexes by, for example, X-ray crystallography

can provide information on an epitope at the atomic level,<sup>7</sup> but such three dimensional intricacy can be technically demanding to reveal. Additional laboratory procedures for epitope delineation are available but they characterize with reduced accuracy and usually necessitate substantial experimental effort.<sup>7-9</sup> Computational methods for epitope prediction originally aimed at recognizing antigenic determinants that may be recognized by any antibody epitope, and are thus not specific to a given antibody.<sup>10-13</sup> More recently, immunoinformatics for antibody-specific epitope prediction (the prediction of the epitope targeted by an antibody of interest) has been developed.<sup>7,14-17</sup> In particular, new protocols combine antibody-antigen neutralization data with antigen sequence information with the purpose of predicting peptide sequences that may comprise part of an epitope which stimulates an antibody of interest to vaccinologists; this is usually because its production correlates with a putatively protective immune response to a pathogen.<sup>7,14,15</sup>

## The need for and nature of an HIV/AIDS vaccine

HIV/AIDS is a global health concern which, through suppression of the human CD4+ T-cell immune response, renders an individual more susceptible to co-infection with tuberculosis, malaria and a number of opportunistic pathogens. In 2013, the most recent year for which statistics are available, an estimated 35 million people worldwide were living with HIV, 2.1 million persons were newly infected with HIV and there were around 1.5 million AIDS-related deaths.<sup>18</sup> Cell-mediated immunity is important to human host defences against HIV-1 infection. In particular, studies of immune responses of HIV-infected patients indicate that CD8+ T-cells perform a crucial role in controlling virus load. In this regard, the onset of HIV-specific CD8+ CTL activity coincides with clearance of viraemia during primary HIV-

1 infection,<sup>19,20</sup> and a reduced CTL response correlates with disease progression in infected individuals.<sup>21,22</sup> On this basis, potentiation of a putatively protective CTL response forms the basis for rational vaccine design. However, the development of a vaccine that boosts the elicitation of a broad and effective anti-viral neutralization response remains a challenge after three decades of investigation.<sup>23,30</sup> One of the drawbacks of traditional, inactivated vaccine candidates is a lack of broad cross-protective B- and T-cell-mediated responsiveness to HIV-1. The efficacy of those tested has been limited due to the genetic variation of the virus.<sup>31</sup>

## Pros and cons of traditional methods for HIV/AIDS vaccine development

Vaccine design in the pre-omics eradependedheavily on biochemical and immunological techniques such as phage display library, overlapping peptides, ELISA, immunohistochemistry, immunofluorescence, radioimmunoassay, Western blotting, NMR spectroscopy, X-ray crystallography of antibody-antigen structure, and attenuation of wild type pathogens by random mutation and serial passage, a process that is very expensive, time-consuming, with poor immunogenicity and the possibility of reversion of attenuation. Now, with the aid of epitope predictive software and databases, it is possible to narrow the focus more rapidly on a protein of interest, and thereby reduce drastically the requirement for laboratory experimentation. Hence, vaccine construction is made both cheaper and faster.<sup>1</sup> Given the potential importance of epitope identification in producing vaccines against infectious, immune and other antigen-related diseases, peptide sequences are studied by researchers in a wide range of biomedical fields, and as a consequence there has been a large expansion of databases, predictive methods and related software.

Structure determination, by Xray crystallography or NMR spectroscopy, may provide atomic level resolution of epitopes and interactions in antibodyantigen complexes, but such data can be difficult or even infeasible to obtain.<sup>7</sup> Cryoelectron microscopy may also be used to identify general epitope regions, but this method is frequently associated with lower resolution structures and in most instances cannot provide atomic level information.<sup>32</sup> Epitope residue mapping may also be subjected to a range of other experimental techniques, although these are typically laborious and are restricted by various factors including sensitivity to effects from distal residues not part of the direct antibodyantigen interactions (e.g. alanine scanning) or dependence on the presence of substantial antibody interactions in a sequentially continuous region of the antigen (e.g. peptide scanning).<sup>8</sup> The latter example is particularly limiting since most antibody epitopes are discontinuous, i.e. involve multiple sequential non-contiguous regions.<sup>7</sup>

