A purely computational method is proposed for reducing the antigenicity of a protein molecule, except that of a chosen region, so that the modified molecule, when used as an immunogen, would be expected to elicit an antibody response that is primarily directed at the chosen region. The chosen region could be a site that is critical to the structure or biological activity of the molecule, for example, a binding site for specific ligand, a catalytic site, a region that undergoes structural change for effective function, a neutralizing antibody epitope, etc. The method consists of (1) identifying the antibody epitopes that include the chosen region or part of it and (2) by judicious replacement of the amino acid residues, reducing the antigenicity of all the antibody epitopes of the molecule, except for those which include the chosen region. The method is useful in the design of vaccines, or of immunogens for the production of antibodies for various biochemical and medical applications. The method can also be used to design a molecule whose antigenicity is generally suppressed while preserving a critical region, e.g., when "humanizing" a nonhuman antibody by reducing the antigenicity of the framework regions while preserving the structure and properties of the antigen-binding region.

KEYWORDS
antigenicity, antibody, epitope, vaccine design, allergen, critical region, immunogen, de-Antigenization, neutralization

INTRODUCTION

When we are exposed to a foreign substance (an antigen), our immune system reacts by producing molecules and cells that are specific for the substance. Antibodies are molecules produced by our immune system which bind the antigen with high specificity and affinity, neutralizing or immobilizing it, and, thereby, rendering it more susceptible to elimination by normal processes.

Examples of antigens are molecules on the surface of pathogens or secreted by them, allergens, venom, and other molecules that are not normally part of our body. Those foreign molecules have regions that are critical to their function or activity and those could be specifically targeted by our immune system for more effective neutralization. Those critical regions include receptor binding sites, enzyme active sites, regions that undergo structural change for effective function, and epitopes of neutralizing antibodies, among others.

The binding of antibodies to the critical region of an antigen will effectively neutralize its activity. An antigen that has been modified so that the antigenicity of the antibody epitopes which
include the critical region is emphasized would be expected to elicit an antibody response that is primarily directed at the critical region. An antigen so modified would be useful as a vaccine, or as an immunogen for the production of antibodies that could be used for the neutralization of the activity of the original antigen. Antibodies that bind to a particular site on a molecule would be useful in biochemical applications, for example, in the detection, isolation, purification, and quantitation of molecules. Further, such antibodies would be useful in immunotherapy and other medical applications. An immunogen so designed could also be used for the production of antibodies that are particularly specific for a protein that shares antibody epitopes with other proteins, thereby minimizing, or altogether eliminating, cross-reactivity.

While every accessible region of an antigen could be an antibody epitope (Benjamin et al. 1983; Davies et al. 1988), the antigenicity of each epitope depends on the physicochemical properties of the amino acid residues which constitute the epitope (Padlan 1985).

If we are able to modulate the antigenicity of antibody epitopes, we may be able to redirect the antibody response from one region to other regions of an antigen. That possibility was originally suggested by Fazekas de St. Groth (1977) and has been explored by various groups (e.g., Jones et al. 1991; Shafferman et al. 1991; Scheerlink et al. 1993; Temoltzin-Palacios et al. 1994; Garrity et al. 1997; Martinet et al. 1998; Nara and Garrity 1998; Cleveland et al. 2000; Padlan 2008a; Padlan 2008b; Selvarajah et al. 2008; Tobin et al. 2008) using methods involving excision of immunodominant antibody epitopes, change in glycosylation patterns, replacement of amino acids, etc. But those methods do not purposefully direct the antibody response to a particular region of an antigen.

One attempt to elicit an immune response to a chosen region of a protein antigen makes use of isolated peptides which represent part of a suspected antibody epitope (see, for example, Hopp and Woods 1981; Küpper 1984; Hopp 1993; Horváth et al. 1998; Bianchi et al. 2005). However, antibody epitopes are generally conformational, i.e., they include peptide segments from different, and not necessarily nearby, parts of a molecule (Davies et al. 1988). Moreover, a peptide may assume an entirely different structure when removed from the parent molecule (see, for example, Stanfield et al. 1990). The method described herein presents an antibody epitope in the context of the native structure of the antigen, with its obvious advantages.

