Immune responses in mice following immunization with chimeric synthetic peptides representing B and T cell epitopes of measles virus proteins

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The immunogenicity of chimeric peptides produced by collinear synthesis to contain both T and B cell epitopes from the fusion protein and the haemagglutinin of measles virus was studied in mice. The T cell epitope used was from the fusion protein (residues 288 to 302), which has been shown to be promiscuous in its binding to mouse major histocompatibility complex molecules. This epitope was coupled by (i) a glycine-glycine spacer to a B cell epitope from the fusion protein (residues 404 to 414) and (ii) either its amino or carboxy terminus to a neutralizing antibody epitope from the haemagglutinin (residues 188 to 199). The results obtained show that such chimeric peptides can indeed function as complete immunogens in a range of mouse strains of different H-2 haplotype, and can induce the production of antibodies which bind to the fusion protein and to measles virus. Furthermore, it was shown that the orientation of the T cell epitope with respect to the B cell epitope had a significant effect upon the immunogenicity and antigenic specificity of the chimera. This work gives further support to the concept of rationally designed synthetic peptide vaccines.

Introduction

It is now widely accepted that antibodies to the two surface glycoproteins of measles virus, haemagglutinin (H) and the fusion protein (F), are of critical importance in immunity to infection. Antibodies to H inhibit haemagglutination by the virus and adsorption to host cells, and antibodies to F inhibit haemolysis and membrane fusion (Varsanyi et al., 1984; Merz et al., 1980). The importance of anti-F antibodies in vivo is highlighted by the development of atypical measels in recipients of a formalin-inactivated virus in whom no anti-F antibodies could be demonstrated (Norrby et al., 1975). The development of a synthetic peptide vaccine based on the mimicking of antigenic structures in F and H would thus appear to be a valid new strategy for the control of measles.

Work reported from several laboratories over the last few years has indicated that immunization of experimental animals with synthetic peptides representing specific regions of viral proteins can induce immune responses specific to those proteins (Milich, 1989). Such an approach has obvious implications for the rational design of future vaccines and is being actively pursued by many research groups (Steward & Howard, 1987). The success of this strategy depends upon the successful identification of appropriate regions of the protein for subsequent synthesis, a task which has been greatly assisted by recent developments in fundamental immunology. As a result of these developments, it is now apparent that immunogens are recognized not only by immunoglobulin receptors on B cells but also by T cell receptors which bind nominal antigen in association with class II major histocompatibility complex (MHC) molecules on antigen-presenting cells (Berzofsky, 1987).

For synthetic peptides to act as effective immunogens, they need to contain two distinct sites, one to promote B cell interaction and a second to induce cognate T helper (Th) cell activity (Milich, 1989).

Considerable attention has been focused on the difficult problem of identifying the structural characteristics of B cell epitopes, which may be either linear or conformational in nature. A number of empirical approaches have been employed to identify linear B cell epitopes from the primary amino acid sequence of proteins, including the identification, for example, of regions of hydrophilicity, solvent accessibility, protrusion, atomic mobility and secondary structures (e.g. β-turns). However, the identification of conformational determinants is clearly very difficult in the absence of crystal structures. Despite the conceptual and practical problems, a number of important B cell epitopes has been identified by these simple approaches on proteins from viruses, bacteria and parasites.

The identification of potential T cell epitopes is theoretically simpler than that of B cell epitopes because it appears that T cells recognize short linear sequences of the antigen with lower order structural properties.
Empirical analysis of the regions of proteins recognized by T cells has shown that a high proportion have the characteristic of being amphipathic \( \alpha \)-helices (DeLisi & Berzofsky, 1985; Spouge \textit{et al.}, 1987). In addition to these secondary structure correlations, Rothbard \& Taylor (1988) have observed the association of a primary amino acid sequence motif with T cell recognition sites. Thus a sequence of a charged amino acid or a glycine followed by two or three hydrophobic residues and a charged or polar residue seems to be a characteristic feature for T cell recognition. These two approaches are not mutually exclusive because the postulated motif corresponds to one turn of an \( \alpha \)-helix and both have been shown to have predictive value in identifying T cell epitopes (Lamb \textit{et al.}, 1987; Cease \textit{et al.}, 1987). A further primary amino acid sequence motif has been described which is associated with DR1-restricted, influenza-specific Th cell activity and is made up of a cluster of a positively charged amino acid and three hydrophobic residues in relative positions 1, 4, 5, 8 (Rothbard \textit{et al.}, 1988). In an \( \alpha \)-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, and the opposite face would bind to the T cell receptor. This motif has been shown to be present in the T cell epitopes of the influenza virus H and matrix (M) protein, and the existence of the motif has been used successfully to predict a DR1 determinant in the 19K protein of \textit{Mycobacterium tuberculosis} (Lamb \textit{et al.}, 1988). We have used these algorithms successfully to identify a T cell epitope from F of measles virus (residues 288 to 302) which is immunogenic in a panel of mouse strains of different H-2 types and in seven of 10 humans (Partidos \& Steward, 1990).

