Prediction and identification of a T cell epitope in the fusion protein of measles virus immunodominant in mice and humans

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Amino acid residues 288 to 302 of the fusion protein of measles virus were predicted by a variety of methods to represent a putative T cell epitope. This sequence was synthesized and the peptide was injected into mice of six inbred strains to test this possibility. Lymphocytes from peptide-immunized mice from all six H-2 disparate strains were able to mount a proliferative response following in vitro culture with the peptide. In addition, lymphocytes from three strains also proliferated in the presence of live measles virus. The peptide also behaved as a B cell epitope in that immunization with free peptide in adjuvant resulted in anti-peptide antibody production in all mouse strains. However, these antibodies did not react with the virus in either a solid-phase immunoassay or a virus neutralization assay. Peripheral blood lymphocytes from 10 laboratory personnel with a prior history of exposure to measles virus were tested in a proliferation assay with the peptide and with the virus. Lymphocytes from all 10 individuals proliferated in response to culture with the virus and those from eight responded to the peptide. These results give further support to the concept of permissive interaction of antigenic peptides with a wide range of class II major histocompatibility complex molecules both in mice and man and indicate the possibility of designing peptides that could be used as components of a synthetic vaccine for use in man.

Introduction

Measles virus is a member of the morbillivirus subgroup of the paramyxovirus family of negative-stranded RNA viruses. It has two major surface glycoproteins, the haemagglutinin (H) and the fusion protein (F). It is generally accepted that the H polypeptide has a receptor-binding activity while the F protein is involved in membrane fusion. The F protein is synthesized as an inactive precursor (F₀) which is cleaved by a host protease to yield two disulphide-bonded polypeptides, F₁ (MVF1) and F₂ (MVF2). The new N terminus on the F₁ polypeptide generated by the cleavage is involved in membrane fusion. Antibodies to the H and F proteins can neutralize the virus in vitro. Antibodies to H inhibit haemagglutination by the virus and adsorption to host cells whereas antibodies to F inhibit haemolysis and membrane fusion. The importance of antibodies to the F protein is illustrated by the observation (Merz et al., 1980) that the immunopathological response of atypical measles following immunization with formalin-inactivated virus is associated with the absence of antibodies to the F protein. The development of a synthetic peptide vaccine based on antigenic structures within the F protein would thus appear to be a valid strategy for the control of measles. The recent availability of the complete amino acid sequence of the F protein (Richardson et al., 1986) has made it possible for this approach to be directly tested.

As a result of work in several laboratories on the molecular basis of antigen recognition, it has become apparent that both cytotoxic and helper T cells recognize processed antigen in association with a major histocompatibility complex (MHC) gene product. Furthermore, it appears that both types of T cell employ similar mechanisms for this recognition which is thought to involve the association of peptide from the protein antigen with molecules of the MHC. Support for this view has come from experiments demonstrating the binding of immunogenic peptides by purified class II and class I molecules (Babbitt et al., 1985; Chen & Parham, 1989), the binding of peptide to class II molecules on living B lymphoid cells (Cepellini et al., 1989), and that class II molecules have a single antigen binding site based on functional analysis of antigen competition at the cellular level (Guillet et al., 1986) and with purified class II molecules (Guillet et al., 1987; Buus et al., 1987). The analysis of the three-dimensional structure of the human class I molecule (Bjorkman et al., 1987) has indicated a single site where binding of peptide could occur. Furthermore, a hypothetical model of a class II molecule has suggested structural homology with the
class I molecule and the existence of a single peptide-binding site (Brown et al., 1988); short synthetic peptides can replace protein antigen in both class I- (Townsend et al., 1986; Bastin et al., 1987; Takahashi et al., 1988) and class II-restricted T cell responses (Guillet et al., 1986; Rothbard et al., 1988) and they share common structural motifs (Takahashi et al., 1988; Rothbard et al., 1988). Furthermore, recent studies have shown that purified class I protein pulsed with influenza peptides can induce an antigen-specific degradation of cytotoxic T lymphocytes (Kane et al., 1989). It thus appears that both class I and class II MHC molecules have a single site for the binding of peptide antigens and it is the complex of peptide plus MHC molecule which is recognized by the T cell receptor.

