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A Mimotope from a Solid-Phase Peptide Library Induces a Measles Virus-Neutralizing and Protective Antibody Response

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A solid-phase 8-mer random combinatorial peptide library was used to generate a panel of mimotopes of an epitope recognized by a monoclonal antibody to the F protein of measles virus (MV). An inhibition immunoassay was used to show that these peptides were bound by the monoclonal antibody with different affinities. BALB/c mice were coimmunized with the individual mimotopes and a T-helper epitope peptide (from MV fusion protein), and the reactivity of the induced anti-mimotope antibodies with the corresponding peptides and with MV was determined. The affinities of the antibodies with the homologous peptides ranged from 8.9 $\times 10^5$ to 4.4 $\times 10^7$ liters/mol. However, only one of the anti-mimotope antibodies cross-reacted with MV in an enzyme-linked immunosorbent assay and inhibited MV plaque formation. Coimmunization of mice with this mimotope and the T-helper epitope peptide induced an antibody response which conferred protection against fatal encephalitis induced following challenge with MV and with the structurally related canine distemper virus. These results indicate that peptide libraries can be used to identify mimotopes of conformational epitopes and that appropriate immunization with these mimotopes can induce protective antibody responses.

Synthetic peptides have considerable potential value in biology and medicine as analytical and diagnostic reagents, therapeutics, and new vaccines. The use of peptides as vaccines offers several advantages over conventional vaccine strategies in terms of safety, cost, stability, and relative ease of production. In addition, peptide vaccines have the advantage that they can be rationally designed to contain structures that stimulate appropriate immune responses (e.g., Th cells, B cells, and Tc cells) while avoiding structures that stimulate unwanted responses (e.g., suppressive effects). Although many antibodies are directed toward linear antigenic epitopes, it is likely that a significant proportion of humoral antibody responses to infectious agents are directed toward conformational epitopes. Thus, the identification of peptide structures that mimic linear epitopes from the primary amino acid sequence of a particular protein should be relatively straightforward but the identification of conformational B-cell epitopes is likely to be more problematic.

Combinatorial synthetic peptide libraries, in which peptides are expressed on either the surface of filamentous bacteriophages or an insoluble solid phase, have been used to generate vast numbers of different peptides for biological screening both in vivo and in vitro. For example, the bacteriophage peptide libraries have been used to identify ligands for proteins such as streptavidin, integrins, and the urokinase cell surface receptor (3, 9, 17). In addition, these libraries have been used to identify a linear structural epitope on plasminogen-activating factor inhibitor type 1 (10), an epitope on human immunodeficiency virus type 1 gp120 which induces a neutralizing antibody to human immunodeficiency virus type 1 (11), and an epitope on herpes simplex virus type 1 (22). However, attempts to identify conformational epitopes by using phage libraries have met with only limited success (1, 6, 13). In this case, the peptide mimicking a discontinuous epitope recognized by a monoclonal antibody against *Bordetella pertussis* toxin, although reactive with the monoclonal antibody, could not induce antibodies which were reactive with the original antigen.

Solid phase combinatorial peptide libraries have also been used to identify a number of biologically important peptide sequences such as enzyme inhibitors for human immunodeficiency virus protease (18) and trypsin (5), but the use of the solid phase libraries to generate mimics of conformational epitopes (mimotopes) has been very limited since the methodology was originally defined (7). It should be emphasized that mimotopes of conformational epitopes will not necessarily bear sequence identity with the primary amino acid sequence of the protein but will assume a conformation which mimics that of the epitope.

Measles virus (MV) and the closely related canine distemper virus (CDV) are members of the morbillivirus subgroup of the paramyxovirus family. The surface localization of the hemagglutinin (H) and fusion (F) glycoproteins is important in determining their role in mediating virus-cell membrane interactions during infection. The H protein is responsible for the first step in infection, i.e., the adsorption of the virus to receptors on the host cell, whereas the F glycoprotein is actively involved in the induction of membrane fusion (2). Monoclonal antibodies against both H and F glycoproteins have been shown to neutralize the virus in vitro and to protect animals from infection when administered passively (4, 14). Although the widespread use of attenuated measles vaccine has been successful in developed countries, the virus still remains one of the main causes of infant mortality in many parts of the world. Thus, the development of a synthetic peptide vaccine based on the critical antigenic sites from the structural proteins of the virus is a valid strategy for the control of measles.

In this paper, we describe the use of a solid phase combinatorial peptide library to generate a panel of mimotopes of the epitope recognized by a monoclonal antibody to the F protein of MV (MVF). The ability of these peptides to interact with the monoclonal antibody has been assessed, and the reactivity of anti-mimotope antibodies induced in mice with the corresponding peptides and with MV has been determined. Finally,

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evidence is provided to indicate that immunization of mice with one of these mimotopes induces an antibody response which confers protection against fatal encephalitis induced following challenge with neuroadapted MV or CDV.