The successful identification of both T and B cell epitopes on viral proteins would open the way to the synthesis of chimeric peptides representing both types of epitopes, generating complete immunogens with the potential to elicit antibodies and prime memory responses. In certain circumstances, the use of T cell epitopes from unrelated proteins could also be appropriate (Francis \textit{et al.}, 1988), but such an approach would not of course provide memory T cell responses to the pathogen.

A major concern for the prospects for synthetic peptide vaccine development has been the possibility that, because of genetic restriction or immune response gene control, peptides will only be recognized by a limited number of individuals in the genetically heterogeneous human population. However, a number of peptides with the property of binding to multiple MHC alleles has been described recently (Sinigaglia \textit{et al.}, 1988; Pannina-Bordignon \textit{et al.}, 1989; Partidos \& Steward, 1990; Hale \textit{et al.}, 1989; Kilgus \textit{et al.}, 1991), which suggests that this problem can be overcome by appropriate selection of peptides.

In this paper, we describe studies on the immunogenicity of chimeric peptides constructed by collinear synthesis of a T cell epitope (F;288-302) from F of measles virus with sequences representing B cell epitopes from either F (F;404-414) or H [H:188-199(Y)] of the virus. The results show that such chimeric peptides do function as complete immunogens in mice of a range of H-2 haplotypes, resulting in the production of antibodies which bind to F and to measles virus. In addition, it was shown that the orientation of the B cell epitope in relation to the T cell epitope has a marked effect on the immunogenicity and antigenic specificity of the chimera.

**Methods**

**Selection of B and T cell antigenic sites.** The amino acid sequence of the measles virus F (Richardson \textit{et al.}, 1986) was analysed for potential B cell epitopes using algorithms to identify regions of hydrophilicity (Kyte \& Doolittle, 1982), protrusion (Thornton \textit{et al.}, 1986), mobility (Karplus \& Schultz, 1985) and secondary structure (Wilmot \& Thornton, 1988), and the following regions were identified: residues 43 to 55, 240 to 258, 358 to 373, 404 to 414 and 433 to 443. Of these regions, F;404-414 was chosen as the most appropriate for consideration as a potential B cell epitope because it had the properties of high hydrophilicity, high protrusion index, high mobility and the presence of a \( \beta \)-turn. In addition, this region of F of the related Sendai virus contains a neutralizing epitope (Portner \textit{et al.}, 1987); the sequence is also within a region of the protein which contains a cluster of cysteine residues conserved in all paramyxoviruses and which has been suggested to be critical for the fusion process (Huil \textit{et al.}, 1987). The sequence H:185-195 of the virus H has been identified as a B cell epitope which can induce virus-neutralizing activity (Mäkelä \textit{et al.}, 1989). A peptide representing residues H:188-199 was therefore synthesized, avoiding part of the glycosylation site in the published sequence. A peptide representing residues 288 to 302 of F (Partidos \& Steward, 1990) was chosen as an ideal T cell epitope because it is the only region which contains the 1, 4, 5, 8 motif and shows close sequence homology with three human T cell epitopes (Lamb \textit{et al.}, 1989), is predicted as an amphipathic \( \alpha _{10} \) helix (Margalit \textit{et al.}, 1987), contains a motif predicted by Rothbard \& Taylor (1988) to be a T cell epitope and contains an I-A\(^{\text{a}}\) binding motif (Sette \textit{et al.}, 1989).

**Peptide synthesis.** Peptides representing residues F;288-302 and F;404-414 of F, and residues H:188-199 of H of measles virus were synthesized by manual solid-phase synthesis using Fmoc chemistry. In addition, a chimeric peptide was produced in which the B cell epitope (F;404-414) was collinearly synthesized at the amino terminus of the T cell epitope (F;288-302) using a Gly-Gly spacer (Table 1). Furthermore, two chimeric peptides were produced by synthesizing the H B cell epitope (H:188-199) in either the amino- or carboxy-terminal orientation with respect to the T cell epitope without the spacer residues (Table 1). Fmoc-protected amino acids were converted to the hydroxybenzotriazole-activated esters by treatment with hydroxysbenzotriazole and N,N'-disopropylcarbodiimide in dimethylformamide (DMF). The subsequent coupling reactions were 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-
<table>
<thead>
<tr>
<th>Peptide</th>
<th>Amino acid sequence</th>
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<tr>
<td>H:188-199(Y)</td>
<td>C-S-G-P-T-T-I-R-G-Q-F-S(Y)*</td>
</tr>
<tr>
<td>Chimeric</td>
<td></td>
</tr>
<tr>
<td>H:189/F:1:288(Y)†</td>
<td>C-S-G-P-T-T-I-R-G-Q-F-S(Y)</td>
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* Tyrosine residues included in parentheses were added to facilitate radiolabelling. They do not form part of the native protein sequence. † Cysteine at position 188 of this sequence was eliminated from this chimeric construct to avoid possible polymerization.