*In silico* methods for epitope prediction are also available but the majority focus on predicting protein residues that form part of any epitope and thus do not recognize antibody in particular.<sup>10–13</sup> Only a limited number of antibody-specific epitope prediction protocols have been proposed thus far.<sup>16,17</sup>

The maintenance of HIV in tissue culture in the laboratory is a difficult, skilled, time-consuming process; thus, several immunoinformatics tools have been developed to predict B- and T-cell epitopes of virulence-associated proteins. These programs have emerged on the basis of accessible and validated data that have specific algorithms.<sup>33,34</sup> HIV/AIDS is a major public health problem for which there is a pressing need for sensitive and specific diagnostic methods. In current practice pathologists perform immunoassays as a diagnostic measure that requires purified antigen which is attained only by culture of HIV or recombinant technology. In order to

circumvent this issue, bioinformatics is an alternative approach for prediction of epitopes without wet laboratory practice and which can help to accelerate virology research. For instance, T-cell epitopes may be synthesized chemically to use as antigen in commercial diagnostic kits for HIV detection and also for development of peptide-based vaccines.<sup>35</sup>

## Immunoinformatics tools from the los alamos HIV database

The HIV Molecular Immunology Database that is maintained at the Los Alamos National Laboratory, New Mexico, USA, has collected data on HIV-specific cellular immune responses for several years and the list of targeted regions within HIV peptide sequences has grown steadily.<sup>36</sup>

Investigators may choose between methods, servers and software for prediction of general epitopes, T-cell epitopes, secondary and tertiary structures, and characterization of epitopes. Currently available tools include: QuickAlign which aligns amino acids or nucleotides against known sequences; PeptGen generates overlapping peptides for any protein; PepMap generates peptide maps in Fasta, HTML and PDF formats; Hepitope scans for prospective epitopes based on HLA enrichment; HLA frequency analysis tools calculate HLA frequencies or HLA linkage disequilibrium in a population; ELF epitope location finder; Motif Scan screens alignments for HLA-binding motifs; HLA genotype/serotype, genotype/motif and supertype dictionaries; HIV/SIV Sequence Locator tool, formerly the HXB2 Numbering Engine; HIV BLAST searches peptides against annotated HIV sequences; Mosaic Vaccine Tool Suite assesses polyvalent protein sequences for T-cell vaccines; N-Glycosite finds N-linked glycosylation sites; Highlighter detects matches and mismatches in a set of aligned sequences; Genome Browser displays the HIV genome and proteome.<sup>37</sup>

Analogous approaches have been utilized previously to predict accurately T-cell epitopes for *Mycobacterium tuberculosis*,<sup>38</sup> influenza A virus,<sup>39</sup> tick-borne encephalitis virus,<sup>40</sup> and human papillomavirus.<sup>41</sup> A key element of adaptive immunity is the interaction between foreign antigens and antibodies produced by B cells. The ability to identify and characterize epitopes on antigen surfaces is important for vaccine design, development of antibody therapeutics and immunodiagnostic tests. In the last decade, significant effort has been invested to understand the nature and characteristics of linear epitopes with the goal of developing reliable methods for their prediction. Many bioinformatics analysis tools of varying utility were produced and have been reviewed.<sup>42</sup>

One of the major hurdles to achieving these targets is the extraordinary capacity of the virus for genetic variation, with some genes differing at up to 40% of nucleotide positions among tested isolates. Due to this complication, the genetic foundation for many viral phenotypes, most prominently susceptibility to neutralization by a particular antibody, are troublesome to establish by computational methods.<sup>43</sup> Thus, it is desirable for researchers in this field to apply these tools to their maximum capacity and to use the information they provide judiciously. Only through so doing may it be possible to prepare a 'new age' vaccine against a virus such as HIV-1 that undergoes constant antigenic variation.