MATERIALS AND METHODS

Synthesis of peptide library and selection of mimotopes. The solid phase peptide library was synthesized on NovasynTG, a polystyrene-polyoxyethylene resin functionalized with amino groups, in such a way that each resin bead bears a different 8-amino-acid peptide (12). The resin allows the deprotection of the peptide without its cleavage from the resin, and there are 2.86×10^6 beads per g of resin, with a capacity of 0.24 mol/g. Nineteen reaction vessels containing the resin were used for the synthesis by Fmoc chemistry with all amino acids except cysteine.

The monoclonal anti-MVF antibody (F7-21) used in these studies was kindly provided by P. de Vries, Bilthoven, The Netherlands. The beads were blocked before use with 10% bovine serum albumin–1% Tween 20 in phosphate-buffered saline at room temperature for 2 h. Then 500 μl of a 1:5,000 dilution of F7-21 in blocking buffer was added to 50 mg of the resin library. The beads were incubated for 2 h at room temperature and then overnight at 4°C. Beads bound by the monoclonal antibody were identified after the addition of peroxidase-conjugated rabbit anti-mouse immunoglobulin G antibody (heavy plus light chains; Nordic, Tilburg, The Netherlands) at a dilution of 1:1,000 in blocking buffer followed by diaminobenzidine-chloronaphthol substrate. Darkly stained beads were selected manually with a capillary tube, the peroxidase-labelled antibody was removed by washing with trifluoroacetic acid, and the peptides on the individual beads were microsequenced (Department of Biochemistry, University of Manchester, Manchester, United Kingdom).

Peptide mimotope synthesis. Conventional solid phase peptide synthesis was performed by converting Fmoc-protected amino acids to the hydroxybenzotriazol-activated esters by treatment with hydroxybenzotriazol and diisopropylcarbodiimide in dimethyl formamide. The subsequent coupling reactions were performed in dimethyl formamide, and the Fmoc were groups removed with 20% piperidine in dimethyl formamide followed by a series of washes in dimethyl formamide. After synthesis, side chain protecting groups were removed, and the peptide was cleaved from the support resin with trifluoroacetic acid in the presence of scavengers. After cleavage, the peptides were extracted into diethyl ether and purified by preparative high-pressure liquid chromatography (HPLC), and their purity was assessed by analytical HPLC, amino acid analysis, and fast atom bombardment mass spectrometry.

Mice. Inbred male and female BALB/c (*H-2*^{d}), SWR/J (*H-2*^{q}), and C57BL/6 (*H-2*^{b}) mice were purchased from the Medical Research Council, Mill Hill, London, United Kingdom.

Viruses. Rodent-neuroadapted MV (CAM/RBH strain) was kindly provided by U. Liebert, Institut für Virologie und Immunologie, Wurzburg, Germany, and neuroadapted CDV (Ondersterpoort strain) was kindly provided by F. Wild, Institut Pasteur, Lyon, France. Stocks of virus were prepared by passage in suckling mouse brain. Mouse brain homogenates (25%) from infected suckling mice were used as the source of virus and were stored at −70°C.

Immunization and challenge of mice. Groups of BALB/c and SWR/J mice were immunized intraperitoneally with 50 μg of mimotopes 1 to 6 alone or coimmunized with 50 μg of the mimotope plus 50 μg of MVF 258-277 in Freund's complete adjuvant (FCA) and boosted after 2 weeks with the same dose of peptides in Freund's incomplete adjuvant. We have previously demonstrated that simple coimmunization of B- and T-helper epitope peptides is effective at inducing good anti-B epitope antibody responses (20). Serum samples were obtained weekly.

For protection studies, three groups of six to eight mice were used. (i) Groups of 6-week-old female BALB/c mice were coimmunized intraperitoneally with 50 μg of mimotope 2 and either 50 μg of MVF 258-277, Th epitope SH45 from the SH protein of respiratory syncytial virus (21) in FCA, or 2×10^4 PFU of MV per mouse. The mean virus-neutralizing titer was 1:40 in peptide-immunized mice and 1:180 in virus-immunized mice. Offspring born to mimotope 2-immune mice, offspring born to nonimmune mice, and offspring born to MV-immunized mice were challenged intracranially at 2 to 3 weeks of age with 10^4 PFU of neuroadapted MV or 10^4 PFU of neuroadapted CDV in 25- to 30-μl volumes. At this age, mice are maximally susceptible to intracranial challenge. Since suckling mice receive maternal antibody only via their mother's milk, this approach allows us to answer the question whether antibody alone can confer protection. Mice were monitored daily for the development of clinical signs of disease and were weighed. Cumulative mortality was assessed over a period of up to 8 weeks postchallenge (16). (ii) Groups of 6-week-old female BALB/c mice, immunized intraperitoneally with 50 μg of either MVF 258-277 or respiratory syncytial virus SH45, were challenged with neuroadapted MV as controls. (iii) BALB/c mice, 2 weeks old (five mice per group), received 200 μl of either anti-M2 serum ($\log_{10}$ titer, 3.79) or normal mouse serum intraperitoneally and were challenged intracranially 24 h later with 10^4 PFU of MV in 25- to 30-μl volumes.

Mice were monitored daily for the development of clinical signs of disease and were weighed. Cumulative mortality in immunized and control groups was assessed over a period of up to 2 months after challenge.