A way to emphasize the antigenicity of the antibody epitope or epitopes which include a chosen region would be to reduce the antigenicity of all the other antibody epitopes of the antigen. A purely computational procedure for achieving such a purposeful focusing of the antibody response is presented here and is illustrated using three examples.

In the first example, the catalytic site of an enzyme was chosen as the critical region. The enzyme in this example is hyaluronidase (hereinafter referred to simply as Hyal), a major allergen of bee venom that has enzymatic activity (see, for example, the review by Hoffman 2006) and whose three-dimensional structure has been elucidated (Markovic-Housley et al. 2000).

The second example also makes use of Hyal, but here the epitope of a murine anti-Hyal monoclonal antibody (mAb) (Padavattan et al. 2007), that competes for Hyal binding with IgE (the antibody type that is responsible for the usual allergic reactions) isolated from the sera of individuals known to be allergic to bee stings, was the chosen critical region. The usual immunotherapy for allergy is by desensitization through the gradual introduction of the allergen to the immune system of the patient, with the hope that the immune system would produce non-IgE antibodies that would compete with specific IgE for binding to the allergen. An immunogen that is designed to elicit an antibody response focused on the binding site of specific IgE would be useful as a vaccine against the allergy.

In the third example, the antigen is the ectoplasmic region of the apical membrane antigen 1 (AMA1) of the malarial parasite, *P. vivax*, and the chosen critical region is the epitope of a potentially useful therapeutic antibody (Igonet et al. 2007). This example is used to illustrate the structure modeling that may be required, as well as the need to avoid possible antigenic...
variability due to polymorphism in closely-related molecules.

In the sections that follow, the term "epitope" is used to mean an "antibody epitope", unless otherwise specified.

MATERIALS AND METHODS

(a) The method

Summary of the method:
The procedure to design an immunogen that could be used to elicit an antibody response that is focused on a critical region of a protein molecule is summarized in the following steps:

(Step 1) Identify a region on the protein molecule that is critical to the protein's function or activity.

(Step 2) On the basis of the three-dimensional structure of the molecule, map all the possible epitopes on its surface. Identify the epitopes which include the critical region identified in Step 1, or at least part of it.

(Step 3) Identify the amino-acid residues which, by virtue of their physicochemical properties and their surface accessibility, contribute significantly to the antigenicity of the epitopes which do not include the critical region identified in Step 1, i.e. all the epitopes except the ones identified in Step 2.

(Step 4) Replace the residues identified in Step 3 with amino acids that would be expected to contribute less to antigenicity, while ensuring that the replacements will not significantly alter the structure of the antigen. A possible set of replacement rules shown in Table 1 (reproduced from Padlan (2008a)) could be used. To ensure immunogenicity, it is probably wise to preserve at least one helper T-cell epitope.

(Step 5) The sequence from Step 4 represents a molecule that could be used as an immunogen to elicit antibodies against the protein molecule, with the antibody response focused on the critical region identified in Step 1.
Structural information:
Experimentally-determined structural information is often available from the Protein Data Bank (PDB) (Berman et al. 2000). In the absence of an actual structure, a model could be built based on structural information from a closely related molecule.

Calculation of solvent accessibilities:
On the basis of the three-dimensional structure of the antigen, the solvent accessibilities of the individual amino acid residues can be computed by standard methods using the programs developed by Connolly (1983). Solvent accessibilities could also be obtained using the program DSSP (Define Secondary Structure of Proteins) (Kabsch and Sander 1983). The solvent accessibilities of the amino acid side chains (see, for example, Padlan 1990), are used here as weighting factors in the calculation of the antigenicities. The use of solvent accessibilities as weighting factors de-emphasizes the contribution of residues that are not too accessible and that probably do not contribute much to the interaction with antibody.

