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# Design of synthetic peptides for oral vaccination

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## Abstract

The rational design of effective oral vaccines based on synthetic peptides is a very ambitious undertaking, and involves the solution of huge problems related to protection of the antigens against degradation in the alimentary tract, efficient uptake of the antigens by the relevant cells, and efficient induction of long lasting systemic immunity, local immunity, or both. This paper summarises the steps, necessary to develop such synthetic oral vaccines. These steps involve: (1) the definition of B-cell epitopes; (2) the definition of T-cell epitopes; (3) definition of the carrier or backbone molecule; (4) definition of an immunomodulating element; (5) definition of an adjuvant element; and (6) definition of a targeting element. Good progress is being made with respect to the first three steps, the other steps still provide major challenges, notably the definition of targeting elements. Nevertheless, the first synthetic oral vaccines may become reality in the near future, depending on the speed by which new technology in the area of molecular recognition will develop, i.e. the appropriate chemistry, organic chemistry, molecular modelling, resolution of the molecular interaction of key molecules in microbiology and immunology.

*Keywords:* Synthetic peptide; Oral vaccine; Drug design

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## 1. Introduction

The ultimate vaccine for human use will be an oral vaccine which is cheap, stable, induces long-

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lasting immunity against a spectrum of diseases after a single application. Development of such a vaccine is extremely ambitious and not yet feasible because necessary data and technology is lacking. However, less ambitious undertakings, for instance the development of a synthetic oral vaccine against a single disease which is stable and does not require a “cold chain” would already revolutionize vaccination programs, especially in the developing countries. Such vaccines would negate the need for trained medical personnel and the need to use syringes, which in itself, poses a considerable health risk.

If oral vaccination is used as an alternative for parenteral vaccination then the target would be to establish, in addition to local immunity, systematic immunity [1]. In this paper I limit myself to the possibilities to develop synthetic oral vaccines. Oral vaccines based on live carrier systems, for instance *Salmonella typhimurium* [2] may be equally effective and are probably closer to realisation, however they are beyond the scope of this contribution.

Development of effective synthetic oral vaccines has to take into account three aspects, which are special to this route of application: (1) the antigen should be sufficiently protected from the harsh environment to which it is exposed in the alimentary tract, i.e., extreme differences in pH and the abundance of proteolytic enzymes; (2) due to the large surface of the epithelium of the alimentary tract the antigen should be precisely targeted towards the entry point of the gut associated immune system in order to prevent the need for unrealistic high vaccine doses; and (3) due to the inherent low responsiveness of the gut associated immune system the antigen should be properly adjuvanted. When effective systemic immunity is needed in addition to local immunity, appropriate immunomodulation may be required as well.

To address these aspects properly two basic strategies can be used (Fig. 1). Both strategies are based on delivering molecules carrying a combination of basic functions towards the appropriate site in the gut, i.e. the M cells of the Peyer's patches.

One strategy is based on the delivery of the “naked” molecule which is harnessed against the

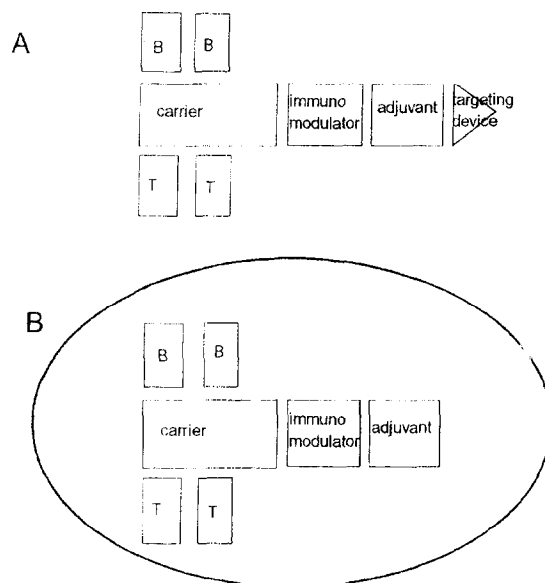


Fig. 1. Two strategies to develop oral vaccines. Strategy A is based on the use of all necessary elements combined into one molecule. Strategy B is based on delivering all necessary elements in an encapsulated form. In contrast to strategy A, a targeting device may not be necessary, because the encapsulated particles, depending on their physical-chemical properties, will be taken up spontaneously by the target cells.

environment, the other strategy is based on delivery of the molecule or molecules shielded from the environment by encapsulation. Both strategies are different and one has to decide at an early stage which one should be followed.