Table 1. Notation and sequences of peptides

Lymphocyte stimulation assays. Eight days after immunization, the draining lymph nodes from groups of four mice were removed aseptically, pooled and the mononuclear cells collected by centrifugation. Viable, unfractionated lymph node cells (4 × 10^6) in 0.2 ml RPMI-1640, supplemented with 1% autologous serum, 2 mM-L-glutamine, 10 mM-HEPES and 100 µg/ml antibiotics, were incubated in the presence of the peptides over a dose range of 0-1 to 20 µg/culture or medium alone in a humidified 5% CO₂ atmosphere at 37 °C for 5 days. The cells were pulse-labelled with 1 µCi [³H]thymidine in 10 µl volumes/culture 16 to 18 h before harvesting. Thymidine incorporation was assessed by liquid scintillation spectrometry and the results were expressed as the mean c.p.m. (±s.d.) from triplicate cultures.

Results

Antibody responses following immunization with uncoupled F:1:404-414 peptide or the F:1:404–G2–288(Y) chimeric peptide from F of measles virus

To study the immunogenicity of the F:1:404–414 peptide and the F:1:404–G2–288(Y) chimeric peptide in mice of various haplotypes, 100 µg doses of each in FCA were injected i.p. into groups of five animals. All groups of mice were boosted i.p. with the same dose of the two peptides in FIA 6 weeks after priming. Serum samples of individual mice were collected 3 weeks after priming and 2 weeks after the boost and tested by ELISA for antibody to peptide F:1:404–414 coated on the wells of the plate. As shown in (Fig. 1 a, b), no antibodies to F:1:404–414 were detected in any of the six strains of mice after immunization with F:1:404–414 alone, i.e. in the absence of the Th cell determinant (F:1:288–302). However, immunization with the chimeric peptide F:1:404–G2–288(Y) produced both primary (Fig. 1 a) and secondary (Fig. 1 b) anti-F:1:404–414 peptide responses in all tested strains of mice. C57BL/6 mice were found to be low responders to the F:1:404–414 peptide compared with the other strains of mice.


The ability of peptide F:1:288–302 to provide an effective Th cell site in the F:1:404–G2–288(Y) chimeric peptide was studied by comparing proliferative T cell responses in CBA mice. Lymph node cells from mice primed in vivo 8 days previously with 100 µg F:1:288–302 peptide were restimulated in vitro with the homologous peptide or the chimeric peptide. As shown in Table 2, F:1:288–302 immune lymphocytes gave equivalent responses upon restimulation in vitro with either the homologous peptide or the chimeric peptide. When mice were primed with 100 µg F:1:404–G2–288(Y) chimeric peptide, lymphocytes again responded equally well upon in vitro stimulation with the homologous peptide or the F:1:288–302 peptide.
Fig. 1. Antipeptide antibody responses of various strains of mice following immunization with the F1:404-414 peptide with (□) or without (■) the addition of the F1:288-302 Th cell determinant. The anti-F1:404-414 responses were assessed by ELISA (a) 3 weeks after the primary and (b) 2 weeks after the secondary immunization. The results shown are the mean (± S.D.) antibody titres from groups of five mice.

Table 2. In vitro proliferative responses of lymph node cells from CBA mice immunized with either F1:288-302 or F1:404-G2-288(Y) peptides

<table>
<thead>
<tr>
<th>Immunogen</th>
<th>F1:288-302</th>
<th>F1:404-G2-288(Y)</th>
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<tr>
<td>F1:288-302</td>
<td>26106 ± 1097*</td>
<td>23172 ± 1780</td>
</tr>
<tr>
<td>(3551 ± 333)†</td>
<td>(3642 ± 234)</td>
<td></td>
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<tr>
<td>F1:404-G2-288(Y)</td>
<td>23751 ± 983</td>
<td>30188 ± 1205</td>
</tr>
<tr>
<td>(4232 ± 447)</td>
<td>(4375 ± 428)</td>
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* Values represent mean c.p.m. (± S.D.) of triplicate cultures in the presence of peptide antigen (20 μg/culture).
† Values in parentheses represent mean c.p.m. (± S.D.) of triplicate cultures in the absence of peptide antigen.