Calculation of antigenicities:
A method had been proposed earlier for quantifying the antigenicity of a protein epitope using the physicochemical attributes of the amino acid residues comprising the epitope (Padlan 1985). That method was used in the proposed de-Antigenization of immunodominant epitopes for vaccine design (Padlan 2008a; Padlan 2008b). That method is used here also to calculate the contribution of the individual residues to antigenicity. Structural parameters describing the physicochemical attributes of the various amino acids have been proposed (for example, Sneath 1966; Grantham 1974; Sandberg et al. 1998) and those can be used in the calculation of the antigenicities. The structural parameters have been shown to provide a good measure of the ability of a given region to participate in protein-protein interactions (see, for example, De Genst et al. 2002; David et al. 2007). Thus, antigenicity computed in this manner is directly correlated with the ability of a particular region to engage in tight binding to antibody.

De-Antigenization of epitopes:
De-Antigenization of epitopes is achieved by the judicious replacement of the residues in those epitopes with amino acids that would contribute less to the total antigenicity values, while preserving the structure of the molecule. By taking into account the physicochemical properties of the amino acids and their propensity to participate in a particular secondary structure (see, for example, Pace and Scholtz 1998; Street and Mayo 1999; Linding et al. 2003; Hutchinson and Thornton 1994 for helix, beta sheet, coil, and turn propensities, respectively), replacement rules could be proposed. The replacement rules used here are presented in Table 1 (extracted from Table 1 of Padlan 2008a). Other replacement rules could be proposed and used provided they result in reduced antigenicity while preserving structure.

Figure 2. Plots of antigenicity vs. residue position for bee venom hyaluronidase before (top) and after (bottom) general de-Antigenization while preserving the highly antigenic Hyal Tyr268 epitope, which includes part of the catalytic site of the enzyme. The antigenicities for the mutated Hyal were computed based on a three-dimensional model generated with the help of the protein modeling server, SWISS-MODEL (Schwede et al. 2003) (implemented in http://swissmodel.expasy.org/), using the Hyal structure as template.
Example 1 - Design of a possible immunogen that could be used to elicit an antibody response focused on the catalytic site of bee venom hyaluronidase

Structural and sequence data:

The three-dimensional structure of bee venom hyaluronidase in complex with a substrate analog has been determined by X-ray crystallography (Markovic-Housley et al. 2000) to a resolution of 2.65 Å and refined to a crystallographic residual of 0.180 (PDB entry 1FCV).

The catalytic site was delineated by identifying the atoms in Hyal which contact the bound ligand (atoms were considered in contact if they are within 4.0 Å of each other). Atoms from 14 Hyal residues, namely: Phe20, Met21, Phe46, Ile53, Tyr55, Asp111, Glu113, Tyr184, Tyr227, Trp267, Gln271, Trp301, Ser303, and Ser304 (the Hyal molecule in PDB entry 1FCV starts with residue 10), were found to contact the ligand and were designated as comprising the catalytic site. The centroid of the catalytic site was determined from the coordinates of those atoms. The catalytic site atom farthest from the centroid was found to be 11.7 Å away.

The sequence of Hyal that is used here corresponds to the sequence in PDB Entry 1FCV and is presented in Figure 1.

Calculation of antigenicities and identification of dominant epitopes that include part of the catalytic site of Hyal:

Antigenicity calculations were performed on Hyal using the structural parameters provided by Sandberg et al. (1998). The antigenicity of a region centered at each alpha-carbon position was computed by taking the sum of the zz1, zz2 and zz3 structural parameters of Sandberg et al. (1988) corresponding to all the residues within 14 Å of the alpha-carbon. In this example, the radius of 14 Å was chosen on the basis of the results of calculations on the known epitopes of hen egg white lysozyme (Padlan 1996). The fractional solvent accessibilities of the side chains obtained above for Hyal were used as weighting factors in the calculation of the antigenicities.