The functions or elements that are combined to form an effective oral vaccine are the following (they are the same in both strategies, however differently combined):

- (1) definition of the appropriate B-cell epitopes
- (2) definition of the appropriate T-cell epitopes
- (3) definition of the carrier or backbone molecule
- (4) definition of an immunomodulating element
- (5) definition of an adjuvant element
- (6) definition of a targeting element

Definition of each element is highly dependent on the strategy that is being followed. If the naked molecule strategy is followed, the whole synthetic molecule, including the epitopes, must be resistant to the environment; this can be

effected using non-natural building blocks, for instance D-amino acids. The consequence is that the epitopes should be mapped using the same building blocks.

Furthermore, the relative high number of elements that need to be combined more or less requires to keep the physical size—the molecular weight—of each element as small as possible; for instance, as small peptides or peptide-like molecules.

## 2. Definition of the elements of a synthetic oral vaccine

The minimal elements necessary to induce a proper immune response are B- and T-cell epitopes. B-cell epitopes are necessary to induce antibodies, T-cell epitopes to switch on the immune response.

### 2.1. Definition of B-cell epitopes

B-cell epitopes are defined as those parts of the molecule which bind antibodies with the appropriate properties, for instance virus neutralizing activity. In the case of proteins they are between 12 and 20 amino acids long. Since multiple, partially overlapping, B-cell epitopes may be located on an antigenic site [3], it is better to use the term “antigenic site” if the object is to define an antigen which will induce the appropriate antibodies. However, for simplicity sake I shall use the term B-cell epitopes instead.

From a practical point of view B cell epitopes can be divided into three groups (Fig. 2 [4,5]):

- (1) Real linear epitopes. These epitopes are easily mapped using peptides, but probably do not occur very often.
- (2) Discontinuous epitopes with relative large linear parts. The linear parts of these epitopes are easily mapped using peptides. Epitopes of this group probably form a large minority of all existing epitopes.
- (3) Discontinuous scattered epitopes. These epitopes probably form the majority of all existing epitopes. They are difficult to map

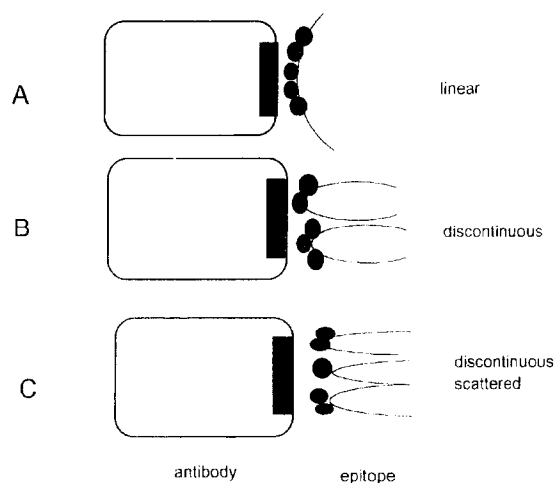


Fig. 2. Schematic representation of the three theoretically possible epitope configurations (epitope: antigenic determinant of a monoclonal antibody). The antibody is represented as a rough outline of the Fab fragment. The hatched area indicates the part of the antibody in contact with the amino acids of the epitope. The amino acids of the epitope are represented by small circles. If the amino acids of the epitope form a single protein strand the epitope is called linear; if the amino acids are located on two or more protein strands which are spatially next to each other due to the conformation of the antigen molecule, the epitope is called discontinuous. The most extreme case has been called discontinuous scattered. (From Ref. [4].)

with peptide based methods. One has to rely on methods like ‘site directed mutagenesis’, or, in the case of replicating agents, on ‘escape mutations’. Often the data will only produce a useful definition of the epitope if the spatial structure of the protein carrying the epitope is known.

The precise definition (i.e., length, core, role of individual amino acids) of epitopes of the first group is relatively straightforward, using PEP-SCAN methods (Fig. 3 [4]) or other peptide-based methods. Also the reconstruction of such epitopes as synthetic peptides is relatively easy and has been shown to induce readily the appropriate antibodies or even protection [5–7]. The precise definition of parts of discontinuous epitopes with relative large linear parts is equally easy.