Reactivity of anti-F1:404-G2-288(Y) sera with F and measles virus

Since peptide F1:404-414 was predicted to be a B cell antigenic site and it has been demonstrated that anti-F1:288-302 antibodies failed to react with either F or measles virus (data not shown), it was of interest to determine whether antibodies raised against the chimeric peptide F1:404-G2-288(Y) would react with F- or measles virus-coated ELISA plates. As shown in Fig. 2, serum samples from all strains of mice reacted with F and, more interestingly, reactivity was observed towards the virus. However, serum samples from C57BL/6 mice reacted weakly with F but did not react with the virus. Furthermore, the anti-F1:404-G2-288(Y) sera were tested for neutralizing and virus fusion-inhibiting activity, and in neither of these assays was in vitro activity of the antipeptide serum towards the virus demonstrable.

Antibody responses to chimeric peptides F1:288/H:188(Y) and H:189/F1:288(Y) in CBA mice

One of the advantages that collinear synthesis of immunogens provides is the flexibility to alter the position of one epitope in relation to another, thus allowing the study of the influence of epitope orientation on antibody specificity. The synthetic constructs F1:288/H:188(Y) and H:189/F1:288(Y) were therefore synthesized to determine the effect of altering the position of the B cell epitope in relation to the T cell epitope (F1:288-302). Preliminary experiments had shown the failure of the uncoupled H:188-199(Y) peptide to induce either primary or secondary responses to H:188-199(Y) peptide in CBA mice. However, as
Mouse immune responses to synthetic peptides

shown in Fig. 3, immunization of CBA mice with 100 μg F₁:288/H:188(Y) chimeric peptide resulted in the induction of primary anti-H:188–199(Y) and anti-F₁:288/H:188(Y) responses as measured by ELISA. These responses were boosted by a second injection of the chimeric immunogen (100 μg) administered 5 weeks later in FIA. No response to the F₁:288–302 component was observed. Immunization of CBA mice with 100 μg H:189/F₁:288(Y) chimeric peptide did not produce a detectable primary antibody response to either component of the chimera. However, the peptide was immunogenic after the boost but the specificity of the antibody response was directed to the F₁:288–302 component and not the H:188–199(Y) component (Fig. 4). When samples of sera raised to each chimeric peptide were tested for antivirus activity, reactivity was observed only with anti-F₁:288/H:188(Y) antibodies (mean log₁₀ titre of 2.5), which had no demonstrable virus-neutralizing activity.

Discussion

Short synthetic peptides representing B cell epitopes are generally poor immunogens and need to be coupled to a carrier protein with Th cell epitopes in order to induce an antibody response. However, such an approach has limited relevance to vaccine production because of the lack of carrier proteins suitable for human use, the phenomenon of carrier-induced suppression and, most importantly, the failure of this strategy to induce priming of a T cell memory response to the pathogen. Therefore, for the rational development of a synthetic peptide vaccine, it is necessary to design chemically defined immunogens representing relevant B and T cell epitopes of the pathogen.

In this paper, we describe the use of this strategy to induce the production of antibodies to the surface proteins of measles virus. The data presented here show that although peptide F₁:404–414 is non-immunogenic in all strains of mice tested, the addition of the Th cell epitope F₁:288–302, previously shown to be immunogenic in a range of strains of inbred mice and to be recognized by virus-primed lymphocytes in seven of 10 humans tested (Partidos & Steward, 1990), renders it immunogenic. Moreover, this ability was found not to be MHC-restricted because in all six strains of mice tested, an anti-F₁:404–414 response was observed after immunization with the chimeric peptide. The results of the proliferation assays in CBA mice suggest that all the Th cell activity of the F₁:404–G₂:288(Y) chimeric peptide was associated with the F₁:288–302 moiety. Lymph node cells immune to the F₁:288–302 peptide proliferated equally well upon in vitro stimulation with either the homologous peptide or the F₁:404–G₂:288(Y) chimeric peptide. In addition, when lymph node cells were primed with the chimeric peptide the proliferative responses observed upon in vitro stimulation with the homologous peptide or F₁:288–302 were found to be the same. Although the proliferative responses to the F₁:404–414 peptide have not been studied, the antibody data reveal that this peptide is not immunogenic, providing further evidence for the Th cell activity of the F₁:288–302 peptide within the chimeric construct. Thus, it can be argued that the N-terminal orientation of the B cell epitope (F₁:404–414) in relation to the T cell epitope
The results have also shown the ability of antipeptide antibodies to recognize F and the measles virus. This finding shows that residues 404 to 414 of F, which were empirically predicted to be surface-exposed, do represent an antigenic region on the surface of F. The results also confirm the work of Mäkelä et al. (1989) indicating that residues 188 to 199 of measles virus H contain an important B cell epitope. However, the antibodies produced to both B cell epitopes had no demonstrable in vitro virus-neutralizing or fusion-inhibiting activity in spite of their ability to bind to the virus. A number of potential explanations for this failure can be given, including the possibility that the region does not represent the complete epitope or that the region is simply not a neutralizing or fusion-inhibiting epitope. An alternative explanation may be that the antibody response elicited is qualitatively poor for these effector functions, i.e. antibodies are of an inappropriate immunoglobulin class or subclass, or are of too low affinity. If the affinity of the antipeptide antibodies does play an important role in antibody function (Steward & Steensgaard, 1983), then suitable approaches to immunization need to be devised to maximize the affinity of the antibody response, i.e. adjuvants, route of immunization and the form of the immunogen (Holland et al., 1990).