Two epitopes, centered at the alpha-carbon atoms of Hyal residues Tyr268 and Lys269 and whose antigenicity values are greater than two r.m.s. deviations above the mean, were found to be within 12 Å of the center of the catalytic site. Both epitopes include a significant part of the catalytic site (six residues out of the 14 which constitute the site) and antibodies directed against those epitopes should inhibit the catalytic activity of Hyal. The Tyr268 epitope has a higher antigenicity value and was chosen for further analysis.

General de-Antigenization of Hyal except the Tyr268 epitope which includes part of the catalytic site of the enzyme:

All the residues outside the Tyr268 epitope, whose contribution to antigenicity is at least 3% of the total value for their respective epitopes and whose fractional solvent accessibility is at least 40%, were replaced. The replacement rules proposed in Table 1 were applied during de-Antigenization.

Two epitopes, centered at the alpha-carbon atoms of Hyal residues Tyr268 and Lys269 and whose antigenicity values are greater than two r.m.s. deviations above the mean, were found to be within 12 Å of the center of the catalytic site. Both epitopes include a significant part of the catalytic site (six residues out of the 14 which constitute the site) and antibodies directed against those epitopes should inhibit the catalytic activity of Hyal. The Tyr268 epitope has a higher antigenicity value and was chosen for further analysis.

General de-Antigenization of Hyal except the Tyr268 epitope which includes part of the catalytic site of the enzyme:

All the residues outside the Tyr268 epitope, whose contribution to antigenicity is at least 3% of the total value for their respective epitopes and whose fractional solvent accessibility is at least 40%, were replaced. The replacement rules proposed in Table 1 were applied during de-Antigenization.

During de-Antigenization, residues 212-229, which were identified as containing possible helper T-cell epitopes by SYFPEITHI, a T-cell epitope predictor (implemented in http://www.syfpeithi.de/) (Rammensee et al. 1999), were not changed.
Design of a possible immunogen that could be used to elicit an antibody response to bee venom hyaluronidase focused on the epitope of a murine mAb that competes with IgE isolated from the sera of individuals known to be allergic to bee stings.

**Structural and sequence data:**

The three-dimensional structure of the complex of bee venom hyaluronidase and the Fab of a murine mAb that competes with specific IgE has been determined by X-ray crystallography (Padavattan et al. 2007) to a resolution of 2.60 Angstroms and refined to a crystallographic residual of 0.212 (PDB entry 2J88).

The epitope of the murine mAb was delineated by determining the Hyal atoms which contact the murine Fab using the criteria used in Example 1. The Hyal residues which comprise the epitope are Arg138, Glu140, Pro142, Phe143, Trp144, Asp145, Asp146, Gln147, Arg148, and Val149.

The Hyal structure in PDB entry 2J88 (at 2.60 Angstrom resolution) is better determined than that in PDB entry 1FCV (at 2.65 Angstrom resolution), but only marginally. The 1FCV structure, on the other hand, was refined to a significantly better extent (crystallographic residual of 0.180 for 1FCV compared to 0.212 for 2J88). For this reason and for consistency, the 1FCV structure was chosen for this example also. Thus, the epitope atoms of the murine mAb identified above were located in the 1FCV structure. The center of the epitope in 1FCV was determined and the epitope atom farthest from the center was found to be 10.4 Angstroms away.

**Solvent accessibilities:**

The fractional solvent accessibilities of the side chains of the residues of Hyal were those used in Example 1.

**Calculation of antigenicities and identification of the Hyal epitopes which include the epitope of the murine mAb in PDB entry 2J88:**

The antigenicity values obtained in Example 1 for Hyal were used here also.

In the design of a possible immunogen that would elicit an antibody response directed mainly at the epitope of the murine mAb, only Hyal epitopes within 5 Angstroms of the center of the former were considered for preservation. There are three Hyal epitopes within this distance: those centered at the alpha-carbons of Phe143, Trp144, and Asp145. All three include all the residues in the epitope of the murine mAb in PDB entry 2J88.