Considerable interest has centred on the structural characteristics of the regions on proteins which are recognized by T cells, as distinct from those recognized by B cells. Empirical analysis of the regions recognized by T cells has shown that a high proportion have the characteristic of being amphipathic α helices (DeLisi & Berzofsky, 1985; Spouge et al., 1987). In addition to these secondary structure correlations, others (Rothbard & Taylor, 1988) have observed the association of a primary amino acid sequence motif within T cell recognition sites. Thus a sequence of a charged amino acid or glycine followed by two or three hydrophobic residues followed by a charged or polar residue seems to be a characteristic feature for T cell recognition. These two approaches are not mutually exclusive since the postulated motif corresponds to one turn of an α helix and furthermore, both have been shown to have predictive value in identifying T cell epitopes (Lamb et al., 1987; Cease et al., 1987).

An additional primary amino acid sequence motif associated with DR1-restricted influenza virus-specific T helper cell activity has also been described (Rothbard et al., 1988; Lamb et al., 1988). This motif is made up of a cluster of a positively charged amino acid and three hydrophobic residues in the relative amino acid positions 1, 4, 5 and 8. In an α-helical configuration of peptides containing this motif, the discontinuous residues would form a common facade of the helix allowing them to associate with the MHC restriction element whereas the opposite face would bind to the T cell receptor. This motif was shown to be present in the T cell epitopes of influenza virus H protein (307 to 319) and M protein (17 to 29) and the presence of the motif has been used successfully to predict a DR1 determinant in the 19K protein of Mycobacterium tuberculosis (Lamb et al., 1988).

In this paper, we describe studies with a synthetic peptide representing residues 288 to 302 of the F1 polypeptide of the fusion protein (F) of measles virus which is the only sequence in the protein which contains the 1, 4, 5, 8 motif. We show that, as predicted, this peptide indeed functioned as a T cell epitope and, interestingly, it does so in all members of a panel of inbred mouse strains of different H-2 type and in seven out of 10 humans.

**Methods**

**Peptide synthesis.** A peptide representing residues 288 to 302 of the F protein of measles virus (see below) was produced by manual solid-phase synthesis using Fmoc chemistry. Fmoc-protected amino acids were converted to hydroxynbenzotriazol-activated esters by treatment with hydroxynbenzotriazol and diisopropylcarbodiimide in dimethylformamide (DMF). The subsequent coupling reaction was performed in DMF and the Fmoc groups were removed with 20% piperidine in DMF, followed by a series of washes in DMF. After synthesis, side-chain-protecting groups were removed and the peptide was cleaved in trifluoroacetic acid in the presence of scavengers. After cleavage, the peptide was extracted into diethyl ether and purified by preparative HPLC. The purity of the peptide was assessed by analytical HPLC and by amino acid analysis.

**Mice.** The following inbred strains of mice were purchased from the National Institute for Medical Research (Mill Hill, U.K.): A/J, BALB/c, CBA, C57BL/6, SJL and SWR/J.

**Immunizations.** Female mice, aged 6 to 8 weeks were purchased from the National Institute of Medical Research and were immunized via the hind footpads with 100 μg of the peptide emulsified in Freund's complete adjuvant (FCA). Their lymph node cells were used for lymphocyte stimulation studies. Control mice were injected with an emulsion of FCA and distilled water. For antibody studies, mice were immunized intraperitoneally once with 100 μg peptide emulsified in FCA.

**Virus.** A lymphophilized preparation of the attenuated Schwartz strain (10⁶ TCID₅₀) was resuspended in 1 ml of sterile phosphate-buffered saline for use in the lymphocyte stimulation assays with mouse lymph node cells. For the human lymphocyte studies, the virus was heat-inactivated at 56 °C for 2 h as the virus is known to have an immunosuppressive effect on human lymphocytes.

**Lymphocyte stimulation assays.** Eight to 10 days after immunization, the popliteal and inguinal lymph nodes from groups of four mice were removed aseptically, pooled and the mononuclear cells collected by centrifugation.

