Identification of helper T cell antigenic sites in mice from the haemagglutinin glycoprotein of measles virus

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The aim of this study was to define the helper T cell epitopes on the haemagglutinin (H) of measles virus (MV) in BALB/c (H-2d) and TO (H-2S) mice. A panel of 55 synthetic peptides (15-mers, overlapping by five amino acids) representing 92.2% of the H protein were synthesized and tested for immunogenicity and ability to stimulate MV-primed lymphocytes in vitro. The results obtained show that mouse lymphocytes respond to defined regions of the H protein which differ according to mouse strain. Virus-primed lymphocytes from BALB/c mice responded in vitro to peptides 7, 38, 39 and 44 whereas lymphocytes from virus-primed TO mice responded only to peptide 39. When mice of both strains were immunized with the peptides, a number of peptides induced proliferative responses, showing that the T cell repertoire for epitopes on the H protein is broader than that following immunization with virus. In BALB/c mice, lymphocytes primed to peptides 37, 39, 40, 42 and 43 responded in vitro to MV and in TO mice, lymphocytes primed to peptides 14, 32, 39, 40 and 49 responded to the virus. Thus in both strains of mice peptide 39 behaved as a dominant T cell epitope following immunization with virus or peptides. When the results obtained experimentally were compared with sequences predicted to be T cell epitopes by a number of algorithms, the concordance was limited.

Introduction

Measles virus (MV) is a member of the morbillivirus subgroup of the paramyxovirus family (Kingsbury et al., 1978). The surface localization of the haemagglutinin (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, the adsorption of the virus to receptors on the host cell, whereas the F glycoprotein is actively involved in the induction of membrane fusion (Choppin et al., 1981). 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 (Drillien et al., 1988; Malvoisin & Wild, 1990).

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. Several factors such as maternal antibodies, instability of vaccines in the tropics and early age of infection have been identified as contributing to the high mortality rate in developing countries. 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. The recent availability of the complete amino acid sequences of MV proteins (Alkhatib & Briedis, 1986; Richardson et al., 1986) has made it possible for this approach to be directly tested. Over the last few years much effort has been focused on the development of synthetic peptide vaccines against various pathogens (Milich, 1989). Such vaccines should be highly immunogenic and able to stimulate broadly reactive neutralizing antibodies as well as a vigorous cell-mediated immune response to eradicate both free virus and infected cells.

T cell immunity is a major defence against viral infections and T-helper lymphocytes are essential for both antibody production and the induction of cytotoxic T cell responses. Thus the inclusion of T-helper cell epitopes in the peptide-based vaccines is thought to be essential for the induction of both humoral and cellular immunity. On the basis of studies mapping T-helper cell epitopes on a range of protein antigens both in mice and in humans it has become apparent that T cells recognize only a limited number of regions on an antigen (Adorini et al., 1989; Berzofsky, 1987; Brett et al., 1988). Several factors such as (i) availability of the appropriate sized peptide after antigen processing (Gammon et al., 1987; Adorini et al., 1989), (ii) binding to major histocompatibility complex (MHC) class II molecules (Babbitt
et al., 1985; Buus et al., 1987), (iii) in vivo competition between self and non-self peptides for binding to MHC class II molecules (Adorini et al., 1989) and (iv) the composition of the expressed T cell repertoire after thymic selection (Blackman et al., 1990) have all been shown to influence the selection of the epitopes. Several methods have been employed to predict the likely immunodominant T-helper epitopes by analysing the amino acid sequence of the protein antigens. These predictions are based on the observation that the majority of immunodominant T cell epitopes tend to be either amphipathic α-helices (Margalit et al., 1987) or have common motifs of amino acid sequences (Rothbard & Taylor, 1988; Sette et al., 1989).

H is a major viral antigen and has been shown to induce proliferative lymphocyte responses in BALB/c mice (Makela et al., 1989). In this paper we have used a series of overlapping synthetic peptides covering 92.2% of the MV H sequence to define the T-helper cell antigenic sites on the H protein recognized by two strains of mice (BALB/c and TO). These experimentally determined sites were compared with those identified by the predictive approaches to provide an assessment of the accuracy of the computer-based methods.

