

De-antigenization of immunodominant epitopes: a strategy for designing vaccines against constantly mutating pathogens and other applications

Eduardo A. Padlan

Marine Science Institute, College of Science
University of the Philippines Diliman
Quezon City, 1101 Philippines

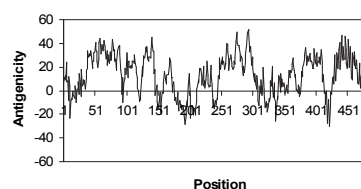
Our immune system counters the invasion by viruses, bacteria, parasites and other pathogens, with specialized molecules and cells that eliminate or neutralize those invaders or the toxins that they produce. An important molecular arm of the immune system is composed of the antibodies which bind tightly and specifically to foreign substances (antigens). The specificity of an antibody molecule is so exquisite that it can distinguish between, for example, two protein antigens that differ by only one amino acid residue.

Very importantly, our immune system has memory and we are able to mount an immediate response to infection by a pathogen that we had encountered before. Indeed, we are able to prevent many diseases by vaccination, i.e., by exposing our immune system to non-infective versions of the pathogen, or to its antigen(s). However, many pathogens, like the flu virus, the cold virus, and the virus that causes AIDS, are able to evade the immune response by constantly mutating. Sometimes, evasion is accomplished by a single nucleotide change in an antigen that is coded by more than a thousand bases.

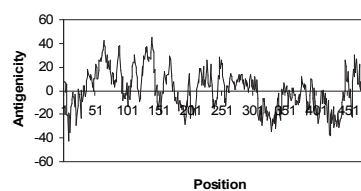
Our immune system is capable of reacting to essentially all parts of an antigen, although some parts, the so-called immunodominant epitopes, elicit most of the response. The clever pathogens, of course, localize their mutations in those immunodominant epitopes. A strategy for thwarting those pathogens then is to use as vaccines versions of their antigens, in which the antigenicity (the ability to attract an antibody response) of their immunodominant epitopes had been reduced so that our immune system would react vigorously to the other (less reactive) parts of the antigens also. Implementation of the strategy is done in three steps: first, the immunodominant epitopes are located; second, the residues which are responsible for the high antigenicity of those epitopes are identified; and third, those residues are replaced with amino acids that are expected to contribute less to the antigenicity, while preserving structure. The strategy is illustrated here using the design of a possible vaccine against an H3N2 flu virus as an example.

The antigen

The antigen is the hemagglutinin of the influenza A virus, (A/New York/55/2004(H3)), (entry ABO37541 in the NCBI database ([Antigenicity plot for NY-55-2004 HA
plus ex 1ha0 Sandberg zz1-zz3 16A](http://</p>
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Antigenicity plot for NY-55-2004 HA
plus ex 1ha0 S3 16A DeAn1



Antigenicity plot for NY-55-2004 HA
plus ex 1ha0 S3 16A DeAn2

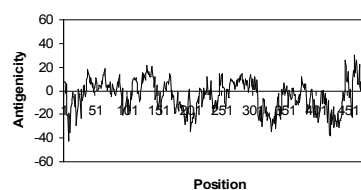


Figure 1. Plot of antigenicity vs. position for the hemagglutinin of (A/New York/55/2004(H3)) virus before (top), after one cycle (middle), and after two cycles (bottom), of de-Antigenization using Sandberg et al. (1998) values. Antigenicities are in arbitrary units.

www.ncbi.nlm.nih.gov). A three-dimensional model for this molecule was generated using the protein modeling software, SWISS-MODEL (Schwede et al. 2003) (<http://swissmodel.expasy.org/SWISS-MODEL.html>), and using the Protein Data Bank entry 1HA0 (Chen et al. 2002) as template.

Excerpted from the Severino and Paz Koh Lectureship Award for Science talk delivered by the author at the 28th Annual Meeting of the Philippine-American Academy of Science and Engineering held at Georgetown University, in Washington D.C., USA, on May 22-24, 2008.