Viable, unfractionated lymph node cells (4 × 10⁵) in 0.2 ml RPMI 1640 medium supplemented with 1% syngeneic serum, 2 mM-L-glutamine, 10 mM-HEPES and 100 μg/ml penicillin/streptomycin were incubated in the presence of either peptide 288-302 over the dose range of 0.1 to 20 μg/ml or human measles virus (dose range of 80 to 10⁴ TCID₅₀/ml) in a humidified 5% CO₂ atmosphere at 37 °C for 5 days. The cells were then pulse-labelled with 1 μCi [³H]thymidine per well and cells harvested 16 to 18 h later. Thymidine incorporation was assessed and the results are expressed as stimulation indices (SI) which represent the ratio of the mean c.p.m, in quadruplicate wells containing cells plus peptide or virus to the mean c.p.m. in quadruplicate wells containing cells and medium only. Results are also expressed as Δ c.p.m. which represents the mean c.p.m. of cells in quadruplicate wells cultured with peptide or virus minus the mean c.p.m. in quadruplicate wells cultured in medium only.

Peripheral blood mononuclear cells from a panel of 10 laboratory personnel who had been previously exposed to measles virus were isolated by Ficoll-Hypaque (Pharmacia) centrifugation. 1 × 10⁶ cells/ml in RPMI medium supplemented with 2 mM-L-glutamine,
5% autologous plasma, 100 μg/ml penicillin/streptomycin and 20 mM-HEPES were cultured for 6 days in the presence of either the 288–302 peptide (dose range 0.1 to 20 pg/well), heat-inactivated measles virus (dose range 80 to 10^4 TCID₅₀/well) or control peptide (MVF2 43–55). Cultures were then pulse-labelled with 1 μCi [³H]thymidine and the cells harvested after 18 h. Results of these assays were expressed as for those from the assays of mouse lymphocytes.

**Antibody assays.** Mice were bled from the retro-orbital venous plexus under anaesthesia 14 and 21 days after immunization and every 14 days thereafter up to 120 days. The presence of anti-peptide antibody in serum samples was assessed by a solid-phase enzyme immunoassay employing microtitre plates coated with peptide. Bound antibody was detected by the addition of peroxidase-conjugated rabbit anti-mouse immunoglobulin antibody. Titres are expressed as the logarithm of the dilution of the serum sample that gave an absorbance reading at 492 nm corresponding to that given by a 1:10 dilution of a control serum from each mouse strain.

**Results**

**T cell epitope analysis of measles virus fusion protein**

The identification of peptides with similar structural features which are restricted by the same MHC class II molecule has suggested the presence of allele-specific subpatterns in T cell epitopes. In three known DR1-specific T cell epitopes (influenza virus H and M proteins and the 19K protein of *M. tuberculosis*), a common cluster was observed of a positively charged amino acid and three hydrophobic residues in the relative positions of 1, 4, 5, 8 (Lamb *et al.*, 1989). The sequence of the fusion protein of measles virus (MVF1) was scanned and it was found that the sequence containing residues 288 to 302 was the only one which contained this motif. Furthermore, this motif showed close sequence homology to the three human DR1 determinants (Fig. 1), is predicted as an amphipathic 3_10 helix when analysed by the algorithm described by Margalit *et al.* (1987) and also contains a structural motif shown by Rothbard & Taylor (1988) to be predictive of a T cell epitope [glycine:(hydrophobic)₃:charged]. When this peptide sequence was oriented in a helical wheel structure, residues 1, 4, 5 and 8 were located on the same face of the helix (Fig. 2). Thus it is possible that this face could allow interaction with the MHC restriction element and the opposite face of the helix could interact with the T cell receptor.

In addition, the MVF1 288–302 sequence was aligned to the six amino acid core region (327 to 332) which has been shown to be critical for I-A^d^ binding of the ovalbumin peptide 323–339 (Sette *et al.*, 1987). Using the algorithm described by these authors (Sette *et al.*, 1989), the MVF1 sequence has been shown to contain a six-residue motif (291 to 296) with a score of 486 which suggests that the predicted sequence may bind well to I-A^d^ (Table 1).