**General de-Antigenization of Hyal except the three epitopes which include the epitope of the murine mAb in PDB entry 2J88:**

![Antigenicity plot for hyaluronidase before epitope focusing](image1)

![Antigenicity plot for hyaluronidase after focusing on the anti-Hyal mAb epitope](image2)
All the residues outside the three epitopes identified above were replaced following the criteria used in Example 1 and using the same replacement rules. As in Example 1, residues 212-229, were not changed.

(d) Example 3 - Design of a possible immunogen that could be used to elicit an antibody response to the ectoplasmic region of the apical membrane antigen 1 (AMA1) of the *Plasmodium vivax* malarial parasite (hereinafter, the isolated ectoplasmic region is referred to simply as *PvAMA1*), focused on the epitope of an antibody while avoiding the positions which are polymorphic in the AMA1 of closely-related strains of the parasite

Structural and sequence data:

AMA1 is a molecule that is partly exposed on the surface of the malarial parasite and is a leading candidate vaccine against malaria. It is composed of an ectoplasmic region, a transmembrane region and a small cytoplasmic region. The structures of the ectoplasmic regions of the *P. vivax* and *P. falciparum* AMA1 have been determined using x-ray crystallography (Bai et al. 2005; Pizarro et al. 2005; Igonet et al. 2007; Coley et al. 2007) and are available from the Protein Data Bank (PDB entries 1W8K and 1W81 for the *P. vivax* molecule, and 1Z40, 2Q8A and 2Q8B for the *P. falciparum* molecule). A whole ectoplasmic region is not available from any of these x-ray structures, but can be modeled. The *P. vivax* and *P. falciparum* structures are sufficiently similar so that the segments that are missing in the *P. vivax* structures, but present in the *P. falciparum* structures, could be used as modeling templates. The modeled PvAMA1 corresponds in its extent to the structure in PDB entry 1W8K and its sequence is presented in Figure 5 (labeled (1)).

The crystal structure of a complex of the Fab of the murine monoclonal antibody, F8.12.19, which had been raised against *P. vivax* AMA1, and a polypeptide that includes the F8.12.19 epitope, has been determined (Igonet et al. 2007) and is available (PDB entry 2J4W). The epitope of F8.12.19 comprises 12 residues, at positions: 385, 386, 389-395, and 408-410 (numbering follows that in Figure 5).

The 148 partial sequences of the AMA1 from *P. vivax*, that had been isolated from the Philippines and which were available in the NCBI database on 5/14/2009, were used in the study of the polymorphism in the molecule. This sample is among the biggest among those isolated from the same strain of the parasite and from the same locality. The partial sequences correspond to the N-terminal part of the molecule where polymorphism is most observed (see, for example, Bai et al. 2005).

Modeling of the AMA1 structure:

A three-dimensional model for PvAMA1 was generated with the help of the protein modeling server, SWISS-MODEL (Peitsch 1995; Guex et al. 1997; Schwede et al. 2003) (implemented in http://swissmodel.expasy.org/), using the 1W8K and 1W81 structures as templates and, when segments in these structures were missing, the corresponding segments in the 1Z40 and 2Q8A and 2Q8B structures were utilized.

Solvent accessibilities:

Fractional solvent accessibilities of the side chains of the...
residues in PvAMA1 were obtained based on the model obtained above and using the procedure described in Padlan (1990).

**Analysis of the variability of related apical membrane antigen 1; identification of polymorphic positions:**

The 148 partial sequences of the AMA1 from *P. vivax*, that had been isolated from the Philippines and which were available in the NCBI database on 5/14/2009, were compared.

Polymorphism was observed at positions: 5, 23, 25, 33, 34, 38, 82, 83, 86, 103, 111, 120, and 121 (numbering follows that in Figure 5).