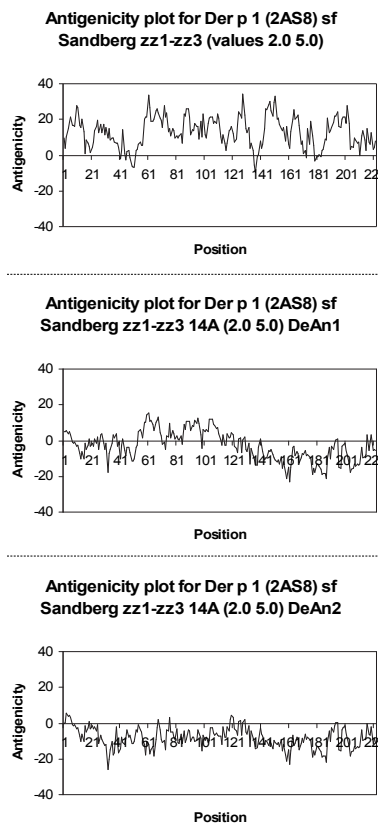


Figure 2. Plot of antigenicity vs. position for the Der p 1 antigen from European house dust mites before (top), after one cycle (middle), and after two cycles (bottom), of de-Antigenization using Sandberg et al. (1998) values. Antigenicities are in arbitrary units.

Location of the immunodominant epitopes

The strength of the binding of antibodies to antigens is determined by the reactivities of both the paratope (the antigen-binding site) of the antibody and of the epitope (the site to which the antibody binds) of the antigen. In the case of protein antigens, the antigenicity of an epitope is ultimately determined by the physicochemical properties of the amino acids which constitute the epitope. Several measures of the physicochemical properties of the various naturally-occurring amino acids are available (for example, Grantham 1974, Sandberg et al. 1998) and such measures can be used to estimate the antigenicity of protein antigens (see, for example, Padlan 1985).

Here, an epitope is defined as the cluster of amino acids within a certain distance from a chosen point in the three-dimensional structure of an antigen (for example, the alpha-carbon position nearest the geometric center of the cluster) and the antigenicity of the epitope is defined as the sum of the contributions of the constituent amino acids. The contribution of each amino acid residue is the chosen physicochemical measure weighted by the solvent exposure of the residue.

The antigenicity values of the epitopes of (A/New York/55/2004(H3)), calculated using parameters proposed by Sandberg et al. (1998), are plotted against residue position in Figure 1 (top plot). Several peaks are observed to be significantly above the average; they are taken to represent the “immunodominant epitopes”.

Amino acid replacements and recalculations

The residues, which contribute significantly to the “immunodominant epitopes”, were replaced by amino acids that are expected to reduce antigenicity. A model of the molecule, with the suggested sequence

changes incorporated, was built using SWISS-MODEL and the antigenicities recalculated. After two cycles of amino-acid replacements, remodeling, and recalculation of antigenicities, no additional replacements were suggested. The antigenicity plots resulting from these two cycles are included in Figure 1 (middle and bottom plots). It is observed that after one cycle (middle plot), many of the prominent peaks in the original plot (top) are greatly reduced in size; the “immunodominant” peaks are essentially all gone after the second cycle (bottom plot)

Possible vaccines against this particular influenza A virus

Either of the sequences (not shown), which produced the middle and bottom plots in Figure 1, could represent a possible vaccine against influenza A virus, (A/New York/55/2004(H3)).

Further applications of the strategy

The de-antigenization of immunodominant epitopes can also be used in designing hypoallergenic molecules useful in allergy desensitization (allergy shots). Allergy is caused by the binding of allergens to a type of antibody, IgE, that is bound to mast cells in connective tissue and basophils in the blood. Allergen-IgE binding triggers the cells to release histamines and other compounds responsible for allergy symptoms including anaphylaxis. Allergens also have immunodominant epitopes, called dominant IgE epitopes, to which specific IgE binds. Allergy desensitization is done by introducing increasing amounts of allergen into the patient to elicit an IgG rather than an IgE response. De-antigenization of the dominant IgE epitopes will render the existing specific IgE incapable of binding to the modified allergen, so that larger doses of the modified allergen could be administered with lessened chance of anaphylaxis.

Applying the strategy of de-antigenization to Der p 1 (NCBI entry Po8176 (Chua et al. 1988)), the major allergen of the European house dust mite, *D. pteronyssinus*, results in the antigenicity plots shown in Figure 2. Either of the sequences which generated the middle and bottom plots in Figure 2 and those which would result from the application of the strategy to the other allergens of *D. pteronyssinus* represent molecules that should be useful in the desensitization against house dust mite allergy.

Patent applications for the use of the strategy in designing vaccines against constantly mutating pathogens and in designing molecules useful in allergy desensitization have been submitted (US11/645,448 and US11/823,330, respectively).

References

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