

## EDITORIAL

# Synthetic peptide vaccines

The explosion in knowledge of the sequence and structure of proteins from pathogenic organisms over the last decade or so led to high expectations that it would be possible to use this information in the development not only of improved vaccines but also of vaccines for diseases for which no vaccines currently exist, e.g., malaria and AIDS. The theoretical attractions of synthetic peptides as vaccines, whether chemically synthesised or as the products of recombinant DNA technology, to substitute for the complex mixture of proteins in conventional vaccines, include targeting to specific sequences, commercial advantages and major improvements in product quality. However, the design of a peptide vaccine requires full understanding of the nature and extent of the immune response and as a consequence, progress in the development of successful synthetic vaccines has been disappointing, reflecting limitations in this understanding.

The protective immune response to a pathogenic micro-organism or parasite usually involves the induction of both humoral and cell-mediated responses. Whereas neutralising antibodies are considered to be the major defence against the majority of viral, bacterial and parasitic organisms, the cellular immune response, mediated by cytotoxic T lymphocytes (CTL), also plays an important role in host resistance to disease, through lysis of infected cells. Antigens can also activate helper T cells ( $T_h$ ) which secrete various mediators (lymphokines) that stimulate the production of B cells synthesising specific antibodies.

In many cases, the peptides used for producing synthetic vaccines were selected only on their likelihood of forming surface epitopes. However, such vaccines are usually non-protective. This is because such an approach ignores the function of the target protein. In practice, the construction of an effective synthetic vaccine requires the identification of the specific epitopes within the protein antigen responsible for eliciting the protective immune response. Unfortunately, the majority of protein epitopes recognised by neutralising antibodies are discontinuous. Short synthetic peptides are linear, and hence do not normally form neutralising epitopes. This requires alternative strategies to be adopted. One such approach includes synthesis or expression of peptides sufficiently large that they can adopt a tertiary conformation. It may also be possible to construct artificial conformational epitopes. There are several examples where this has been successful, including the first demonstration, over 10 years ago, of two peptides from the VPI coat

protein of foot and mouth disease virus (FMDV) that could elicit protective immunity in target species.<sup>1</sup> The first synthetic peptide vaccine to malaria in man to undergo large field trials was based on a similar approach.<sup>2</sup> Three short peptides, representing sequences from three blood-stage antigens, were used to construct a polymer in which the sequences adopted a tertiary conformation. Results of these trials in Colombia, involving 1548 volunteers, are encouraging with reported protective efficacy of 38.8%.<sup>3</sup> Whereas a linear peptide comprising the loop region of influenza virus haemagglutinin (HA), which according to crystallographic data is the most accessible site on HA, did not lead to neutralising antibodies, protective immunity was achieved when the peptide was fixed into a loop structure (cyclised), thus more closely resembling the conformation of the natural epitope.<sup>4</sup>

There have been a number of reports on the production of neutralising antibodies against peptides corresponding to sequences of bacterial toxins. However, in almost all of these instances neutralisation was incomplete, at best.<sup>5,6</sup> This is perhaps not surprising, as each toxin (which is a globular protein) possesses several neutralising epitopes, most of which are likely to be conformational. This will clearly limit the usefulness of a single peptide vaccine for the induction of a neutralising response. It may be necessary to use a mixture of conformational epitopes to produce a successful antitoxin vaccine. A detailed knowledge of the structure and function of the toxins will be essential to the success of any such approach.

Unlike neutralising epitopes, the epitopes recognised by  $T_h$  cells are sequential or continuous, the products of processed or partially degraded proteins.<sup>5</sup> Hence, synthetic peptides should be better suited to stimulating a  $T_h$  cell response. Thus, with an FMDV peptide, neutralising activity of the antibody was considerably enhanced by the presence of a synthetic  $T_h$  cell epitope.<sup>1</sup> This has led to the concept of chimeric peptides, comprising neutralising and  $T_h$  cell epitopes. One approach that has been investigated is the combination of multiple copies of a single peptide on a polylysine backbone, forming both neutralising (B-cell) and  $T_h$ -cell epitopes in a defined orientation,<sup>7</sup> although recent evidence suggests that T-cell-dependent B-cell activation can occur without covalent linkage of the B- and  $T_h$ -determinants.<sup>8</sup> Such short synthetic peptides corresponding to linear  $T_h$ -cell epitopes may be of considerable value in priming the immune response to subsequent challenge with otherwise sub-immunogenic doses of toxin, such as cholera

toxins. This may be particularly important in areas of endemic diseases, where the population is continuously exposed to low levels of the infectious organism.<sup>6</sup>

Like T<sub>h</sub>-cell epitopes, CTL epitopes are linear sequences of processed immunogens. Unfortunately, the prediction of such epitopes is currently not possible. However, such epitopes have been identified by sequencing peptides presented to CTL receptors. This has led to the identification of a conserved nonapeptide, representing a putative CTL epitope, from the liver stage specific antigen of the malaria parasite. Currently, this peptide is being investigated as the basis of a multiple peptide vaccine against malaria.<sup>9</sup> Several other examples of the use of CTL epitopes in vaccines have been described, such as those from the nucleoproteins of influenza and Sendai virus.<sup>6</sup> It is important to keep in mind the fact that synthetic vaccines based on these CTL epitopes will also require peptides that are capable of inducing a humoral immune response. An example of such a hybrid immunogen, consisting of a macromolecular assembly of neutralising, T<sub>h</sub>-cell and CTL epitopes has been described as a possible vaccine to HIV.<sup>10</sup>

Early expectations that it would be relatively simple to produce synthetic vaccines against a wide variety of organisms were clearly over-optimistic and to some extent naive. Indeed, there are those who now hold a contrary view, that it is improbable that synthetic vaccines will ever be produced to the majority of pathogens,<sup>11</sup> including HIV.<sup>12</sup> Perhaps a more realistic position is that of others, who feel that although there is a long way to go, much has been learned from the so-called "first generation" vaccines.<sup>13</sup> A rational approach is essential. Neutralising epitopes are largely conformational and this must be taken into account in vaccine design. Identification of the relevant epitopes will require detailed understanding of the relationship between tertiary structure and function. Finally, the immune response is complex and multi-faceted. Successful synthetic vaccines will comprise components that stimulate the appropriate elements of the immune system.

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