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Epitope-Based Vaccines : One Step at a Time

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Though the potential of the epitope-based strategy for vaccine development is undeniable it nevertheless has its own host of associated problems. While some of these problems can be anticipated, there may be others of which we may be presently unaware. Only time and experience will tell. Clearly this is a strategy that has to be developed one step at a time, which often will translate into resolving one problem at a time.

Key Words: Epitopes, Poly-epitope constructs, Vaccine design

Introduction

There is no doubt that the conceptual leap in understanding which led to the realization that one may use individual epitopes rather than whole microorganisms or its protein antigens as vaccines has paved the way for more versatile and flexible strategies. Indeed this approach now offers the possibility of eventually circumventing hurdles posed by some of the more intractable pathogens with respect to eliciting protective immunity. Thus the problem of antigenic variation (as in the case of HIV-1) may conceivably be tackled by using a cocktail of a spectrum of epitope variants. Furthermore complications arising due to the presence of immunodominant decoy sequences or sub-dominance or masking of neutralization epitopes can potentially be eliminated with using individual 'pure' epitopes which will not be expected to have any of these attendent problems.

However inspite of the multifarious advantages that epitope-based strategies offer there are nevertheless some limitations that hamper their applicability. It is our opinion that the eventual success of this strategy will be contingent upon the successful resolution of these issues.

Individual Epitopes

At the level of individual epitopes there are three principal drawbacks which dominate the issue. The first is that of genetic restriction. Because epitopes represent only a fraction of Th cell determinants on pathogens it is likely that they will lack the ability to induce Th cell activation in the context of a variety of MHC class II alleles. Since all vaccines are always intended for out-bred populations this may pose a serious problem. However the recent discovery of 'promiscuous' T cell epitopes offers a solution to the problem. The second hurdle is that of conformation. The overall conformational rigidity of native proteins entails that antigenic determinants are described not only by primary amino acid sequence but also by secondary and, in many cases, tertiary structures of proteins. Thus a mimetic of a native epitope must represent not only the sequence but also the native

conformation. This problem is an extremely daunting one and has not proved very easy to resolve.

The third hurdle is that of immunogenicity. It is a truism that smaller peptide sequences are less immunogenic than larger ones as a result of which epitope-based constructs are less likely to induce potent, long-lasting immune responses in the host. This is again not a trivial problem since any successful vaccine is expected to induce long-lasting immunity.

A Candidate Peptide Vaccine for Hepatitis B

It is apparent from what has been discussed so far that the limitations described above will need to be overcome to validate the epitope-based strategy for vaccine development. Our initial efforts were therefore aimed in this direction. For this we chose the hepatitis B virus (HBV) as our working system for the following reasons:

Several studies have demonstrated that protection against HBV is mediated purely by antibodies and an excellent vaccine consisting of the major envelope protein of HBV surface antigen (HBsAg) is commercially available.

The neutralization epitope(s) on HBsAg is a group specific one (also known as 'a' determinant) and has been shown to be encoded by disulfide-restricted conformational stretches of sequence.

Antibodies against the 'a' determinant provide long-lasting (> 8 years) immunity against hepatitis B.

We first addressed the issue of reconstructing a mimetic of conformational 'a' epitope. Based on literature studies and some speculation on our part we were able to generate cysteine-rich twenty four amino acid sequence from HBsAg that was shown to spontaneously self-oligomerize via disulfide bonding to recreate the 'a' epitope of HBsAg (Manivel et al. 1992a). A variety of experiments showed that our oligomeric or self-assembling peptide (peptide OS) represented a high fidelity mimic of the native determinant and that the fidelity was stringently dependent upon the integrity of the disulphide bonds (Manivel et al. 1992a). This epitope was also shown to represent an immunodominant constituent of the HBsAg epitope-repertoire in the context of the human immune system (Manivel et al. 1992a, Manivel et al. 1992b, Tripathy et al.1992). Subsequently we demonstrated that peptide OS could induce predominantly 'a'-specific antibodies in animal models which were able to immunoprecipitate HBV virions *in vitro* (Manivel et al. 1992a).

Fine mapping studies revealed that the 'a' epitope presented by peptide OS was a discontinuous one encoded by a methionine and lysine side-chains that were specially proximal in the oligomer (Manivel et al. 1992b). This also coincided with the fine-specificity of human anti-HBsAg responses (Manivel et al. 1992b).

Having thus resolved the problem of conformational epitopes, at least for the present example, we next addressed the issue of genetic restriction. Surprisingly this did not pose a serious problem since we found that peripheral T lymphocytes humans either infected with HBV or vaccinated with HBsAg could all be induced to proliferate on challenge with peptide OS *in vitro* assays (Mishra et al. 1993). A more detailed analysis indicated that the twenty four amino acid sequence encodes a multiplicity of Th determinants that are differentially recognized by various individuals and which collectively account for the universal T cell responses (Mishra et al. 1993).