Antibody assays. (i) Enzyme-linked immunosorbent assay (ELISA): anti-peptide antibody. Anti-peptide antibody activity in serum samples obtained from immunized mice prior to and after virus challenge was assessed by a solid phase immunoassay with microtiter plates (Nunc, Roskilde, Denmark) coated overnight at 4°C with a 5-μg/ml solution of the appropriate peptide in 0.1 M carbonate-bicarbonate buffer (pH 9.6) at 50 μl per well. Plates were washed with tap water, and the wells were blocked by incubation at 37°C for 2 h with 1% gelatin in phosphate-buffered saline (pH 7.3). Serial twofold dilutions of the sera in phosphate-buffered saline–0.05% Tween 20–0.25% gelatin were added to the wells of the plates and incubated for 1 h at 37°C. The wells were washed, 50 μl of a 1:1,000 dilution of peroxidase-conjugated rabbit anti-mouse immunoglobulin G (heavy plus light chains) was added to each well, and the wells were incubated for 1 h at 37°C. Unbound conjugate was removed by washing, and bound enzyme was detected by the addition of a solution of 0.04% *o*-phenylenediamine–0.004% hydrogen peroxidase in citrate-phosphate buffer at 50 μl per well. After 10 min, the reaction was stopped by the addition of 25 μl of 2 M sulfuric acid per well and absorbancies (A_{492}) were determined on a Titertek Multiskan (Dynatech MR5000). Titers are expressed as $\log_{10}$ of the reciprocal of the dilution of the antiserum giving an A_{492} of 0.2.

(ii) ELISA: affinity of anti-mimotope antibodies. The relative affinities of the anti-mimotope antibodies for their homologous peptides were assessed by a solid phase enzyme inhibition assay (24). ELISA plates were coated with mimotopes 1 to 6 at 5 μg/ml, doubling dilutions of each anti-mimotope antibody were added, and the assay was performed as described above. The dilution of each antibody giving an A_{492} of 0.6 was determined and used in the second stage of the assay. We used 0.5 $\log_{10}$ dilutions of a 3-μmol/ml solution of each mimotope to inhibit the binding of the homologous anti-mimotope antibody. The relative affinity of the antibody was calculated as the reciprocal of the concentration of mimotope which gave 50% inhibition of the binding in the absence of inhibitor peptide.

(iii) ELISA: anti-virus antibody. The reactivity of anti-mimotope antibodies with MV was assessed by using two ELISA systems. The first was a commercial ELISA kit (Enzygnost; Behring) in which infected and noninfected cells were used as solid phase antigens. As specified by the manufacturer, a difference in A_{492} of greater than 0.2 obtained with test and normal sera bound to infected and mock-infected cells, respectively, was regarded as positive in the commercial ELISA. A pooled serum sample from MV-immunized BALB/c mice was included in the assays as a positive control. In the second system, ELISA plates were coated with 2×10^2 PFU of virus per well to serve as the solid phase in the assay performed as described above.

The reactivity of the anti-mimotope antibodies with CDV was assessed by an ELISA in which plates were coated with 2×10^2 PFU of virus per well (kindly provided by C. R. Howard, Royal Veterinary College, London, United Kingdom).

(iv) ELISA: affinity of the monoclonal anti-MVF antibody for the mimotopes. The monoclonal antibody could not be shown to bind to the mimotopes in direct binding assays on a solid phase or a fluid phase radioimmunoassay (data not shown). However, the reactivity of the mimotopes with the monoclonal antibody could be shown by their ability to inhibit its binding to MV. The relative affinity of the binding of the monoclonal antibody to each mimotope was expressed as the reciprocal of the concentration of each mimotope required to inhibit by 50% the binding of the monoclonal antibody to MV in the Behring Enzgnost assay.

(v) Virus neutralization. MV neutralization was carried out as described by Giraudon et al. (8). Briefly, 50 PFU of virus was mixed with a range of dilutions of heat-inactivated serum. The virus-serum mixtures were incubated for 1 h at 37°C in 96-well microtiter plates. Vero cells (2×10^4 per well) were added, and the plates were examined 3 to 6 days later for any cytopathic effect. End points were determined as the dilution of serum which reduced the plaque number to 50% of the mean normal mouse serum control value. A pooled serum sample from MV-immunized BALB/c mice was included in the assays as a positive control.

Analysis of peptide sequences. The structural similarity or dissimilarity of the amino acid sequences of the mimotopes with respect to sequences in the fusion protein of MV was assessed with a computer program for calculating average structural dissimilarity (PROASD), kindly provided by C. Hackett and D. Horowitz, Wistar Institute, Philadelphia, Pa. This program compares a peptide segment with a larger amino acid sequence, taking into account the structural dissimilarity between the two sequences by using the average structural dissimilarity (ASD) formula (19) and a dissimilarity table (23). The sequences having the lowest ASD score are most similar, whereas those with the higher ASD scores are least similar.