The precise definition of epitopes of the third

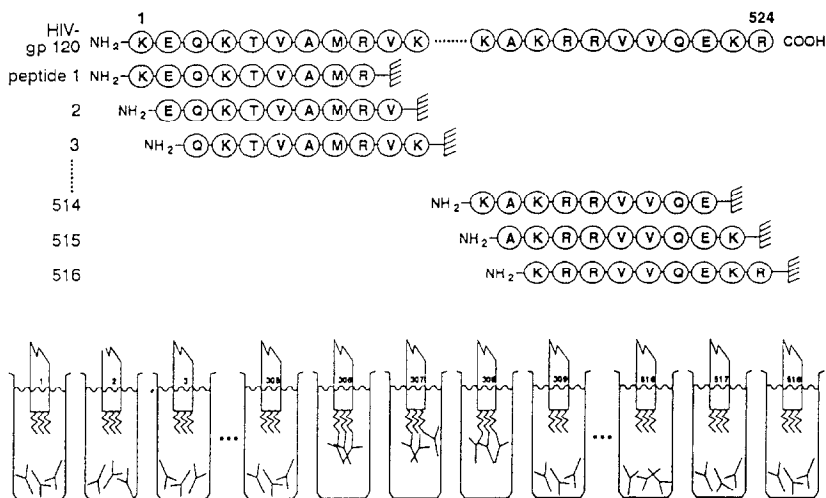


Fig. 3. Schematic representation of the PEPSCAN method. On top is shown the primary amino acid sequence of gp120 of HIV-1, the major glycoprotein of HIV-1. Each circle represents an amino acid. For the amino acids the single character code is used (i.e. A, alanine; C, cysteine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; Y, tyrosine). The amino acid sequence of gp120 of HIV-1 is then divided into overlapping peptides, as indicated. Peptide no. 1 is the peptide which starts with amino acid no. 1 and ends with amino acid no. 9, peptide no. 2 is the peptide starting at amino acid no. 2 to amino acid no. 10, etc. The peptides are synthesized on polyethylene rods, as shown at the bottom of this picture; the peptides are indicated as  $\sim$ . (The rods are in such a configuration that they fit into microtiter wells; this greatly simplifies handling of the rods and data manipulation.) All rods with peptides are then contacted with the same antibody (indicated as  $\leftarrow$ ). Some peptides will bind this antibody. After the rods are taken out of the antibody solution and have been washed, the antibody still present on the rods (bound to the peptide) can then be tested with anti-antibody conjugate for the presence of antibody. This directly produces the sequence of the peptide which bound the antibody. After this process the antibody can be removed from the peptides and the peptides can be reused. One technician can easily test 2000 different peptides daily for reactivity with a given antibody. Over 2000 different peptides are being synthesized on a routine basis at our laboratory each month. (From Ref. [4].)

group requires different methods and such epitopes are difficult or even impossible to reconstruct as linear epitopes. Their resolution needs different approaches, for instance the use of (random) peptide libraries which would allow the definition of peptide mimics of complex conformational epitopes [8–14]. Results are promising and the first useful examples may materialize soon. Nevertheless, a significant number of peptides has been defined which readily induce antiviral, anti-bacterial, anti-parasite, anti-hormone or anti-allergen antibodies.

The viruses include: foot-and-mouth disease virus [4,15–21], parvoviruses [22,23], herpes viruses [24,25], retro viruses [26–43]; corona viruses [44–50], and others [51–55]. The bacteria include: mycobacteria [56]. The parasites include:

malaria, trypanosoma and chlamydia [57–63], the hormones include: hCG and inhibin [64,65]. The allergens include: house dust mite major allergen der p II [66]; cat allergen Fel d I [67]. Others include alpha-bungarotoxins [68], fat globules [69], tumour necrosis factor-alpha [70].

Before starting to define B-cell epitopes to be used in an oral vaccine it is necessary, as discussed above, to decide which strategy (Fig. 1) will be followed. If the “encapsulated” strategy is selected, natural amino acids can be used; furthermore it may not be necessary to have all elements combined into one large molecule. If the naked molecule strategy is chosen than the epitopes have to be mapped using the same building blocks as used in the synthesis; because the use of non-natural amino acids may be

mandatory to counteract the action of proteolytic enzymes, the same amino acids have to be used for the epitope mapping (using PEPSCAN or other methods based on synthetic peptides this is hardly a problem).

## 2.2. Definition of T-cell epitopes

T-cell epitopes are necessary to switch on the immune response. Without T-cell epitopes poor responses, if any, are obtained. Thus T-cell epitopes must be present in a vaccine.

Preferentially T-cell epitopes should be from the same agent for which the vaccine is designed. Sometimes however T-cell epitopes can be borrowed from other proteins [6]. Pathogens normally carry numerous T-cell epitopes; it depends however on the genetic make-up of the vaccinated individual which epitope will be used [71,72]. For the development of peptide vaccines this forms an unsolved problem. Solutions are sought using “promiscuous” T-cell epitopes, i.e. epitopes that can be used by the immune system of individuals with different genetic make-up [73].

T-cell epitopes are linear peptides approximately between 9 and 15 amino acids long. Since large sets of free linear peptides can be made [74] systematic definition of T-cell epitopes, at the level of single amino acids, is equally simple and straightforward as that for linear B-cell epitopes [5]. Just as in case of B-cell epitopes one has to decide at the very beginning which strategy (Fig. 1) will be followed, because this determines how the T-cell epitopes must be mapped.

