Heterogeneity of linear B cell epitopes of the measles virus fusion protein reacting with late convalescent sera

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B cell epitopes of the measles virus fusion protein were mapped, by reacting sera from late convalescent donors with synthetic overlapping pentadecapeptides, segments covering the whole F protein sequence. Unselected individual sera recognized 7 to 20% of the total sequence. Cumulation of the binding patterns of 30 sera identified eight to 10 clusters of antibody-binding peptides spread over most of the sequence. The B cell epitopes included regions of transition between the more hydrophobic (including the N-terminal end of the F1 and F2 protein) and hydrophilic sequences. When the regions of antibody binding were compared with the predicted secondary structure of the F protein, no detectable pattern became apparent. Exposed sequences as well as sequences hidden in the viral membrane or in the protein core of both the F1 and F2 polypeptides were recognized by the antibodies. The heterogeneity of the binding patterns was not merely dependent on the anti-measles virus titre. The importance of antibodies recognizing linear epitopes of the measles virus fusion protein for the immune protection is presently not known.

Introduction

Antibodies play an important part in immune protection against measles. This is highlighted in newborn and infant children who are protected by transplacentally acquired maternal antibodies (Albrecht et al., 1977; Black, 1989). The protective role of antibodies is confirmed by the successful prevention of measles outbreaks with infusions of immunoglobulins with elevated anti-measles virus activity (Janeway, 1945). Two membrane proteins of the measles virus, the haemagglutinin (HA) and the fusion (F) protein are known to mediate a protective immune response (Giraudon & Wild, 1985; Makela et al., 1989). There is evidence that a humoral response to the F protein is required for complete protection (Norrby et al., 1975; Merz et al., 1980). In the present study, the authors mapped the linear antibody-binding sites of the measles virus F protein using overlapping synthetic pentadecapeptides covering the whole sequence.

Methods

Sera. Sera were collected from 30 healthy adult blood donors. The measles virus titres were determined using a commercial complement fixation kit (Virion, CH-6330 Cham).

When the regions of antibody binding were compared with the predicted secondary structure of the F protein, no detectable pattern became apparent. Exposed sequences as well as sequences hidden in the viral membrane or in the protein core of both the F1 and F2 polypeptides were recognized by the antibodies. The heterogeneity of the binding patterns was not merely dependent on the anti-measles virus titre. The importance of antibodies recognizing linear epitopes of the measles virus fusion protein for the immune protection is presently not known.

Peptides. One-hundred and eight overlapping pentadecamer peptides covering the whole sequence of the F protein of the measles virus (strains Edmonston and Hallé; Richardson et al., 1986; Buckland et al., 1987) were synthesized on a multiple peptide synthesizer (Zinsser SMPS 350; Zinsser Analyt). p-Benzoyloxybenzyl alcohol resin (30 mg) loaded with the first Fmoc-amino acid was filled in separate small tubes (Eppendorf cups). Fmoc deprotection was carried out with 50% piperidine in N,N-dimethylformamide (0·5 ml) for 5 min and repeated for 15 min. Single couplings were performed with Fmoc-amino acids in 10-fold excess and 1-hydroxybenzotriazole/diisopropyl-carbodiimide activation in N,N-dimethylformamide within 1 h. After coupling and after deprotection the tubes were washed with N,N-dimethylformamide (0·5 ml), 10 times each. N-Fmoc- and side chain-protected pentadecapeptide resins were washed with dichloromethane, N,N-dimethylformamide and methanol, dried in vacuo and treated with 0·5 ml of trifluoroacetic acid :thioresol :thioanisole (95:2:3) for 3 h. The N-Fmoc peptides were washed twice by sonication in ether. The precipitates were dissolved in tertiary butanol and this solution was lyophilized. Peptide samples were dissolved in 6 M-HCl and hydrolysed for 24 h at 110 °C. Amino acids were separated as trifluoroacetyl-amino acid propylesters by gas chromatography on a glass capillary coated with Chirasil-Val. The D-amino acid content was determined by enantiomer labelling (Frank et al., 1978). Amino acid analysis produced results according to the expected data, and no racemization could be detected. The peptides were designated by running numbers starting with the C-terminal peptide KDPLTGTSKSYVRSL. One-hundred and four peptides overlap with 10 amino acids with their adjacent peptides (Fig. 1). For chemical reasons, peptides 21, 41, 91 and 99 were shifted by one amino acid position towards the N terminus and overlapped by nine amino acid residues with the previous peptides.