RESULTS

Amino acid sequences of mimotopes and their comparison with the MVF protein by using the PROASD program. The amino acid sequences of the peptides on the six resin beads selected with the monoclonal anti-MVF antibody are shown in Table 1. These sequences were compared with the sequences of MVF and CDVF proteins (they share 82% sequence homology) by using the PROASD program. The mimotopes had

TABLE 1. Amino acid sequences of mimotopes of the monoclonal anti-MVF antibody

Mimotope	Sequence
1	TVHTQQNY
2	NIIRTKKQ
3	QFFRRMDY
4	TRRAGPMQ
5	GRRLPVVI
6	IAAQKPKV

some structural similarity to restricted regions of both MVF and CDVF. Mimotopes 1, 2, 4, 5, and 6 had structural similarity to the region from 153 to 181 of MVF, and mimotopes 1, 4, 5, and 6 had structural similarity to the region from 173 to 206 of CDVF. These sequences occur in a region of the F proteins of the two viruses (145 to 210) which have 68% sequence identity and 91% homology.

Reactivity of polyclonal mouse anti-MV antibody with mimotopes. A pooled anti-MV antiserum from a group of five MV-immunized BALB/c mice was used in an ELISA with either mimotope 1 or 2 on the solid phase. The serum reacted with both mimotopes 1 and 2 with titers of 1:40 and 1:80, respectively.

Affinity of monoclonal anti-MVF antibody for mimotopes. It was not possible to demonstrate direct binding of any of the mimotopes to the monoclonal anti-MVF antibody by solid-phase ELISA or fluid-phase radioimmunoassays (data not shown). However, binding of the mimotopes by the monoclonal antibody was demonstrated by the ability of the mimotopes to inhibit the binding of the monoclonal antibody to MV in an ELISA. This allowed the determination of relative affinity values of the binding of each mimotope by the antibody. There were marked differences in the relative affinity values obtained, which ranged from 1.1×10^2 to 1.26×10^6 liters/mol (Table 2).

Immunogenicity of mimotopes in BALB/c and SWR/J mice. No anti-mimotope antibody activity could be demonstrated in the serum of groups of BALB/c mice immunized with the mimotopes alone in FCA. Accordingly, groups of BALB/c mice were coimmunized (18) with mimotopes 1 to 6 and MVF 258-277 (a Th epitope) and SWR/J mice were coimmunized with mimotopes 1 and 2 and the Th epitope. All mimotopes induced antibody responses to the homologous peptides (Fig. 1 and 2) with affinities which ranged from 8.9×10^5 to 4.5×10^7 liters/mol in BALB/c mice (Table 3).

Reactivity of anti-mimotopes with MV. All anti-mimotope antisera were tested in the Enzygnost assay for anti-MV reactivity and in the MV plaque inhibition assay. Only anti-mimotope 2 antibodies in BALB/c and SWR/J mice had demonstrable anti-MV activity by these assays (Table 4).

Protection following challenge of mimotope-immunized mice with CDV and MV. A group of five female BALB/c mice were coimmunized with mimotope 2 and the Th epitope peptide MVF 258-277. They and a group of control unimmunized females were then mated, and their offspring were challenged intracerebrally at 3 weeks of age with neuroadapted CDV. Cumulative mortality in the offspring of mimotope 2-immunized mothers was markedly slowed compared with that seen in offspring of mothers in the control group (Fig. 3), although all mice eventually died.

A further challenge experiment was performed with the off-

TABLE 2. Relative affinity of monoclonal anti-MVF antibody for the six mimotopes assessed by a solid phase inhibition ELISA

Mimotope no.	Concn (μM) for 50% inhibition	Relative affinity (liters/mol)
1	2.65	3.78×10^5
2	2.36	4.23×10^5
3	10.0	1.00×10^5
4	0.79	1.26×10^6
5	500.0	2.00×10^3
6	8,900.0	1.10×10^2

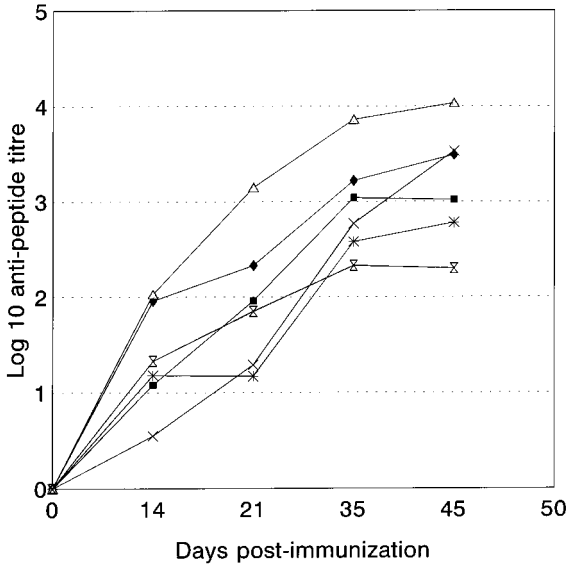

FIG. 1. Immunogenicity of mimotopes 1 to 6 in BALB/c mice. Groups of four to six 6-week-old BALB/c mice were coimmunized intraperitoneally with 50 μg of each of the mimotopes 1 to 6 mixed with 50 μg of MVF 258-277 (to provide T-cell help) in FCA and boosted after 2 weeks with the same dose of peptides in Freund's incomplete adjuvant. Serum samples were obtained weekly. Values represent $\log_{10}$ anti-peptide antibody titer at various times. Symbols: ⧖, mimotope 1; △, mimotope 2; ∗, mimotope 3; ▨, mimotope 4; ×, mimotope 5; ◆, mimotope 6.