**Calculation of antigenicities and identification of the computed PvAMA1 epitopes which include all the residues in the observed epitope of antibody F8.12.19 but which do not include the polymorphic positions:**

Antigenicity calculations were performed on the model of PvAMA1 using the structural parameters provided by Sandberg et al. (1998). The antigenicity of a region centered at each alpha-carbon position was computed by taking the sum of the zz1, zz2 and zz3 structural parameters of Sandberg et al. (1988) corresponding to all the residues within 15 Angstroms of the alpha-carbon. In this example, the radius of 15 Angstroms was chosen on the basis of the results of calculations on the known epitopes of three anti-neuraminidase Fabs (PDB entries 2AEP, 1NMB, and 1NCD). The fractional solvent accessibilities of the side chains obtained above for the PvAMA1 model were used as weighting factors in the calculation of antigenicities.

One epitope, centered at the alpha-carbon atom of PvAMA1 residue Ile381 and whose antigenicity value is greater than two r.m.s. deviations above the mean, was found to include all of the 12 residues comprising the F8.12.19 epitope, but not any of the positions found above to be polymorphic in the collection of AMA1 from the Philippines.

**General de-Antigenization of PvAMA1 except for the epitope which includes the F8.12.19 epitope:**

All the residues outside the Ile381 epitope identified above were replaced following the criteria used in Example 1 and using the same replacement rules.

**RESULTS**

The sequence of bee venom hyaluronidase before and after general de-Antigenization (labeled (1) and (2), respectively), while preserving the antigenicity of the catalytic site of the enzyme, is shown in Figure 1. The plots of antigenicities computed for Hyal, before and after the amino-acid replacements, are presented in Figure 2. It is seen from Figure 2 that a marked reduction in the general antigenicity of the molecule has been achieved, except in some regions of the molecule; those regions include part of the catalytic site of Hyal.

![Antigenicity plot for PvAMA1 before epitope focusing](image1)

![Antigenicity plot for PvAMA1 after focusing on the mAb F8.12.19 epitope](image2)

Figure 6. Plots of antigenicity vs. residue position for PvAMA1 before (top) and after (bottom) general de-Antigenization while preserving the epitope of antibody F8.12.19 and while avoiding variable positions in related AMA1 molecules. The antigenicities for the mutated PvAMA1 were computed based on a three-dimensional model generated with the help of the protein modeling server, SWISS-MODEL, using the unmutated PvAMA1 structure as template.
Sixty five out of the 324 total residues in Hyal, or 20%, were replaced during the general de-Antigenization of the molecule while retaining the Tyr268 epitope. Since it is generally accepted that 30% sequence identity implies similarity in tertiary fold of homologous proteins, this 80% retention of residues during the de-Antigenization of Hyal strongly suggests that the three-dimensional structure of Hyal will be preserved in the mutated version.

The sequence of Hyal before and after general de-Antigenization (labeled (1) and (2), respectively), while preserving the antigenicity of the epitope of the murine anti-Hyal monoclonal antibody that competes for Hyal binding with IgE isolated from the sera of individuals known to be allergic to bee stings, is shown in Figure 3. The plots of antigenicities for Hyal are presented in Figure 4. It is seen from Figure 4 that here also a marked reduction in the general antigenicity of the molecule has been achieved, except in one region - the region which includes the epitope of the murine mAb. Here also, the high retention rate (79.7%) ensures that the three-dimensional structure of Hyal will be preserved.

Sixty nine residues of Hyal, or 21.3%, were replaced during the general de-Antigenization of Hyal while preserving the three epitopes which include the epitope of the murine mAb. Here also, the high retention rate (79.7%) ensures that the three-dimensional structure of Hyal will be preserved.

The sequence of PvAMA1 before and after general de-Antigenization while preserving the Ile381 epitope (labeled (1) and 2), respectively) is shown in Figure 5. The plots of antigenicities for the PvAMA1 in this example are presented in Figure 6. It is seen from Figure 6 that here also a marked reduction in the general antigenicity of the molecule has been achieved, except in the region which includes the residues in the epitope corresponding to that of F8.12.19.

One hundred twenty one residues of the modeled PvAMA1, or 27.9%, were replaced during the general de-Antigenization while preserving the epitope which includes the F8.12.19 epitope and while avoiding the polymorphic positions. Here also, the high retention rate (72.1%) ensures that the three-dimensional structure of the molecule will be preserved.