As might be anticipated immunogenicity proved a drawback for peptide OS. Whilst peptide OS was highly immunogenic in Freunds Adjuvants, its immunogenicity, in alum - the only adjuvant presently considered for human use - left much to be desired. However we were subsequently able

to resolve the problem by simple attaching a lipid tail to the peptide sequence. This hydrophobic tail induced non-covalent aggregation of oligomer OS molecuels into large structures via micellar interactions (Manivel et al. 1993). The immunogenicity of these lipidated derivatives was shown to be comparable to that of recombinant HBsAg in the monkey model (Manivel et al. 1993).

Apart from providing a candidate peptide vaccine for hepatitis B we believe that these studies also suggest that problems associated with individual epitopes can be resolved.

Poly-epitope Constructs as Vaccines

In spite of what has been said earlier it must be realized that in many if not most cases immunization with a single epitope is unlikely to provide an effective immune cover for the host. This is especially true of multi-stage pathogens (eg. the malaria parasite plasmodium falciparum) or pathogens showing a high degree of antigenic diversity (eg. HIV-1). The prospect of designing multiple-epitope constructs therefore needs to be considered. From a generic standpoint the problem poses itself as - 'How does one design such poly-epitope molecules in a manner that will be immunologically productive?' This again is not necessarily a trivial question since there are several potential problems which can complicate idealized immunological behaviour. Some of these are:

Creation of irrelevent junctional epitopes

fine-specificity of antibody response

selective immunodominance of B cell epitopes

To address these issues we recently expressed a designed, chemically synthesized and assembled gene coding for a hundred amino acid polypeptide MEP-1 (Kumar et al. 1992). This polypeptide included select determinants from the large protein of HBsAg among which was the conformation-dependent 'a' epitope described earlier (figure 1). We were successfully able to regenerate this 'a' epitope in the context of MEP-1 by chemically induced disulphide rearrangement and also demonstrated the integrity of all included determinants (figure 2). A preliminary analysis indicated that MEP-1 was highly immunogenic in a variety of mouse strains and therefore held promise

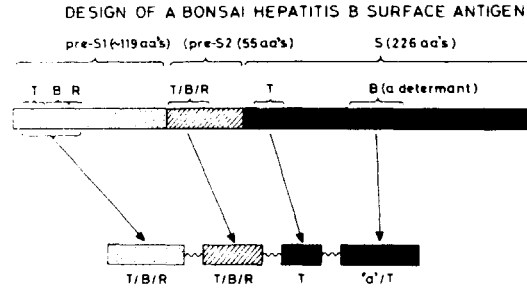

Figure 1 Design of a 'Bonsai' polypeptide derived from the large protein of hepatitis B virus (HBV). [The large protein of HBV is schematically illustrated in the top half of the figure. The lower half represents MEP-1 where the HBV-derived domain are indicated. **T**, T cell epitope; **B**, B cell epitope; **R**, receptor binding segments].

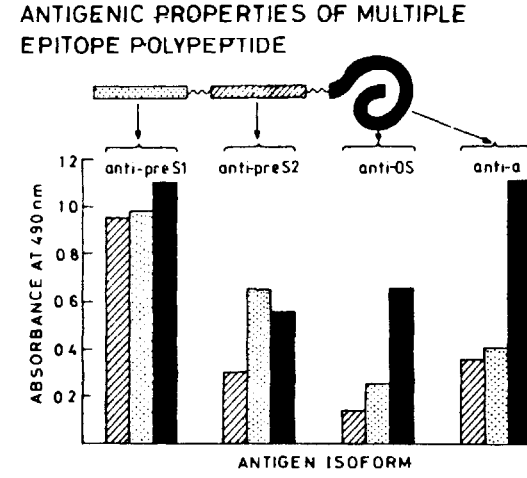

Figure 2 Immunoreactivity of MEP-1 segments by ELISA. [Integrity of the various HBV-derived domains in MEP-1 were assessed using antibodies raised against the corresponding synthetic peptides in an ELISA assay. In addition to this, antibodies specific for the 'a'-determinant of HBsAg were also employed]. ■, BME-reduced; ▨, gel extracted; ■, chemically treated for disulphide-bond formation.

(Kumar et al. 1994). The potential problems listed above were addressed as follows:

Creation of irrelevent junctional epitopes: Tandem linkage of two distinct epitopes always creates the possibility of inadvertantly generating a third epitope that spans the junction of the first two. Such epitopes, if immunodominant, could complicate matters by generating antibodies in host that are irrelevent with respect to the original pathogen. We have, in the past, demonstrated that this problem can be easily overcome by introducing a spacer of two glycine residues between epitopes (Rao & Nayak 1990, Kironde et al. 1991). This strategy also proved successful for MEP-1 where antibodies induced were exclusive for HBV-derived determinants in the absence of specificities directed against intervening regions (Kumar et al. 1994).