**Methods**

**Peptide synthesis.** Peptides were synthesized by the RAMPs (Rapid Multiple Peptide Synthesis, Du Pont) solid-phase synthesis method using Fmoc-chemistry with the 4-[2',4'-dimethoxyphenyl-Fmoc-(amino-methyl)-phenoxy resin] (Novabiochem). Each peptide was 15 amino acids long and overlapped by five residues with the following peptide (apart from peptide 1 which is a 23-mer) (Fig. 1). Cysteine was replaced with alanine to prevent dimerization and tyrosine was added at the C terminus to peptide sequences that lacked tyrosine for radiolabelling purposes. Fmoc-protected amino acids were converted to the 1-hydroxybenzotriazole-activated esters by treatment with 1-hydroxybenzotriazole and N,N'-diisopropyl-carbodiimide in DMF and the Fmoc-groups were removed with 50% piperidine in DMF followed by a series of alternate washes in DMF and methanol. After synthesis, side-chain protecting groups were removed and the peptides were cleaved in trifluoroacetic acid in the presence of scavengers. After cleavage, peptides were extracted into diethyl ether and purified by gel filtration using a Sephadex G-10 column (Pharmacia) and by HPLC. Purified peptides were analysed by fast atomic bombardment mass spectrometry and had the expected amino acid composition.

**Virus.** The MV vaccine (Schwartz strain, Connaught Laboratory) was used as a source for the virus. Briefly, Vero cells grown in roller bottles were infected with the virus at an m.o.i. of 0.1. When the c.p.e. reached 95%, the medium was harvested and the cell debris was removed by centrifugation at 900 g for 15 min and stored at −70 °C. Virus was heat-inactivated at 56 °C for 30 min prior to use for immunization.

**Mice.** BALB/c (H-2k) mice were purchased from the National Institute of Medical Research (Mill Hill, London, U.K.). TO (H-2b) mice were obtained from the Biological Services Unit of the London School of Hygiene and Tropical Medicine.

**Immunization.** Groups of three mice were injected intraperitoneally with 100 μg of free peptide emulsified in Freund's complete adjuvant (FCA). Three weeks later mice were boosted by the same route with 100 μg of the peptide emulsified in Freund's incomplete adjuvant (FIA). After three weeks, mice were killed and the proliferative responses of spleen cells were assessed following culture with the homologous peptide or MV. When virus was used as an immunogen, groups of three to five mice were injected intraperitoneally with 2 × 106 p.f.u./mouse of heat-inactivated virus in FCA. A week later mice were boosted with the same dose in FIA and after 7 days were killed and the proliferative responses of their spleen cells were assessed. In the experiments designed to study the effect of co-immunization on the immunogenicity and antigenic specificity of peptides 14 and 39, a group of TO mice was injected intraperitoneally with an equimolar amount of both peptides, 0.5 μg of each peptide mixed in distilled water and emulsified in FCA (1:1). Control mice received the same dose of each peptide injected separately in FCA by the same route. After 3 weeks, all mice were boosted by the same route and with the same concentration of each peptide in FIA. After a further 3 weeks, mice were killed and spleen cells were assessed for proliferative responses to the homologous peptide(s) or virus in vitro.

**Lymphocyte stimulation assays.** Spleen cells were removed aseptically, pooled and teased. Cells were collected by centrifugation and red blood cells were lysed using ACD lysis buffer (0.15 M NaCl, 0.01 M Na2EDTA and 1 M KHCO3). Lymphocytes were collected by centrifugation and red blood cells were lysed using ACK lysis buffer (0.15 M NH4Cl, 0.01 M Na2EDTA and 1 M KHCO3). Lymphocytes were washed twice in RPMI-1640 medium and then resuspended in complete medium (RPMI-1640 supplemented with 1% autologous serum, 2 mM l-glutamine, 10 mM HEPEs buffer and 100 international units/ml penicillin/streptomycin). Viable mononuclear cells were counted using a haemocytometer.