Peptide ELISA. Flat-bottomed microtitre plates (Immunoplate Maxisorb F96; Nunc) were coated overnight at room temperature with
Results

Binding of unselected sera

Sera from healthy donors with anti-measles virus antibody titres ranging from 1:4 to 1:128 were reacted with immobilized Fmoc–pentadecapeptide conjugates in an ELISA. Fig. 2 shows the reaction of two individual sera (no. 176, titre 1:128; no. 264, measles virus titre 1:4) with 108 peptides. The sera with the higher titres reacted with particular peptides loosely clustered in groups of two or more peptides. This can partially be attributed to sequences shared by adjacent peptides. Overall antibody binding of serum no. 176 was more prominent than with the low titre serum no. 264. However, only a few peptides generated signals twofold above background. Both sera markedly differed in the peptides which they recognized, an observation extended in Fig. 3 to 30 unselected donors, grouped by anti-measles virus titres. Binding was very heterogeneous, but some sera showed similar patterns. As shown in Fig. 3, binding patterns were independent of the measles virus titres. Individual sera
reacted with up to 24 peptides (> threefold background; one serum reacted with 32 peptides) or up to 44 peptides (> twofold background). Fig. 4 shows the proportion of sera that reacted with a given peptide (> threefold background). Most peptides reacted with less than one-third of the sera. Peptides 6 and 78 reacted with more than half of the sera. When the cutoff for a positive reaction was lowered to twice the background, nine peptides (peptides 6, 8, 26, 32, 39, 51, 78, 81 and 100) reacted with more than 50% of the sera (data not shown). Sera with a titre of 1:128 appeared to bind to a few peptides only. However, as for serum no. 176 (Fig. 2a) this is partially due to the background defined by the 50% lowest values.

Fig. 4 shows that the antibody-binding peptides are loosely clustered in about eight to 10 groups covering most but not all of the protein sequence. On the basis of the cumulated data of Fig. 4, we have tentatively defined nine regions containing B cell epitopes, covering peptides 6 to 12, 24 to 32, 39 to 44, 48 to 54, 68 to 75, 75 to 82, 84 to 86, 90 to 95 and 99 to 104. Overlapping sequences explain why most of the positive peptides were within two peptides from other positive peptides. However, the size of the clusters was larger than could be explained by binding to sequences shared by adjacent peptides.
The N-terminal ends of F1 (peptides 84 to 86) and F2 (peptides 101 to 104) are both reactive. About 25% of the sera reacted with the C-terminal peptide of F1. Mouse antibodies to this peptide were found to react with the F protein (Vialard et al., 1990). The strong reactivity with peptides 6 and 7 may be due in part to covalent binding to two cysteine residues. Peptides 8 to 11 and 81 to 86 correspond to putative transmembrane sequences. Peptides 50 and 51 correspond to a T cell epitope described by Partidos & Steward (1990). This sequence also induced antibodies in mice which, however, did not react with the protein. Peptides 13 to 20, 33 to 38, 55 to 67, 87 to 89, 96 to 98 and 105 to 108 correspond to regions of enhanced binding gave a similar result: maximal binding was mostly associated with anti-measles virus titres of 1:64 but not with sera with higher titres.

In addition, acrophilicity, the hydrophilicity and hydrophathy patterns of the sequence were analysed according to Kyte & Doolittle (1982) and Hopp & Woods (1981). Fig. 6 schematically summarizes the latter structural features and shows where the antibody binding sites are located. The five C- and N-terminal amino acids of each epitope have been omitted on the basis of the expected reactivity with overlapping sequences. In summary this comparison showed the following. (i) Antibody-binding sites are preferentially but not exclusively located in regions of transition of the most prominent hydrophatic with adjacent hydrophilic sequences. (ii) The hydrophatic signal sequence is not reacting. (iii) Three epitopes (peptides 39 to 44, 68 to 75, 90 to 95) do not contain hydrophatic sequences. (iv) The epitopes include the putative transmembrane regions (amino acids 113 to 147 and 489 to 517). (v) The potential glycosylation sites at amino acid positions 61 and 67 are probably not reactive. (vi) The epitopes do not coincide with acrophilic regions.