## 2.3. Definition of the carrier or backbone molecule

Normally protein molecules, like bovine serum albumin, ovalbumin or keyhole limpet haemocyanin, are used as carrier molecules for peptides. They provide two functions: firstly they make the peptides immunogenic due to the larger molecular weights of the peptide carrier molecule combination (peptides shorter than 18 amino acids are often non-immunogenic); secondly the carrier molecules provide the necessary

T-cell epitopes to switch on the immune response.

However, for oral vaccines these carrier molecules have disadvantages. For instance they are vulnerable to proteolytic attack; furthermore if they are being encapsulated their relative bulk may be disadvantageous. In addition protein carrier molecules suffer from the general disadvantage that the linkage and exposure of the peptides coupled to the carrier molecule are ill defined.

These disadvantages could be overcome by coupling the B- and T-cell epitopes to a synthetic backbone molecule [75]. Thus multiple peptides forming B- and T-cell epitopes combined into a single molecule, have been shown to form good immunogens [76]. This approach allows the use of both natural or small synthetic building blocks and is equally well applicable in both strategies.

## 2.4. Definition of the immunomodulating element

The object is to switch on the appropriate immune response (local, systemic or both). Although it is not yet completely clear how this can be done, it appears that by influencing the cytokines that regulate the development of the immune response, the outcome may be influenced [77–79]. Thus the incorporation of certain cytokines, cytokine inducers or cytokine antagonists into the vaccine, may help to influence the outcome of the vaccination. The use of cytokines, especially in oral vaccine, is probably not very practical due to proteolytic attack by environmental enzymes and due to the cost. However it has recently been shown that peptides derived from cytokines may still be bioactive [80,81; unpublished observations), which would make them excellently suited to be incorporated into a vaccine. Theoretically such peptides could be made proteolysis resistant by using non-natural amino acids; the cost of such peptides would compare very favourably with the cost of whole cytokines.

## 2.5. Definition of the adjuvant element

Adjuvants are probably highly necessary for oral vaccines because normally the local immune

system is known to produce transient responses of short duration or even the induction of a suppressor response [82]. Unfortunately, the working mechanism of adjuvants are poorly defined. Adjuvants probably work among other things, by inducing cytokine cascades. It is therefore possible that adjuvant and immunomodulating activities overlap. It remains to be seen whether they can be separated.

Relative small molecules have been described that exert adjuvant activity [83]. One of them is a peptide derived from IL-1 [84], well suited to be incorporated into a synthetic vaccine. Such synthetic molecules may be used directly mixed into the vaccine or hooked up to the backbone molecule.

### 2.6. Definition of a targeting element

In order to counteract the huge dilution and continuous movement of the vaccine within the alimentary tract, the encapsulated vaccine or naked vaccine molecule needs to be targeted to receptors of the appropriate cells in the gut. Unfortunately, such receptors are still ill defined. Nevertheless if such receptor binding peptides can be defined they can be exposed on the outer layer of the encapsulate vaccine or hooked on to the naked vaccine molecule. Candidate peptides could perhaps be designed as peptides which mimic the binding site of cholera toxin for its cell receptor, ganglioside GM1 [85]. Pending the development of such peptides one could use cholera toxin B subunit (CTB) as a carrier molecule [86] in case of the naked vaccine strategy. In case of the encapsulated strategy a targeting molecule may not be necessary because antigen containing microspheres, of the appropriate size and appropriate physical-chemical make-up, are taken up spontaneously by the appropriate cells in the gut, after oral ingestion [87].

### 3. Conclusion

The rational design of effective oral vaccines based on synthetic peptides is a very ambitious undertaking, and involves the solution of huge

problems related to protection of the antigens against degradation in the alimentary tract, efficient uptake of the antigens by the relevant cells, and efficient induction of long-lasting systemic immunity, local immunity, or both.

Rational approaches need additional data with respect to the details of the antigen uptake by M cells, the mechanism by which the internalized antigens induce a response, and the mode of action of adjuvants.

On the other hand, microorganisms exist which efficiently induce immune responses via the oral route. Furthermore, systematic methods are available to define the necessary epitopes, cell attachment sites and immunostimulating molecules in the form of simple synthetic natural or non-natural peptides. Also the chemistry is being developed to combine these components into biological active degradation-resistant vaccines. Thus, oral vaccines may soon be reality, while the ultimate oral vaccine, which is cheap and stable and induces longlasting immunity against a series of diseases after a single application, may one day be feasible as well. The speed of this development will depend on the speed by which new technology in the area of bio molecular recognition will develop, i.e. the appropriate peptide chemistry, organic chemistry, molecular modelling, resolution of the molecular interaction of key molecules in microbiology and immunology.

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