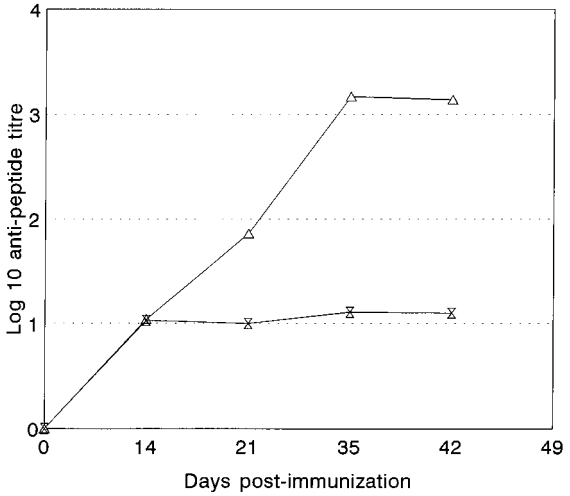

FIG. 2. Immunogenicity of mimotopes 1 and 2 in SWR/J mice. Groups of four to six 6-week-old SWR/J mice were coimmunized intraperitoneally with 50 μg of either mimotope 1 or 2 mixed with 50 μg of MVF 258-277 (to provide T-cell help) in FCA and boosted after 2 weeks with the same dose of peptides in Freund's incomplete adjuvant. Serum samples were obtained weekly. Values represent $\log_{10}$ anti-peptide antibody titer at various times. Symbols: ⧖, mimotope 1; △, mimotope 2.

TABLE 3. Relative affinity of anti-mimotope antibodies raised in BALB/c mice for their homologous peptides

Mimotope no.	Concn (pM) for 50% inhibition	Relative affinity (liters/mol)
1	ND[a]	ND
2	22.4	4.5×10^7
3	1,120.0	8.9×10^5
4	141.2	7.1×10^6
5	223.9	4.5×10^6
6	501.1	2.0×10^6

[a] ND, no detectable antibody binding.

spring from BALB/c mice which had been coimmunized with mimotope 2 and Th epitope peptide 45-60 from the SH protein of respiratory syncytial virus, and immunized and control offspring were challenged with neuroadapted MV. Offspring from mimotope 2-immune mothers challenged with MV showed a delayed cumulative mortality compared with that seen in control animals, and, as with the CDV challenge, all mice subsequently died (Fig. 4).

Antibodies induced following immunization with the MVF 258-277 and the respiratory syncytial virus SH 45-60 Th epitopes did not bind to MV. Furthermore, offspring from helper epitope-immunized mice were not protected against MV-induced encephalitis (data not shown).

To assess the effect of the passively transferred anti-mimotope 2 antibodies on the survival of MV-challenged BALB/c mice, two groups of 2-week-old BALB/c mice received either anti-mimotope 2 serum or normal mouse serum and were challenged intracranially with neuroadapted MV. The passive transfer of anti-mimotope 2 serum conferred significant protection against MV encephalitis to BALB/c mice (day 18, $P = 0.047$; Fisher's exact test) (Fig. 5).

DISCUSSION

The use of combinatorial synthetic peptide libraries has the potential for the identification of peptide structures (mimotopes) which mimic both linear and conformational epitopes recognized by monoclonal antibodies. As mimics of conformational epitopes, mimotopes will not necessarily bear any identity to the primary amino acid sequence of the protein but will represent a structure which can be recognized by the immune system in the same way as the original epitope. In this paper, we report the use of a solid phase random peptide library to identify the mimotopes of the epitope recognized by a monoclonal antibody specific for the F protein of MV.

TABLE 4. Reactivity of anti-mimotope antibodies raised in BALB/c and SWR/J mice

Mouse strain	Mimotope no.	Log_{10} anti-mimotope titer	Anti-MV titer[a]	Plaque inhibition titer[b]
BALB/c	1	2.36 ± 0.19	−ve[c]	−ve
	2	4.03 ± 0.34	1:80	1:40
	3	2.78 ± 0.57	−ve	−ve
	4	3.04 ± 0.49	−ve	−ve
	5	3.53 ± 0.24	−ve	−ve
	6	3.48 ± 0.39	−ve	−ve
SWR/J	1	1.28 ± 0.15	−ve	−ve
	2	2.99 ± 0.64	1:40	1:40

[a] Dilution positive in the commercial Engynost assay. The titer of the positive control serum from mice immunized with MV was 1:640.
[b] The titer of the positive anti-MV serum was 1:160.
[c] −ve, negative.