## Antigen presentation and induction of a CTL response

The generation of a peptide from its precursor polypeptide is a prerequisite for the induction of a CTL response. The major cytosolic

proteases associated with generating antigenic peptides, in particular the C-terminal end, reside within proteasomes.<sup>44-48</sup> After proteasomal cleavage the peptide may be trimmed at the N-terminal end by other peptidases in the cytosol.<sup>49</sup> The next step is the translocation of the peptide from the cytosol to the interior of the ER, which is facilitated by binding to TAP. Once inside the ER, further N-terminal trimming of the peptide may occur,<sup>47,50</sup> as well as binding to MHC class I. The complex thus formed is transported to the cell surface, where it may be recognized by CTL.

The most restrictive step involved in antigen presentation is binding to MHC class I. It is estimated that only 1 in 200 peptides binds well enough to a given MHC class I molecule to elicit a CTL response.<sup>51</sup> However, it has been shown that proteasomal cleavage and TAP transport efficiency both show a degree of specificity.<sup>52,53</sup> Reliable predictions of immunogenic peptides can reduce significantly the experimental chore in identifying new epitopes. Hence, effort has been invested to predict the outcome of the antigen presentation pathway. Several protocols have been developed that predict with great reliability the binding affinity of peptides to different MHC class I molecules.<sup>54-57</sup> Likewise, programs have been designed that predict the efficiency with which peptides of arbitrary length are transported by TAP.<sup>58,59</sup> Assorted algorithms have also been created that aim to predict the proteasomal cleavage pattern of proteins.<sup>52,60-62</sup> In one report, an integrative approach was used to enhance CTL epitope identification.<sup>63</sup>

## New methods for identification of epitope regions of HIV antigens

Various novel methods have been developed by researchers to predict epitope residues and to determine antibody specificity. Broadly neutralizing antibodies against, for example, HIV1 Env glycoprotein,<sup>27,64-66</sup> can have utility as therapeutics in the context of passive transfer<sup>67</sup> and as templates for the design of epitope-specific vaccines.<sup>68</sup> The identification of an epitope targeted by an antibody can aid understanding of virus resistance and escape mutations,<sup>3</sup> provide pointers to improve antibody affinity,<sup>4,5</sup> and direct immunogen design to focus better the immune response on virus-neutralizing epitopes.<sup>6</sup>

Computational docking may be used to predict epitope residues by means of generating a structural model of the antibody-antigen complex. However, docking depends on the existence of separate, accurate structural models of antibody and antigen, and scoring functions are, in general, suboptimal<sup>69,70</sup> and in most cases a poor predictor of the epitope of interest.<sup>71</sup> Recently, a computational method was proposed for predicting the epitopes of putative HIV-1 antigens based on the similarity of their neutralization fingerprints to those of antigens with known epitopes.<sup>72</sup> This technique, however, does not provide residue level information and is not applicable to antibodies that bind to novel epitopes. Another contemporary study utilized HIV1 antibody neutralization panels to identify antigen residues that are functionally important for binding of specific antibodies.<sup>14</sup> In another study, researchers described an efficient computational method to predict antibody-specific HIV1 Env epitopes at the residue level based on neutralization panels of diverse viral strains.<sup>9</sup> This algorithm is intrinsically relevant to antigens that demonstrate sequence diversity and its accuracy was shown to correlate inversely with sequence conservation of the examined epitope.