![Image](https://example.com/image1.png)

**Table 1. Comparison of the ovalbumin 327–332 master sequence for I-A^d^ binding with residues 291 to 296 of the fusion protein of measles virus**

<table>
<thead>
<tr>
<th>Residue number</th>
<th>Peptide</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>V</td>
</tr>
<tr>
<td>2</td>
<td>H</td>
</tr>
<tr>
<td>3</td>
<td>A</td>
</tr>
<tr>
<td>4</td>
<td>H</td>
</tr>
<tr>
<td>5</td>
<td>A</td>
</tr>
<tr>
<td>6</td>
<td>I</td>
</tr>
</tbody>
</table>

*From Sette *et al.* (1987).
† "Motif number" for the measles peptide is 486, suggesting that the predicted sequence may bind well to I-A^d^.

**Proliferative T cell responses in inbred mouse strains**

Mice from a panel of six inbred strains were immunized with peptide 288–302 in FCA and 8 to 10 days later, lymphocytes were obtained from inguinal and popliteal lymph nodes and cultured in the presence of the peptide or live measles virus.
(i) In vitro proliferation in the presence of peptide
Lymph node cells from all six inbred mouse strains responded to the peptide in a linear dose–response fashion and the corresponding curves (A c.p.m. versus peptide concentration) are given in Fig. 3. In all strains, responses of lymph node cells from peptide-immunized mice were significantly greater than those of lymph node cells from control mice of the same strain injected with FCA alone.

(ii) In vitro proliferation in the presence of live virus
Lymph node cells from peptide-immunized mice were cultured in the presence of increasing TCID₅₀ of live human measles virus and the SI thus obtained were compared with those from control mice of the same strain injected with FCA alone (Fig. 4). Of the five strains tested, BALB/c, SJL and CBA strains showed a SI of greater than 2-0. SI in control mice were all below 2-0 and indicated that the virus did not non-specifically stimulate the cells. These results indicate that peptide 288–302 represents a structure which can prime for a T cell response to the virus or processed viral protein.

Proliferative responses to MVF1 288 peptide and measles virus of human peripheral blood lymphocytes
Peripheral blood lymphocytes were isolated from a panel of 10 laboratory personnel who had previously been exposed to measles virus. These lymphocyte preparations were cultured in vitro in the presence of a range of doses of either heat-inactivated virus or peptide 288–302 and the proliferative responses assessed. Lymphocytes from all 10 donors proliferated in the presence of the virus in a dose-dependent fashion (Fig. 5a) with SI ranging from 2-6 to 13-5; significant proliferation (SI > 2-0) was also observed in eight of the donors following culture with the 288–302 peptide (Fig. 5b), with SI ranging from 2-0 to 5-0 (Table 2). The specificity of the proliferation to MVF1 288–302 was confirmed by the observation that lymphocytes from all individuals failed to proliferate following culture with a peptide from the measles virus F protein (representing residues 43 to 55 of MVF2) which contains none of the known structural features associated with T cell epitopes, and does not behave as a T cell epitope in BALB/c, SWR/J and CBA mice (Fig. 5c).

Antibody responses in MVF1 peptide-primed mice
Antibody responses following immunization with MVF1 288–302 were assessed by ELISA. Mice of all strains mounted an anti-peptide response following immuniz-
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Fig. 5. Proliferative responses of peripheral blood lymphocytes from 10 laboratory personnel. Dose–response curves following culture with (a) heat-inactivated measles virus, range 0.4, 2.0, 10.0 TCID50 per culture; (b) MVF1 288–302 peptide, range 0.1, 1.0, 10.0 μg per culture; (c) control MVF2 43–55 peptide, range 0.1, 1.0, 10.0 μg per culture.

Fig. 6. Anti-peptide antibody responses in MVF1 288–302 peptide-primed mice. Results represent values from pooled sera from groups of four to six mice.

<table>
<thead>
<tr>
<th>Donor</th>
<th>C.p.m. in medium</th>
<th>MVF1 288–302 peptide</th>
<th>HI-MV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C.p.m.</td>
<td>μg*</td>
<td>SI</td>
</tr>
<tr>
<td>1</td>
<td>2178 ± 663</td>
<td>3363 ± 996</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>3195 ± 892</td>
<td>6331 ± 447</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>804 ± 53</td>
<td>2740 ± 54</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>855 ± 60</td>
<td>2226 ± 251</td>
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<td>5</td>
<td>1673 ± 233</td>
<td>5247 ± 453</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>3538 ± 233</td>
<td>11834 ± 1012</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>873 ± 229</td>
<td>4000 ± 509</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
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<td>6241 ± 206</td>
<td>10</td>
</tr>
<tr>
<td>10</td>
<td>3449 ± 1307</td>
<td>4982 ± 1423</td>
<td>1</td>
</tr>
</tbody>
</table>

* Values represent dose (μg or TCID50) giving maximum proliferation.
† Underlined values represent significant proliferation.