Fig. 1. Primary amino acid sequence of H of MV, Edmonston strain. Synthesized peptides were 15 amino acids long (apart from peptide 1, which is 23 amino acids long; underlined), and overlapped by five residues with the following peptide (except peptide 1). The peptides represented 92.2% of the H sequence.
cosin stain (0.2% w/v) and the number of cells was adjusted to 10⁶/ml. Cell suspension (200 µl) was placed in each well of a 96-well round-bottomed microtitre plate and incubated in the presence of various concentrations of peptide (0.1 to 20 µg/well) or of heat-inactivated MV (0.2 x 10⁵ to 2 x 10⁴ p.f.u./well) in a humidified 5% CO₂ atmosphere at 37 °C for 5 days. Eighteen hours before the end of the culture, cells were pulse-labelled with 1 µCi of tritiated thymidine. Cells were harvested using an Ilacon harvester and [³H]thymidine incorporation was assessed using a β-spectrometer (Beckman). To be considered positive a peptide had to fulfil two criteria: that the stimulation index (mean c.p.m, in triplicate wells with antigen divided by mean c.p.m. in six to 12 replicate wells without antigen) was greater than twice the background and that the mean was significantly different from control (P < 0.01). All peptides were tested for mitogenicity or cross-reactivity with adjuvant using lymphocytes from mice immunized with FCA alone. Results presented represent the average stimulation index of two separate experiments at the peptide or virus concentration in the dose–response curve giving the maximum stimulation.

Results

Response of MV-primed spleen cells to overlapping H peptides

Since the recognition of an antigen by T cells is under genetic control, all the experiments were carried out in two strains of mice (BALB/c and TO). Mice were immunized intraperitoneally with inactivated MV in FCA and boosted 1 week later via the same route with virus in FIA. After 7 days, spleen cells were collected and in vitro proliferative responses to synthetic peptides and MV were assessed.

As shown in Fig. 2 four peptides (7, 38, 39 and 44) induced significant (P < 0.01) proliferative responses in BALB/c mice. In TO mice only peptide 39 was found to induce weak but significant (P < 0.01) proliferation (Fig. 3). All the responses were dose-dependent and none of the peptides stimulated spleen cells from mice immunized with FCA alone (results not shown). In both strains of mice, in vivo MV priming led to good proliferative responses following in vitro stimulation with MV. Thus it appears that the recognition of the H peptides differs according to the H-2 haplotype. However, in both strains peptide 39 was found to be stimulatory.

Immunogenicity of H peptides

In order to study the potential T cell repertoire for recognition of epitopes on the H molecule, BALB/c and TO mice were immunized with each peptide intraperitoneally in FCA and boosted 3 weeks later via the same route with the peptide in FIA. After 3 weeks, proliferative responses of spleen cells to the homologous peptides were assessed.

In contrast to the responses seen after MV immunization, the number of T cell epitopes recognized on the H molecule was greater when peptides were used as immunogens: 32 peptides in BALB/c (Fig. 4) and 33 in TO mice (Fig. 5) out of the 55 tested peptides were able to induce proliferative responses. Twenty peptides were immunogenic in both strains of mice suggesting the presence of common structural characteristics in the peptides. All the responses were dose-dependent (results not shown).

Proliferative responses of spleen cells from peptide-primed mice to MV in vitro

The helper activity of peptide immune splenocytes was tested further by measuring their in vitro proliferative responses following culture with MV. Spleen cells from BALB/c mice immunized with peptides 37, 39, 40, 42 and 43 show significant (P < 0.01) proliferation in the presence of the virus. In TO mice significant (P < 0.01) proliferation to the virus was observed only after immunization with peptides 14, 32, 39, 40 and 49 (Table 1).

In vivo competition between H protein peptides for interaction with class II molecules

Peptides 14 and 39 primed spleen cells from TO mice for subsequent in vitro stimulation with the MV (Table 1). However, after MV immunization only peptide 39 was able to recall virus memory cells in vitro (Fig. 3). Thus the possibility exists that the in vitro competition between these two peptides for presentation by I-A<sup>α</sup> class II molecules may account for the differences in their ability to stimulate MV-immune T cells. To test this possibility, TO mice which express only I-A<sup>α</sup> class II molecules were immunized with either peptide 14 or peptide 39 or with an equimolar amount of both peptides 14 and 39. As shown in Fig. 6(a), both peptides induced an anti-peptide proliferative response when each was injected separately. However, when peptide 14 was injected with an equimolar amount of peptide 39, the proliferative cell response to the former was abolished whereas the response to peptide 39 was not affected (Fig. 6b) suggesting the immunodominance of peptide 39. When spleen cells were restimulated with MV, there were no significant differences in the proliferative response to the virus of spleen cells from mice co-immunized with the two peptides and the control groups (data not shown).

