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Towards synthetic antiviral vaccines

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First successful attempts to mimick immunogenic sites of viral surfaces by synthetic peptides date back to 1963 when Anderer was able to demonstrate that antibodies to short fragments of the tobacco mosaic viral protein interact with the intact virion (ref. 1). Further studies led to the concept of synthetic vaccines and resulted in a number of active peptide preparations related to such major infections as influenza (refs. 2,3), hepatitis B (refs. 4-6), poliomyelitis (ref. 7).

According to modern views an efficient peptide vaccine must contain segments responsible for interaction with major subpopulations of immunocompetent cells: B-epitopes for B-lymphocytes, T-epitopes for T-helpers and so called agretopes recognized by the Ia antigen at the surface of antigen presenting cells (Fig. 1) (Note a).

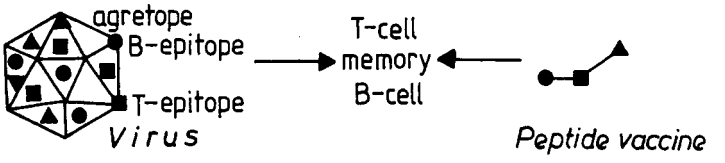

Fig. 1 . Principal construction of the antiviral peptide vaccine.

In the mid-80ies we began our studies of synthetic vaccines. Foot and mouth disease (FMD) was chosen as the first object, the choice being influenced by the recent progress in two groups (refs. 8,9). We synthesized two large series of peptides from the main immunogenic 130-160 sequence of the VP_1 protein of O_1K and A_{22} viral strains and found among them peptides which in the free state (i.e. without high molecular carrier) were able to protect various animal species from the infection. The best protective peptides are shown by thick lines in Fig. 2. Moreover, we were able to locate by immunochemical means approximate B- and T-epitopes in these peptides, the results being complementary to data of other investigators (refs. 10,11). Results of these studies are summarized in the reviews (refs. 12,13). It is of importance that some of the peptides obtained were active not only in small laboratory animals but also in agricultural ones (Table 1).

In summary, the principal feasibility of efficient anti-FMD peptide vaccine is now beyond doubt, its commerial value being dependent upon such parameters as duration of protection, cost, stability on storage, etc. Still, there is a long way to go before the optimal synthetic construction with respect to all these properties is reached.

On the other hand considerable advances achieved in the induction of protection against FMD by synthetic peptides are at least partially explained by the unique structure of the FMD virus. It is the only member of the family of picorna-viruses which has a sequential immunodominant region exposed on the surface of the virion (ref. 15). Furthermore, a characteristic feature of the virus is that the main immunogenic region contains a site for the binding of virus to the host cell (the 145-148 sequence of protein VP_1, ref. 16), so that the high antiviral activity of the antibodies induced by the synthetic peptides might be due to blocking of the sequence involved in binding the virus to the cell receptor.

Note a: Since agretopes and T-epitopes are usually located at the same region of the protein molecule the term "T-epitope" offen refers to both sites

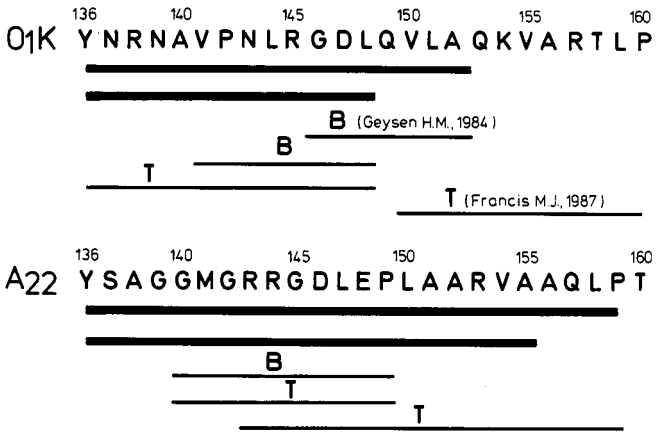

Fig. 2. Experimental peptide vaccines to O_1K and A_{22} strains of FMD virus and their respective epitopes.

TABLE 1. The protective activity of the 135-159 peptide of protein VP_1 from strain A_{22} of the FDM virus in natural hosts for FMD (for experimental details see refs. 13,14)

Peptide	Animal	Dose (mg)		TVA[a]	Protection
		Immunization I	Immunization II		
135-159	heifer	1	1	5.76	+
	heifer	1	1	6.00	+
136-152	heifer	1	1	2.32	-
	heifer	1	1	<1.00	-
Control	heifer	-	-	<1.00	-
	heifer	-	-	<1.00	-
135-159	sheep	1	0.1	6.17	+
	sheep	1	0.1	5.00	+
	sheep	1	0.1	5.17	+
	sheep	1		5.23	+
	sheep	1		5.12	+
Control	sheep	-	-	<1.00	-
	sheep	-	-	<1.00	-

[a]Titre of virus-neutralizing antibodies on the $-\log_2$ scale.

Difficulties arizing on the way to synthetic vaccines for other viral infections are illustrated on the example of hepatitis A (HAV). The amino acid sequences of the human HAV surface proteins VP_1-VP_4 (ref. 17) were analyzed by usual theoretical approaches (hydrophility, acrophilicity and antigenecity profiles, mobility of the side chains, α-helical and β-turn probabilities; see ref. 18 for a review), the selected peptides synthesized and studied for biological activity in the free state (antigenicity) and as conjugates with protein carriers: bovine serum albumin, keyhole limpet haemocyanin (KLH) or gelatine (immunogenicity).