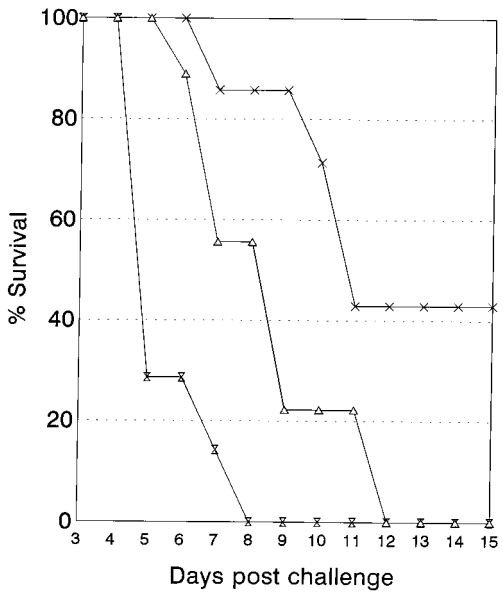

FIG. 3. Effect of challenge with a neuroadapted strain of CDV on the survival of offspring born to mothers immunized with mimotope 2. Six-week-old female BALB/c mice were coimmunized intraperitoneally with 50 μg of mimotope 2 and 50 μg of MVF 258-277 in FCA. Offspring born to these mothers (mimotope 2-immune mothers) and offspring born to nonimmune mothers were challenged intracranially at 2 to 3 weeks of age with CDV. Survival of offspring of MV-immunized mothers is also shown. Values represent the percent survival in the two groups of mice over a period of 20 days following virus challenge. Symbols: △, mimotope 2-immune mothers; ×, MV-immune mothers; ⧖, nonimmune mothers.

The amino acid sequences of the six mimotopes selected by the anti-MVF monoclonal antibody were compared with the primary structure of the MVF and CDVF proteins, and no identity between any of the sequences of the mimotopes and any region in the two proteins could be demonstrated. This suggests that the antibody recognizes a conformational epitope in the MVF protein. When the sequences were compared with that of the MVF protein by using the ASD program, similarity between the mimotopes and amino acid sequences within restricted regions of MVF (residues 153 to 181) and CDVF (residues 173 to 206) was observed, indicating that these mimotopes are mimics of conformational structures in these regions. It should be noted that the region represented by residues 145 to 210 of MVF and CDVF share 91% sequence homology. These observations indicate that these regions may contain all or part of the epitope recognized by the MVF monoclonal antibody.

Although polyclonal mouse anti-MV antibody could react with mimotopes 1 and 2 in ELISA, the failure of the monoclonal anti-MVF antibody to bind to the different mimotopes in either a fluid phase radioimmunoassay or the solid phase ELISA suggests that it recognizes a certain peptide conformation which the mimotopes do not take up in these assays. Presumably, the polyclonal antibody population contained sufficient antibodies with appropriate binding affinity to bind to the conformation of the peptide on the plastic, which the monoclonal antibody did not. The failure to demonstrate the binding of a synthesized mimotope by the antibody used for its selection has been described by others and has led to the use of

inhibition assays to demonstrate specificity (10). In addition, the importance of the way in which peptide mimotopes are conformationally presented is illustrated by recent evidence that hepatitis B surface antigen mimotopes expressed on bacteriophage coat protein were good immunogens whereas mimotopes expressed as a multiple antigenic peptide or coupled to a protein carrier were poor immunogens (15). The mimotopes in our study were, however, able to inhibit the binding of the monoclonal antibody to MV adsorbed to the solid phase of an ELISA, and this allowed the estimation of the relative affinity of the reaction of the antibody to the various mimotopes. The monoclonal antibody bound mimotopes 4 and 2 with the highest affinity, suggesting that the conformation of these two 8-mer peptides represent the best mimics of the epitope recognized by the antibody.

Following coimmunization of mice with the mimotopes and the MVF 258-277 Th epitope peptide, only mimotope 1 failed to induce an antibody response. Antibodies against mimotope 2 and 4 had the highest affinity for their homologous peptides, but only anti-mimotope 2 antibodies reacted with MV and had MV plaque inhibition reactivity. This result is particularly significant since the original monoclonal antibody had no demonstrable virus-neutralizing activity and suggests that when used as an immunogen, mimotope 2 can adopt a conformation capable of inducing a polyclonal antibody response with an affinity and specificity appropriate to achieve virus neutralization. Mimotope 2 thus represents a potential synthetic peptide vaccine candidate.

When this mimotope was used as a vaccine in female mice whose offspring were subsequently challenged intracranially with neuroadapted MV and CDV, partial protection was observed. However, the passive transfer of hyperimmune anti-mimotope 2 antibodies to young mice resulted in significant protection against MV challenge compared with that observed in mice receiving control serum. These results suggest that the amount or perhaps also the affinity of antibody passively transferred to the offspring via the milk was not sufficient to confer total protection whereas the amount given by passive immunization of young mice with the hyperimmune serum was appropriate.