Comparison of predicted epitopes with experimentally defined epitopes

Prediction methods which identify amphipathic segments (Margalit et al., 1987) or the presence of ‘motifs’ (Rothbard & Taylor, 1988; Sette et al., 1989) corresponding to potential T cell epitopes were used to analyse the amino acid sequence of MV H. Since our
Fig. 2. Proliferative responses of MV-primed spleen cells from BALB/c mice to synthetic H peptides. Spleen cells from BALB/c mice immunized with MV in FCA were tested for in vitro proliferative responses to 55 synthetic peptides (peptides 1 to 55) (0.1 to 20 μg/culture), heat-inactivated MV (MV) (0.2 x 10^2 to 2 x 10^2 p.f.u./culture) or medium from mock-infected cells (M). Results presented represent the average stimulation index (ratio of mean c.p.m. of triplicate cultures with the antigen to the mean c.p.m. of six to 12 replicates of control cultures without antigen) of two independent experiments at the peptide or virus concentration that gave the maximum stimulation index.

A series of overlapping synthetic peptides covered 92.2% of the MV H sequence, the experimentally derived data on the immunogenicity and antigenicity of these peptides were compared with sequences predicted as epitopes by the computer-based algorithms. Of the four immunodominant epitopes identified in BALB/c mice after immunization with the virus, peptide 7 was predicted to be amphipathic and peptide 38 predicted to be both amphipathic and to contain a Rothbard motif (Table 2). Five of the panel of peptides, when used as immunogens, primed T cells for significant in vitro response to the virus. Of these, two were predicted to be amphipathic (peptides 40 and 43), peptide 42 contained a Rothbard motif and peptide 37 contained a Rothbard motif, was amphipathic and had an I-A^α motif (Table 1). Peptide 39 was immunodominant in BALB/c and TO mice but was not predicted by any of the algorithms to contain a T cell epitope. Of the five peptides which induced virus-cross-reactive T cells in TO mice, two contained a Rothbard motif (peptides 14 and 49), one was amphipathic (peptide 40) and peptide 32 contained a Rothbard motif and corresponded to an amphipathic segment (Table 1).

Discussion
Recent advances in peptide chemistry have allowed the development of methods for the rapid synthesis of peptides, allowing the detailed mapping of B and T cell epitopes. In addition a better understanding of the molecular mechanisms involved in immune recognition opens the possibility for development of synthetic peptide vaccines (Milich, 1989).

The aim of this study was to define the T-helper cell epitopes on MV H glycoprotein. To identify immunodominant sites, 55 overlapping peptides covering 92.2% of the molecule were synthesized and tested for their
ability to stimulate virus-primed lymphocytes \textit{in vitro} and for their immunogenicity. The results reported in this paper show that mouse T-helper cells respond specifically to defined epitopes of H. In BALB/c mice, T cell responses to peptides 7, 38, 39 and 44 and in TO mice T cell responses to peptide 39 appear to be dominant after virus immunization. It is interesting to note that peptides 38 and 39, to which BALB/c mice respond, overlap by five residues and these two sequences are thus likely to share a common epitope. These data indicate that the number of T cell epitopes in the H molecule is limited although it is possible that these peptides contain more than one T cell epitope. Furthermore, each member of the panel of synthetic peptides used overlapped by five amino acids and thus not every epitope present on the H would necessarily be represented. Extensive studies on eukaryotic and non-eukaryotic proteins have shown that the expressed T cell repertoire is restricted to a limited number of discrete sites or immunodominant determinants (Adorini \textit{et al}., 1989; Berzofsky, 1987). Several mechanisms, such as antigen processing (Gammon \textit{et al}., 1987; Adorini \textit{et al}., 1989), \textit{in vivo} competition between self and non-self peptides for binding to class II molecules (Adorini \textit{et al}., 1989), binding to MHC molecules (Babbitt \textit{et al}., 1985; Buus \textit{et al}., 1987), holes in the T cell repertoire (Blackman \textit{et al}., 1990) and stability of peptides to proteolytic degradation (Eisenlohr \textit{et al}., 1988) could account for epitope selection.

A completely different picture to that seen with MV-primed lymphocytes was obtained when mice were immunized with individual peptides. In both strains of mice it was shown that when injected with FCA, synthetic peptides can be very immunogenic in terms of inducing proliferative responses to their homologous peptide. Thus it appears that the potential T cell repertoire to epitopes on the H molecule is much broader following immunization with peptides than following virus immunization. In addition when peptide-primed spleen cells
were tested for proliferative responses to MV in vitro, peptides 37, 39, 40, 42 and 43 in BALB/c and 14, 32, 39, 40 and 49 in TO mice primed for subsequent in vitro response to the virus. In both strains of mice peptide 39 appeared to be an immunodominant epitope which suggests that there was no difference in the presentation of peptide 39 by spleen cells from virus- or peptide-primed mice. However, the failure of the other tested peptides to generate lymphocytes reactive with the virus would have been due to the generation of different peptides after virus processing (Brett et al., 1988). T cells from these mice could not be stimulated with the virus.