These findings are of particular relevance to the development of synthetic peptide vaccines. A major constraint imposed by immune response genes on T cell recognition of antigen is the dual recognition of antigen and MHC molecules. Thus the effectiveness of a synthetic peptide vaccine may well be limited by restricted recognition of the T cell element by MHC molecules in the human population. Accordingly, peptides which have the property of promiscuous interaction with a wide range of MHC molecules will be necessary to provide helper activity to potential B cell epitopes in the outbred human population. One of the first examples of such a promiscuous T cell epitope to be described was the peptide consisting of residues 378 to 398 of the circumsporozoite protein of Plasmodium falciparum, which can function as a helper determinant in a wide range of mouse strains and is recognized by most human class II MHC molecules (Sinigaglia et al., 1988). The peptide consisting of residues 288 to 302 from F of measles virus initially described by Partidos & Steward (1990) and examined further in the present report represents another promiscuous T cell epitope. Its ability to provide help for B cell epitopes when collinearly synthesized with them is particularly encouraging for peptide vaccine development.

It is likely that the orientation in which the B and T cell epitopes are collinearly synthesized influences their immunogenicity and antigenic specificity. In this study, examination of the orientation of a B cell epitope representing residues 188 to 199 from H of measles virus in relation to the T cell epitope F1:288–302 did show an effect not only on the immunogenicity of the chimeric constructs but also on the specificity of antibody produced. Thus, the N-terminal location of the B cell epitope in relation to the T cell epitope [chimeric peptide H:189/F1:288(Y)] resulted in a synthetic construct unable to induce a primary antibody response. However, after the boost the peptide was found to be immunogenic but the specificity of the antibody response was directed towards the F1:288–302 component instead of towards the H:188–199 component. This observation is in contrast to the results obtained with the F1:404–G2–288(Y) chimeric peptide which elicits an anti-F1:404–414 response as a result of having the B cell epitope at the N terminus of the T cell epitope. Perhaps these differences might be attributed to the presence of two glycine residues between the two epitopes which allow more conformational freedom for interaction with receptors on immunocompetent cells. However, the presence of glycine spacers might not be so important as the actual amino acid composition of the two epitopes which in turn impose conformational constraints to the synthetic immunogen. The latter possibility is supported by findings in a study where a synthetic peptide (residues 65 to 85) from the 65K protein of M. tuberculosis has been shown to provide help for the production of antibodies to the peptide consisting of residues 422 to 436 of the 65K protein of M. lepra when linked by its amino terminus (Cox et al., 1988). In the same study, similar results were also obtained when a peptide consisting of residues 427 to 436 was linked to the N terminus of the foot-and-mouth disease virus peptide consisting of residues 141 to 160, representing both B and T cell epitopes. However, when the peptide consisting of residues 422 to 436 was linked to the C terminus of the peptide consisting of residues 65 to 85, no antibodies to either component of the chimera were observed. This is not the case for the chimeric peptide F1:288/H:188(Y) used in this study. The immunogenicity of this peptide was found not to be affected by the presence of the B cell epitope (residues 188 to 199) at the C terminus of the T cell epitope (residues 288 to 302). Both primary and secondary anti-H:188–199(Y) responses were observed in CBA mice.

These results highlight the importance of careful consideration of the orientation of B and T cell epitopes in chimeric peptide immunogens for the induction of appropriate immune responses with the required specificities.

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References


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