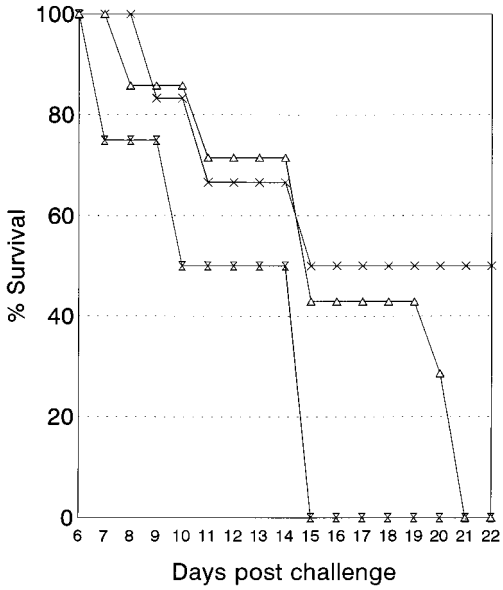

FIG. 4. Effect of challenge with a neuroadapted strain of MV on the survival of offspring born to mothers immunized with mimotope 2. Six-week-old female BALB/c mice were coimmunized intraperitoneally with 50 μg of mimotope 2 and 50 μg of the Th epitope RSV SH45 in FCA. Offspring born to these mothers (mimotope 2-immune mothers) were challenged intracranially with MV at 2 to 3 weeks of age. Survival of offspring of MV immunized mothers is also shown. Values represent the percent survival in the two groups of mice over a period of 22 days following virus challenge. Symbols are as in Fig. 3.

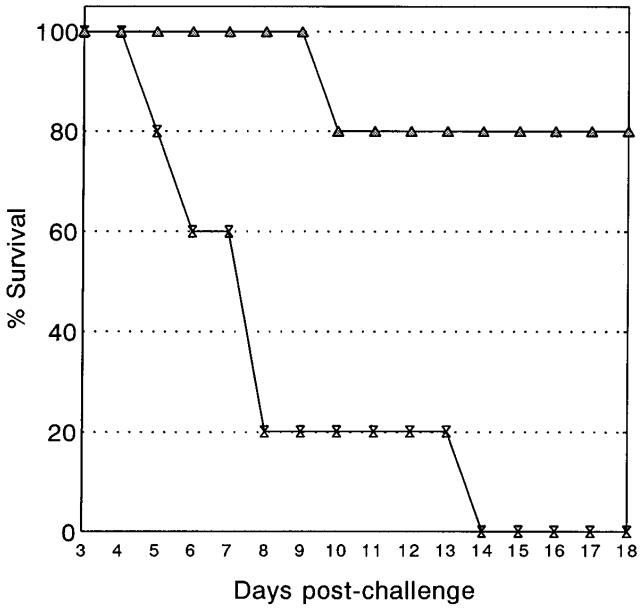

FIG. 5. Effect of passive immunization of BALB/c mice with anti-mimotope 2 antibody on the response to challenge with the neuroadapted strain of MV. Groups of five 2-week-old mice received 200 μl of either anti-mimotope 2 serum ($\log_{10}$ antibody titer, 3.79) or normal mouse serum intraperitoneally and were challenged 24 h later with MV. Values represent the percent survival in three groups of mice over a period of 18 days after challenge. Symbols: △, anti-mimotope 2 antibodies; ⧖, control antibodies.

It is very likely that the affinity of the anti-mimotope antibody is an important factor in determining protective efficacy following passive and active immunization. We have recently shown that immunization with chimeric peptide immunogens in which T- and B-cell epitopes are covalently coupled induces a higher-affinity anti-B-cell epitope response than that obtained following coimmunization with the uncoupled peptides (21). In the work described here, coimmunization with the peptides was used and experiments are in progress to assess the efficacy of immunization with chimeric constructs of mimotope 2 and the T-cell epitope. In addition, we are performing experiments to determine the influence of affinity on the protective efficacy of the anti-mimotope response.

The results presented here illustrate the potential of the use of a solid phase random peptide library to identify peptide sequences which behave as mimics of epitopes on a viral protein and which can be used as surrogate antigens to elicit protective antibody responses. Furthermore, these results indicate that this approach has considerable potential for the design of synthetic peptide vaccines.

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REFERENCES

1. **Balass, M., Y. Heldman, S. Cabilly, D. Givol, E. Katchalski-Katzir, and S. Fuchs.** 1993. Identification of a hexapeptide that mimics a conformation-dependent binding site of acetylcholine receptor by use of a phage-epitope library. Proc. Natl. Acad. Sci. USA **90:**10638–10642.