Some peptides induced virus-specific immune lymphocytes that could not be restimulated in vitro with the homologous peptide (for example 37 and 43 in BALB/c mice and 40 in TO mice) and this may be the result of further processing of the peptide. However, it is also possible that stimulatory activity might not be demonstrable within the range of peptide concentrations tested. In addition, a third possible explanation for this phenomenon is that the peptides could be relatively unstable under in vitro conditions (Buus & Werdelin, 1986), whereas in vivo, in the presence of the emulsion used for immunization their half-life may be long enough to allow appropriate uptake and presentation to lymphocytes. Makela et al. (1989) have shown that H peptides of 10 to 11 amino acids in length, although unable to induce proliferative responses to the homologous peptide, can prime effectively for a subsequent challenge by MV as measured by lymphocyte stimulation or induction of delayed-type hypersensitivity. However, we were unable to confirm these observations with peptides 18, 25 and 31 which correspond to the peptides used by Makela et al.

Explanations for these results could be that there were differences in the immunization protocols used or the size of peptides employed, which may have affected processing and presentation.

Evidence is provided in this paper that in vivo competition between immunogenic peptides for binding to class II molecules might account for epitope selection.
Measles virus haemagglutinin Th epitopes

Fig. 5. Proliferative responses of peptide-primed spleen cells from TO mice to the homologous synthetic peptide from H. Pooled spleen cells from group of three TO mice that were immunized with peptide in FCA were tested for in vitro proliferation to the homologous peptide (peptides 1 to 55) (0.1 to 20 μg/ml). Results presented represent the stimulation index at the peptide concentration that gave the maximum stimulation.

Table 1. Comparison of predicted epitopes and experimentally identified cross-reactive T cell epitopes*

<table>
<thead>
<tr>
<th>Peptide (residues)</th>
<th>Rothbard‡</th>
<th>I-A* Motif</th>
<th>S.I.§ Balb/c</th>
<th>S.I.§ TO</th>
</tr>
</thead>
<tbody>
<tr>
<td>p14 (193–207)</td>
<td>–</td>
<td>±</td>
<td>1.1</td>
<td>4.1</td>
</tr>
<tr>
<td>p32 (373–387)</td>
<td>2.11</td>
<td>+</td>
<td>1.3</td>
<td>3.1</td>
</tr>
<tr>
<td>p37 (423–437)</td>
<td>7.15</td>
<td>±</td>
<td>2.7</td>
<td>1.6</td>
</tr>
<tr>
<td>p39 (443–457)</td>
<td>–</td>
<td>–</td>
<td>2.5</td>
<td>2.3</td>
</tr>
<tr>
<td>p40 (453–467)</td>
<td>4.89</td>
<td>–</td>
<td>3.0</td>
<td>2.6</td>
</tr>
<tr>
<td>p42 (473–487)</td>
<td>–</td>
<td>+</td>
<td>2.6</td>
<td>1.9</td>
</tr>
<tr>
<td>p43 (483–497)</td>
<td>8.99</td>
<td>–</td>
<td>2.2</td>
<td>1.3</td>
</tr>
<tr>
<td>p49 (543–557)</td>
<td>–</td>
<td>+</td>
<td>1.8</td>
<td>3.6</td>
</tr>
</tbody>
</table>

* T cell epitopes were identified as being able to prime for in vitro response to virus in Balb/c and TO mice.

† Each peptide sequence was screened for the presence of amphipathic segment(s) (Margalit et al., 1987), Rothbard (Rothbard & Taylor, 1988) or I-A* motifs (Sette et al., 1989). AS, Amphipathic scores of the peptides as calculated by the AMPHI algorithm.

‡ Motifs in the peptide: (–), no motif, (±), motif to either end of the sequence, (+), motif in the centre of the sequence.

§ Spleen cells from mice immunized with peptide were tested for proliferative responses to heat-inactivated MV in vitro. Results presented represent the stimulation index (S.I.) at the virus concentration that gave the maximum stimulation.