Fine-specificity of antibody responses: Surprisingly this did not pose any problem for us as epitope mapping studies revealed that murine anti-MEP-1 antibodies were specific for those segments of MEP-1 sequence which are functionally relevent for HBV (Kumar et al. 1994).

Immunodominance of B cell epitopes: Partisan recognition by the host immune system of the B cell epitope repertoire displayed by protein antigens has been known for some time (Wicker et al. 1984). While this phenomenon has been termed as relative intramolecular immunodominance its mechanistic basis remains obscure. Nevertheless the ability to put epitopes together in a polyvalent construct can be severely vitiated if only a subset of these are actually recognized during host humoral responses. This was indeed true of MEP-1 where we observed that murine anti-MEP-1 antibodies were predominantly specific for the amino-terminal Pre-S1-derived domain. We therefore investigated the etiology of this selective immunodominance in some detail. Preliminary studies indicated that immunodominance of the Pre-S1 derived region in MEP-1 was not a consequence of primary B cell recognition but was established during maturation of the primary antibody response. This correlated with the inability of B cells directed against alternate epitopes of MEP-1 to interact productively with Th cells and consequently received reduced T cell help. Interaction between epitope-specific B cells and T cells was found to be attenuated in the presence of early primary anti-MEP-1 antiserum and extent of inhibition was directly proportional to the level and affinity of epitope-specific immunoglobulins. Our studies therefore suggested that antibodies to a multi-determinant antigen selectively down-regulate maturation of epitope-specific primary humoral responses (figure 3). Having thus deciphered the basis for selective immunodominance we were subsequently able to circumvent it by altering the mode of antigen delivery (Vijayakrishnan et al. 1994).

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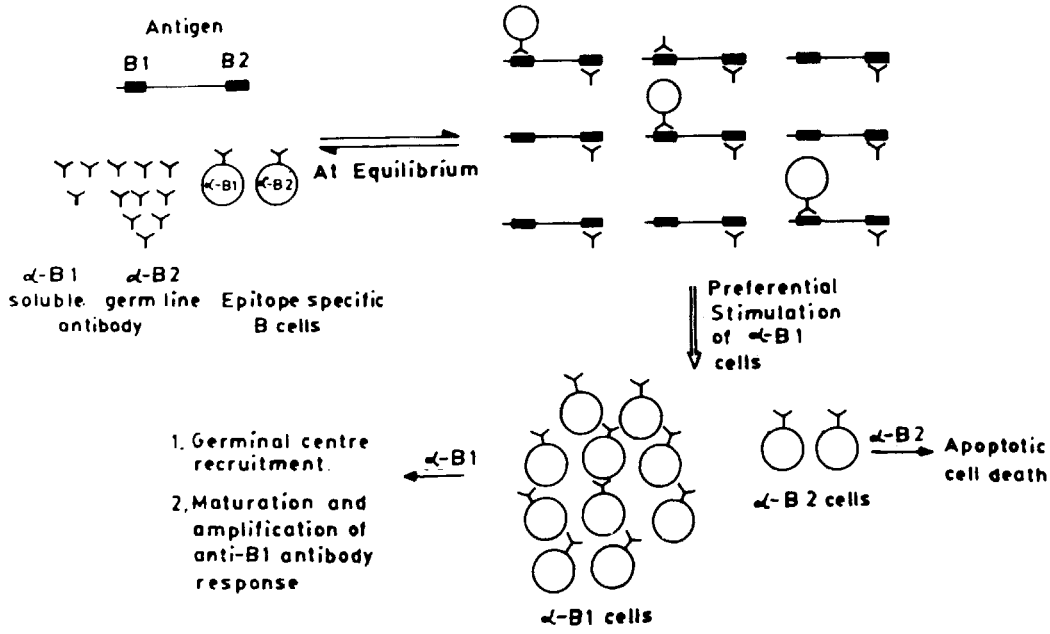

Figure 3 Etiology of relative intramolecular immunodominance. A scheme for germ-line antibody mediated regulation of immunodominance between two B cell epitopes (B1 and B2) on an antigen is presented. Epitope B2 elicitis high affinity (and therefore higher levels) of antibodies which subsequently compete successfully with B2 epitope-specific B cell for antigen recognition. This leads to an attenuation of anti-B2 antibody responses. In contrast, germ-line antibodies to B1 are of lower affinity as a result of which free epitope on antigen is always available for B1 specific B cell recognition, antigen capture, processing and presentation to Th cells. The outcome of these interactions is successful maturation and amplification of anti-B1 humoral responses.

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