**Discussion**

Sera from unselected adult donors were used to map antibody binding to linear epitopes of the measles virus F protein. This approach identifies sequential B cell epitopes both on the protein surface and in its core but ignores conformational epitopes. The binding distribution varied markedly between individual donors and was independent of the measles virus titre. Maximal binding of a serum to a given peptide correlated with the measles virus titre. Most sera recognized between 7 and 20% of the overlapping peptides. The overall reactivity was relatively weak but the cumulation of the binding data from 30 donors defined eight to 10 clusters of antibody binding, which we consider to be B cell epitopes.
These epitopes were located both on F1 and F2 and were found near the N- and C-terminal ends of the two subunits of the heterodimer. The epitopes included all of the more hydrophobic sequences at their transition to adjacent hydrophilic sequences. The codons of the signal sequence (amino acids 1 to 23) correspond to hydrophobic amino acids (1 to 20). Therefore the hydrophobic peptides corresponding to this sequence are adequate negative controls.

Our approach does not discriminate between internal and exposed B cell epitopes. The study shows that in late convalescent donors, antibodies can be detected against most peptides, indicating that both external sequences as well as sequences embedded in the protein or membrane core are reactive. Several other lines of evidence indicate that this is the case. The B cell epitopes did not coincide with acrophilic sequences. Putative transmembrane regions (peptides 6 to 12 and 80 to 87) were recognized by antibodies. The peptide 86 (KRFAGVVLAGAALGV) represents the N terminus of F1 and includes the transmembrane sequence FAGVLAGAALGVATA-AQIV which probably mediates cell fusion (Richardson et al., 1980).

To analyse this further, we have used an algorithm described by Parker & Hodges (1991) which predicts more than 90% of surface sequences. The prediction is based on the presence of tripeptides containing any of the polar residues G, K, S, D, E, N, P, T, A, R and Q. Peptides that do not contain polar tripeptide sequences include peptides 7 to 9, 24, 30, 31, 50 to 53, 61 to 63, 67, 71 and 94 to 98 and are most probably inside the protein, since the Parker and Hodges approach tends to overestimate surface regions of proteins. According to this prediction, the peptides 50 to 53 correspond to an internal sequence of the F protein. Yet antibody reactivity to these peptides is strong. Other putative internal sequences showed little or no reactivity (peptides 30, 31, 61 to 63, 67, 71 and 96 to 98). In mice, peptide 52 induced antibodies, but these were not neutralizing [Partidos et al. (1991) and our own results]. Like this T cell epitope described by Partidos and colleagues (Partidos & Steward, 1990; Partidos et al., 1991), other hidden sequences are also unlikely to be neutralizing. Whether these antibodies contribute to immune protection, and which mechanisms may be involved, remains unclear.

This also raises a question about the role of antibodies recognizing linear sequences of the measles virus F protein in protecting against reinfection. Although antibodies to the F protein can neutralize the virus (Malvoisin & Wild, 1990), it is not clear whether in the F protein linear epitopes can be found which mediate virus neutralization.

Pairing of B cell epitopes with T cell epitopes as found here in peptides 50 to 52 was also observed in other pathogens such as influenza A virus (Barnett et al., 1989). In influenza virus HA, B cell epitopes played a role in defining T cell epitopes (Graham et al., 1989). Therefore, antibody-binding studies with synthetic peptides may not only be useful in identifying linear B cell epitopes but may also help in the search for T cell epitopes.

Presumably, the antibodies detected here are transmitted passively to newborns (Albrecht et al., 1977). It is possible to speculate, on the basis of the heterogeneity of the inter-individual binding pattern, that it may be possible to elicit an antibody response even in the presence of measles virus antibodies by using synthetic peptides. This could be of interest in inducing measles virus antibodies in newborns despite persisting maternal antibodies. Sequences spared by antibodies of natural immunity are potential candidates for an antibody-resistant fractionated vaccine if such sequences can induce neutralizing antibodies. In human immunodeficiency virus infections, it was shown that natural immunity does not involve all possible immunogenic peptides, leaving some capable of by-passing natural antibodies (Rossi et al., 1989). Further studies however are necessary to show, firstly, whether synthetic peptides can elicit neutralizing antibodies against measles virus F protein and, secondly, whether a peptide cocktail can be designed which is (partially) resistant to (maternal) anti-measles virus antibodies.

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References


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