As seen from Table 2 sera taken from the human recovered after HAV infection and containing high titers of antiviral antibodies recognizes none of the peptides tested. Moreover, none of the antipeptide sera binds to the intact virus or neutralizes the viral infectivity in the cell culture. It is also known (ref. 20) that antibodies to denatured proteins VP_1, VP_2 and VP_3 do not bind the virus and do not inhibit its growth *in vitro*. In all probability the sites of viral recognition and neutralization with antibodies in case of HAV, contrary to FMD belong to the class of conformational B-epitopes, i.e. they are made up of a number of peptide segments separated along the amino acid sequence or even belonging to different proteins of the viral particle.

Modeling such sites presents a considerable challange to a synthetic chemist. A hypothetic approach to the problem which is currently under study in our laboratory is depicted in Fig. 3. Relatively short peptide segments representing the putative parts of conformational B-epitopes are anchored in the lipid bilayer with the help of long hydrophobic radicals. Due to mobility of the lipid phase they can freely assemble forming structures resembling the

TABLE 2. Immunochemical properties (titers) of HAV synthetic peptides[a]

Protein	Peptide	Antigenicity		Immunogenicity		
		Binding to anti-native HAV sera	Binding to anti-denatured HAV sera	Anti-peptide sera	Binding of antipeptide sera to native HAV	Virus neutralization *in vitro*
VP_1	11- 25	<10	10^2	10^3	<10	<10
	76- 86	<10	-	10^3	<10	<10
	98-112	<10	10^3	10^4	<10	<10
	102-107	<10	10^2	10^2	<10	<10
	113-123	<10	-	10^2	<10	<10
	290-299	<10	10^2	10^3	<10	<10
	276-298	<10	-	-	<10	<10
VP_2	42- 62	<10	-	-	<10	-
VP_3	62- 75	<10	-	-	<10	<10
VP_4	1- 23	<10	-	10^3	<10	<10

[a]Details will be given in ref. 19.

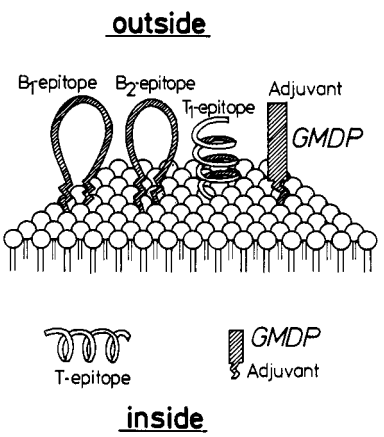

Fig. 3. Peptide components of synthetic vaccine in the lyposome (see the text for the explanation).

native antibody binding sites. T-epitopes can be incorporated into the membrane in a similar way although from immunological point of view we might prefer to have such peptides inside the liposome. In this case the peptide will enter the antigen presenting cell without eliciting unnecessary self-antibodies. Other immunoactive components introduced to such construction might also add to its efficacy. A lipophilic derivative of a powerful immunoadjuvant, N-acetylglucosaminyl-N-acetylmuramyl-D-alanyl-D-isoglutamine (GMDP) discussed by us at one of the previous IUPAC meetings (ref. 21) is shown in the figure as an example.

The family of flaviviruses seems to elicit antibodies to conformational epitopes of their envelope proteins, called E proteins (ref. 22). Amino acid sequence of such protein from the virus of tick-born encephalitis, a dangerous, often lethal infection common to forestry areas of the eastern parts of the Soviet Union was recently deciphered (ref. 23). This allowed us to synthesize a set of peptides from the established sequence and to study biological properties of these peptides and of respective KLH-conjugates (Table 3).

Free peptides except for 35-51 were not antigenic, i.e. they did not bind antibodies to protein E. They were also nonimmunogenic (did not induce antibodies), they did not inhibit the viral growth *in vitro* and had no protective activity *in vivo*. As expected, the KLH-conjugates were better immunogens and, moreover, two of them induced virus neutralizing antibodies. Further studies revealed a surprisingly broad spectrum of biological activities for the 98-113 peptide. This peptide inhibits viral entry to the macrophages, stimulates the phagocytic activities of macrophages and strongly inhibits the immune reaction (8-10 fold suppression of antibody forming spleen cells to sheep erythrocytes) at intrabrain application. We believe that these data are indicative of an important role of the 98-113 segment in the virus-host interactions

Apparently, studies of synthetic peptide vaccines besides serving the main purpose might provide valuable information on the molecular mechanisms of viral pathogenicity, suggesting new ways for treating viral discases.

TABLE 3. Immunochemical properties (titers) of synthetic segments of the protein E from tick-born encephalitis virus

Peptide	Antigenicity	Immunogenicity		
	Binding to anti-protein E sera	Antipeptide sera	Binding of anti-peptide sera to protein E	Virus neutralization *in vitro*
35-51	$10^{3.7}$	<10	<10	<10
(35-51)·KLH	-	$10^{2.4}$	<10	<10
50-61	<10	<10	<10	<10
(50-61)·KLH	-	$10^{2.1}$	<10	<10
98-113	<10	10	<10	<10
(98-113)·KLH	-	$10^{3.8}$	$10^{3.1}$	$10^{2.4}$
118-129	<10	-	-	-
(118-129)·KLH	-	$10^{2.4}$	<10	<10
130-143	<10	10	<10	<10
(130-143)·KLH	-	$10^{2.4}$	$10^{2.2}$	<10
362-372	<10	-	-	-
(362-372)·KLH	-	$10^{2.1}$	<10	<10
394-403	<10	<10	<10	<10
(394-403)·KLH	-	$10^{3.4}$	$10^{2.2}$	$10^{2.8}$
Protein E	$>10^5$	-	$>10^5$	$10^{4.4}$

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