2. **Choppin, P. W., C. D. Richardson, D. C. Merz, W. W. Hall, and A. Scheid.** 1981. The functions and inhibition of the membrane glycoproteins of paramyxoviruses and myxoviruses and the role of the measles virus M protein in subacute sclerosing panencephalitis. J. Infect. Dis. **143:**352–363.
3. **Devlin, J. J., L. C. Panganiban, and P. E. Devlin.** 1990. Random peptide libraries: a source of specific protein binding molecules. Science **249:**404–406.
4. **Drillien, R., D. Spehner, A. Kirn, P. Giraudon, R. Buckland, F. Wild, and J. P. Lecocq.** 1988. Protection of mice from fatal measles encephalitis by vaccination with vaccinia virus recombinants encoding either the hemagglutinin or the fusion protein. Proc. Natl. Acad. Sci. USA **85:**1252–1256.
5. **Eichler, J., and R. A. Houghten.** 1993. Identification of substrate-analog trypsin inhibitors through the screening of synthetic peptide combinatorial libraries. Biochemistry **32:**11035–11041.
6. **Felici, F., A. Luzzago, A. Folgori, and R. Cortese.** 1993. Mimicking of discontinuous epitopes by phage-displayed peptides. II. Selection of clones recognized by a protective monoclonal antibody against the Bordetella pertussis toxin from phage peptide libraries. Gene **128:**21–27.
7. **Geysen, H. M., S. J. Rodda, and T. J. Mason.** 1986. A priori delineation of a peptide which mimics a discontinuous antigenic determinant. Mol. Immunol. **23:**709–715.
8. **Giraudon, P., and T. F. Wild.** 1985. Correlation between epitopes on the haemagglutinin of measles virus and biological activities: passive protection by monoclonal antibodies is related to their haemagglutination inhibition activity. Virology **144:**46–56.
9. **Goodson, R. J., M. V. Doyle, S. E. Kaufman, and S. Rosenberg.** 1994. High-affinity urokinase receptor antagonists identified with bacteriophage peptide display. Proc. Natl. Acad. Sci. USA **91:**7129–7133.
10. **Hoess, R. H., A. J. Mack, H. Walton, and T. M. Reilly.** 1994. Identification of a structural epitope by using a peptide library displayed on filamentous bacteriophage. J. Immunol. **153:**724–729.
11. **Keller, P. M., B. A. Arnold, A. R. Shaw, R. L. Tolman, F. Van Middlesworth, S. Bondy, V. K. Rusiecki, S. Koenig, S. Zolla-Pazner, P. Conard, et al.** 1993. Identification of HIV vaccine candidate peptides by screening random phage epitope libraries. Virology **193:**709–716.
12. **Lam, K. S., S. E. Salmon, E. M. Hersh, V. J. Hruby, W. M. Kazmierski, and R. J. Knapp.** 1991. A new type of synthetic peptide library for identifying ligand-binding activity. Nature (London) **353:**82–84.
13. **Luzzago, A., F. Felici, A. Tramontano, A. Pessi, and R. Cortese.** 1993. Mimicking of discontinuous epitopes by phage-displayed peptides. I. Epitope mapping of human H ferritin using a phage library of constrained peptides. Gene **128:**51–57.
14. **Malvoisin, E., and F. Wild.** 1990. Contribution of measles virus fusion protein in protective immunity: anti-F monoclonal antibodies neutralize virus infectivity and protect mice against challenge. J. Virol. **64:**5160–5162.
15. **Meola, A., D. Delmastro, A. Luzzago, A. Nicosia, F. Felici, R. Cortese, and G. Galfre.** 1995. Derivation of vaccines from mimotopes. Immunologic properties of human hepatitis B virus surface antigen mimotopes displayed on filamentous phage. J. Immunol. **155:**3162–3172.
16. **Obeid, O. E., C. D. Partidos, C. R. Howard, and M. W. Steward.** 1995. Protection against morbillivirus-induced encephalitis by immunization with a rationally designed synthetic peptide vaccine containing B- and T-cell epitopes from the fusion protein of measles virus. J. Virol. **69:**1420–1428.
17. **O'Neil, K. T., R. H. Hoess, S. A. Jackson, N. S. Ramachandran, S. A. Mousa, and W. F. DeGrado.** 1992. Identification of novel peptide antagonists for GPIIb/IIIa from a conformationally constrained phage peptide library. Proteins **14:**509–515.
18. **Owens, R. A., P. D. Gesellchen, B. J. Houchins, and R. D. DiMarchi.** 1991. The rapid identification of HIV protease inhibitors through the synthesis and screening of defined peptide mixtures. Biochem. Biophys. Res. Commun. **181:**402–408.
19. **Padlan, E. A.** 1977. Structural implications of sequence variability in immunoglobulins. Proc. Natl. Acad. Sci. USA **74:**2551–2555.
20. **Partidos, C. D., O. E. Obeid, and M. W. Steward.** 1992. Antibody responses to non-immunogenic synthetic peptides induced by co-immunization with immunogenic peptides. Immunology **77:**262–266.
21. **Shaw, D. M., C. M. Stanley, C. D. Partidos, and M. W. Steward.** 1993. Influence of the T helper epitopes on the titre and antibody affinity of antibodies to B cell epitopes after coimmunization. Mol. Immunol. **30:**961–968.
22. **Shellekens, G. A., E. Lasonder, M. Feijlbrief, D. G. A. Koedijk, J. W. Drijfhout, A. J. Scheffer, S. Welling, S. Wester, and G. W. Welling.** 1994. Identification of the core residues of the epitope of a monoclonal antibody raised against glycoprotein D of herpes simplex virus type 1 by screening of a random peptide library. Eur. J. Immunol. **14:**3188–3193.
23. **Sneath, P. H.** 1966. Relations between chemical structure and biological activity in peptides. J. Theor. Biol. **12:**157–195.
24. **Steward, M. W., C. M. Stanley, R. Dimarchi, G. Mulcahy, and T. R. Doel.** 1991. High-affinity antibody induced by immunization with a synthetic peptide is associated with protection of cattle against foot-and-mouse disease. Immunology **72:**99–103.