Discussion

A number of approaches have been proposed for the identification of regions in protein sequences which can be recognized by T cells (Rothbard & Taylor, 1988; Rothbard et al., 1988; Kane et al., 1989; DeLisi & Berzofsky, 1985; Spouge et al., 1987) or can interact with MHC molecules (Sette et al., 1987, 1989a). Such procedures are likely to be more successful than those seeking to identify B cell epitopes since for the latter tertiary structure is frequently important.

We have used these approaches for the determination of T cell epitopes in the fusion protein of measles virus and have identified one region in the protein (residues 288 to 302) which has the structural motif (1, 4, 5, 8) predictive for T cell epitopes (Rothbard et al., 1988), the characteristic motif for binding to the I-A^d molecule (Sette et al., 1989a) and the propensity to form an amphipathic 3_10 helix (Margalit et al., 1987).

A synthetic peptide representing this sequence of the fusion protein was synthesized and used to immunize mice of a panel of six H-2 disparate inbred mouse strains. Following footpad immunization with unconjugated peptide in FCA, lymph node cells from all six strains were able to proliferate in vitro in response to the
homologous peptide. This apparent lack of Ir gene control of the response to the 288–302 peptide may indicate that different MHC molecules may be able to interact with the same sequence in the peptide. On the other hand, it is quite possible that the MHC molecules in the various strains interact with different or overlapping residues within the peptide. Indeed, there are several published examples of small peptides containing overlapping determinants with different MHC-binding specificities (Sette et al., 1989b; Sinigaglia et al., 1988). It is also possible that the peptide may adopt a different conformation when associating with different MHC molecules (Bhayani & Paterson, 1989).

In contrast to the response to the homologous peptide, the ability of peptide-primed lymph node cells to proliferate in the presence of measles virus was influenced by the H-2 type of the mouse strain. It is very likely that these differences in responsiveness are due to variations in the presentation of viral antigens by antigen-presenting cells, since differences in processing by antigen-presenting cells have not been shown to underlie genetically controlled responsiveness (Friedman et al., 1983).

Immunization with the uncoupled peptide in adjuvant resulted in the induction of anti-peptide antibody in all of the five mouse strains tested and the antibody thus induced persisted for more than 110 days with no further immunization. These anti-peptide antibodies were unable to react with measles virus in an ELISA and none had virus-neutralizing activity. This is perhaps not surprising as this region of the protein was not predicted to be surface-exposed. The absence of genetic restriction in the induction of an antibody response to peptide 288–302 correlates with the similar observation at the cellular level and suggests the presence of overlapping B and T cell epitopes on the peptide.

Since one of the criteria used for the selection of peptide 288–302 was that it contained the motif for I-A^d binding, its ability to be recognized by lymphocytes from mice expressing only I-A^d molecules (C57BL/6: I-A^b; SWR/J: I-A^a and SJL: I-A^d) is notable. Data have already been published which demonstrate that I-A^a and I-A^d molecules can bind to very similar structures within an antigenic peptide but differ in the fine specificity of binding (Sette et al., 1989b). The data presented here suggest that the class II binding of the peptide 288–302 is likely to be via the I-A molecule as three I-E-negative strains (C57BL/6, SJL and SWR/J) are able to respond to the peptide.

The ability of peptide 288–302 to bind to a range of MHC molecules in mice has also been observed in humans. Peripheral blood lymphocytes from eight of a panel of 10 laboratory personnel who had previously been exposed to the virus proliferated in vitro in the presence of the peptide. These observations suggest that the region containing residues 288 to 302 of the fusion protein of measles virus may contain an immunodominant T cell site and the promiscuous nature of the binding of this peptide to MHC molecules could be advantageous in the design of a synthetic peptide vaccine. Thus it may be possible to link peptide 288–302 to an appropriate B cell epitope(s) with the ability to induce virus-neutralizing antibody to produce a synthetic molecule which would be immunogenic in the genetically diverse human population.

References


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