In further significant progress, researchers integrated global sequence and immunology databases to investigate systematically the connection between HIV-1 amino acid sequences and CTL epitope distributions.<sup>9</sup> CTL responses to five HIV-1 proteins - Env, Nef, Gag

p17, Gag p24 and reverse transcriptase - have been characterized especially well in the published literature. By comparing the distribution of CTL epitopes in these proteins to global protein sequence alignments (such as those retrieved from NCBI GenBank), distinct characteristics of HIV amino acid sequences that correlate with CTL epitope localization were identified. HIV CTL epitopes defined experimentally are concentrated in relatively conserved regions. Highly variable regions that lack epitopes show cumulative evidence of prior immune escape that may render them relatively refractive to CTL reactivity through a scarcity of predicted proteasome-processing sites and an accumulation of amino acids that do not serve as C-terminal anchor residues.<sup>73</sup> Furthermore, CTL epitopes are frequently concentrated in alpha-helical regions of proteins. Based on amino acid sequence characteristics, regions in HIV regulatory and accessory proteins were identified that would be likely to contain CTL epitopes; these predictions were subsequently validated by comparison to new sets of experimentally defined epitopes of HIV-1.<sup>73</sup>

The design of protective immunogens presents a significant challenge because of the great ability of viruses to evade host immunity.<sup>74,76</sup> Ongoing HIV1 vaccine research includes determining and characterizing broadly neutralizing antibodies and the epitopes they target.<sup>77,78</sup> Hence, identifying antigenic targets of such antibodies, in parallel with mapping the immunologically important residues of known epitopes that affect neutralization, is a major objective. Env is a highly variable protein and consequently identification of key residues that affect neutralization is not a straightforward task. A lack of neutralization may sometimes be explained by amino acid changes in known epitopes, but often epitope conservation does not ensure neutralization.<sup>79</sup> Many regions external to known epitopes have been shown to affect neutralization sensitivity.<sup>80</sup> Identification of B- and T-cell epitopes is a crucial step in peptide vaccine design but which does not enjoy sufficient accuracy due to the extent of mutations in HIV-1 observed in regionally distinct isolates. Thus, prediction of epitope structures with a wider range of variation may facilitate more efficient treatment of HIV/AIDS on a global scale.

## Current perspective and future directions

A key consideration when designing an HIV/AIDS vaccine is how its structure may best promote elicitation of a protective immune response. This includes identification of novel CTL epitopes with a high binding affinity for MHC class I. Utilization of bioinformatics for accurate prediction of epitope identity and characteristics enables a next generation vaccine design to incorporate appropriate residues to stimulate antigen-specific B- and/or T-cells. As well as for the development of a vaccine an urgent need exists to establish sensitive antigen-based immunodiagnostics for earlier monitoring of HIV. Ongoing and prospective research should endeavour to design a candidate vaccine structure to include several variations predicated on information combined from computational biology and reliable traditional techniques such as X-ray crystallography. Sequence data required may be retrieved from current genome and protein databases to predict a limited number of functionally important antigen residues of an examined antibody, rather than to identify the antibody epitope *per se*.

The incorporation of data from geographically diverse strains of the same virus may increase the likelihood of observing an association between changes in neutralization potency and variations in antigenic sequence. This suggests that the combination of a neutralization-based method with an immunoinformatics approach that provides structural information could prove a useful strategy for the accurate prediction of antibody epitopes of genetically diverse HIV antigens.

## Conclusion

Immunoinformatics is one of a new wave of computational approaches which are used in identification of antigenic peptides and which can be applied to development of diagnostics, therapeutics and vaccines. In recent years the CTL response to HIV-1 has been studied intensively and hundreds of T-cell epitopes are now defined experimentally, their sequences published and compiled in the HIV Molecular Immunology Database. An increasing volume and complexity of information about conserved epitopes of HIV-1 brings closer the development of a universal epitope-based vaccine. Such a construct should contain both conserved B-cell epitopes that are important for induction of cross-protective antibodies and CTL epitopes for involvement of the cellular immune response. Future research should aim to utilise immunoinformatics as a tool to define characteristics of HIV-1 antigens, to identify T-cell epitopes located at amino acid residues and to evaluate the antigenic capacity of predicted T-cell epitope constructs. The novel insights provided from such bioinformatics application would facilitate the production of locally or globally effective antigen-based diagnostics and peptide-based vaccines designed to combat HIV/AIDS.

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## Conflicts of interest

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