DISCUSSION

The ability to elicit an antibody response to a chosen region of an antigen has many possible applications. For example, antibodies directed against the catalytic site of a harmful enzyme would inhibit its unwanted activity. Antibodies directed against the receptor binding site of a pathogen could prevent its binding to its target cell. Further, antibodies directed against a conserved part of an antigenically unstable virus would be protective even if the virus mutates. Thus, the ability to design an immunogen, that can elicit an antibody response that is focused on a particular region of a molecule, would be very beneficial.

Such purposely designed immunogens would be useful as vaccines. Purposely designed molecules could also be used as immunogens for the production of antibodies for very specific use, like immunotherapy against cancer, against severe allergic reactions, against venoms, etc.

A method to design such immunogens is described in this paper. The method is purely computational and requires nothing more than three-dimensional structural information about a protein antigen. The three examples used to illustrate the application of the method demonstrate the possibility of focusing the antibody response to a particular region of a molecule.

The first example was used to demonstrate the possibility of blocking the activity of an enzyme through the generation of antibodies directed against the catalytic site of the molecule. The second example was used to demonstrate the possibility of preventing the binding of one molecule to another, in this case the binding of IgE to an allergen, through the generation of antibodies that would compete for the site on the allergen that the IgE binds to. The third example was used to demonstrate the possibility of focusing the antibody response to a region of an antigen while avoiding the complications of antigenic polymorphism.

It is recommended that the decision to mutate a particular residue be made on the basis of its exposure to solvent, in other words its availability for interaction with antibody, and its estimated contribution to the binding interaction. In the examples above, 40% exposure of the side chain and an estimated contribution of 3% to the binding interaction were used. This resulted in the suggestion to mutate 20% of the residues in Example 1, more than 21% of the residues in Example 2, and about 28% of the residues in Example 3 - a significant fraction of the residues of the target molecule in all three cases. Despite the large number of mutations, the tertiary structure of the molecule is expected to be maintained. If it is deemed wise to mutate fewer residues, higher values of exposure, or of the estimated contribution to the binding interaction, or both, could be used.

To block a specific function, e.g., binding to ligand, enzymatic activity, etc., it may not be necessary to choose an epitope that encompasses the entire active site. The binding of antibodies to an epitope that covers a part of the site, possibly even just a single amino acid in the site, might suffice to interfere with the activity by steric hindrance.

If several possible epitopes are found to include the chosen critical region, it would be best to select an epitope that is already immunodominant. Nevertheless, an epitope with low antigenicity could still be emphasized. More than one epitope could be emphasized if this is found to be advantageous. When the antigen is known to be variable, e.g., the antigens of highly mutable pathogens, it would be wise to select an epitope that does not include the variable positions. This was illustrated in Example 3.
The ability to design an immunogen, that could elicit an antibody response that would interfere with a critical activity of a constantly-mutating pathogen while avoiding the variable positions, has important medical implications. Since the region that is responsible for the critical activity would most probably be conserved, this could lead to the development of a universal vaccine against the pathogen. Such a vaccine could counteract the decline of the immune response with advancing age (immunosenescence), since the memory immune cells, which a universal vaccine would elicit if administered while the individual is still young and healthy, could persist for a long time, possibly even a life-time.

The method is currently being applied in the development of possible vaccines against various pathogens. Those studies will be described in subsequent publications.

The general de-Antigenization of a molecule, except for a chosen region, has an obvious application also in the "humanization" of antibodies. The method could be used to de-Antigenize the framework regions of an antibody while preserving the structure and properties of the antigen-binding region. This is akin to the "veneering" procedure proposed earlier, where the exposed residues in the framework are replaced by human counterparts (Padlan 1991). The method described here will replace those exposed residues with amino acids that are expected to reduce antigenicity while preserving structure (Padlan 2008a).

CONFLICT OF INTEREST

Patent applications on the method have been filed with the US Patent and Trademark Office and with the Philippine Patent Office.

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