In the case of peptide 14, although it is not dominant in H-2* mice after priming with MV it does induce responses that can be recalled by virus, indicating that the peptide is available after processing. The failure of virus-primed lymphocytes to respond to peptide 14 could result from a low frequency of peptide 14-specific clones in the T cell repertoire or that the T cells induced had a poor proliferative capacity. However, that co-immunization of peptide 14 and the immunodominant peptide 39 inhibits the response to the former supports the view that the simultaneous presence of these two peptides results in in vivo competition when they are injected together at equimolar concentrations. The fact that the response to peptide 14 is suppressed suggests that peptide 39 binds to the I-A* molecule with a higher affinity. Recent studies on mechanisms underlying immunodominance have shown that peptides derived from the same or different proteins can compete in vivo for binding to the MHC molecule. This suggests that only those peptides which bind with relatively high affinity to the class II molecule are able to
obtained.

concentration that gave the maximum stimulation.

set the peptides as calculated by the AMPHI algorithm.

pathic segment(s) (Margalit et al., 1988) or I-A~ motifs (Sette et al., 1989) we attempted to predict the likely immunodominant regions on the molecule. However, the results obtained indicate the limitation of this approach although the amount of data available did not allow a proper statistical evaluation. Of particular importance in this context was peptide 39 which was immunodominant in both strains of mice but each of the three predictive approaches used indicated the absence of a T cell epitope on the peptide. It should be emphasized that prediction methods do not take into consideration host factors and thus the possibility exists that the predicted epitopes might well be functional in other strains of mice.

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Table 2. Comparison of predicted and experimentally identified immunodominant T cell epitopes in BALB/c and TO mice*

<table>
<thead>
<tr>
<th>Peptide (residues)</th>
<th>Rothbard‡</th>
<th>AS†</th>
<th>I-A§ Motif</th>
<th>S.I.$</th>
<th>S.I.§</th>
<th>S.I. $</th>
</tr>
</thead>
<tbody>
<tr>
<td>p7 (123-137)</td>
<td>7.07</td>
<td>-</td>
<td>-</td>
<td>4.1</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>p38 (433-447)</td>
<td>4.08</td>
<td>±</td>
<td>2.6</td>
<td>2.6</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>p39 (443-457)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3.7</td>
<td>2.3</td>
<td></td>
</tr>
<tr>
<td>p44 (493-507)</td>
<td>-</td>
<td>-</td>
<td>4.1</td>
<td>43</td>
<td>1.3</td>
<td></td>
</tr>
</tbody>
</table>

* T cell epitopes were identified after immunization with virus.
† Each peptide sequence was screened for the presence of amphipathic segment(s) (Margalit et al., 1987). Rothbard (Rothbard & Taylor, 1988) or I-A§ motifs (Sette et al., 1989). AS, amphipathic scores of the peptides as calculated by the AMPHI algorithm.
‡ Motifs in the peptide: (−), no motif; (+), motif to either end of the sequence; (+), motif in the centre of the sequence. All responses were dose-dependent and values given represent maximum values obtained.
§ Results presented represent the stimulation index (S.I.) at peptide concentration that gave the maximum stimulation.

occupy the number of binding sites required for T cell activation (Adorini & Nagy, 1989).

In addition to host factors, certain properties related to the structure of the antigen itself have been suggested to influence the selection of T cell epitopes (Berzofsky et al., 1987). By screening the amino acid sequence of H for amphipathic segments (Margalit et al., 1987) or the presence of motifs (Rothbard & Taylor, 1988; Sette et al., 1989) we attempted to predict the likely immunodominant regions on the molecule. However, the results obtained indicate the limitation of this approach although the amount of data available did not allow a proper statistical evaluation. Of particular importance in this context was peptide 39 which was immunodominant in both strains of mice but each of the three predictive approaches used indicated the absence of a T cell epitope on the peptide. It should be emphasized that prediction methods do not take into consideration host factors and thus the possibility exists that the predicted epitopes might well be functional in other strains of mice.

Fig. 6. In vivo competition between peptides 14 and 39 for interaction with I-A§ molecules. TO mice were immunized intraperitoneally with 0.5 μM/mouse peptide 14 (X) or 39 (a). In addition, further groups of mice of the same strain were co-immunized with equimolar amounts (0.5 μM/mouse) of peptides 14 and 39 (b). Three weeks after the boost, spleen cells were cultured with different concentrations of either peptide 39 (■) or peptide 14 (X). Results are expressed as the mean stimulation index from two separate experiments.

References


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