Activation of T cells by the ragged tail of MHC class II-presented peptides of the measles virus fusion protein

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Abstract

The efficient and sustained immune response of an antigen requires T cell epitopes, capable of inducing a long lasting T cell memory. To detect T cell epitopes of the measles virus fusion protein (MV-F), the proliferation of lymphocytes from late convalescent donors in response to overlapping pentadecapeptides covering the whole protein sequence was studied. Three major immunodominant regions (F51-70, F121-135 and F211-225) containing promiscuous peptides induce proliferation in peripheral blood lymphocytes in ~50% of the donors. Potential DR1-restricted epitopes were mapped using an MHC competition binding assay. Both the proliferation and the binding data identified a DR1-restricted T cell epitope (F51-65). Contact sites of the peptide HQSLVIKLMPNITLL with MHC were characterized using substitution analogs. Alanine substitutions at most positions did not interfere with F51-65 binding. These analogs were therefore useful for studying the residues which were recognized by the TCR of MV- and F51-induced T cells lines. In addition to amino acid residues of the core of peptide F51-65 both the C-terminal and the N-terminal amino acids were essential for T cell interaction. Since peptides presented by class II molecules vary in length, these findings suggest that residues of the ragged tail are important for T cell activation. It is speculated that in late convalescent donors the length of the flanking sequence of MHC II-restricted peptides may play a role in controlling the heterogeneity of MV-specific T cell clones recruited as T helper/memory cells.

Introduction

In the measles virus a protective immune response involves both cellular (1-4) and humoral reactions (5-7) against the hemorrhagglutinin (MV-H) (8,9) and/or the fusion protein (MV-F) (10-12). A number of authors have investigated the T cell response to inactivated MV using human peripheral blood lymphocytes (PBL) (13-15). Van Binnendijk and colleagues (1,4) have demonstrated the importance of class II-restricted T cells in measles virus infection on a clonal level.

The immune response is triggered by processing of viral proteins by antigen-presenting cells (APC) and the presentation of antigenic peptides. Recognition of antigenic viral peptides bound to MHC class II molecules induces Th cell proliferation and helps for effector cells (for review see 16). T cell proliferation is generally in response to a limited number of defined sequences of the protein (for review see 17).

A popular approach to identify Th cell epitopes is to stimulate the T cells with relevant peptides in a proliferation assay (e.g. 18,19). The natural length of MHC class I-restricted peptides was determined to be 8-11 amino acids long (20,21). In contrast, peptides eluted from MHC class II molecules were considerably longer containing 12-17 residues or more (22). In this study, pentadecapeptides were reacted with human
PBL from HLA-typed donors to study T cell epitopes of the MV-F protein. Similar studies have been conducted with other human pathogens such as the meningococcal class 1 outer membrane protein (18) or the human respiratory syncytial virus (19).

We found a number of T cell epitopes spread over the whole sequence of the F protein, some of which appeared to be restricted by more than one class II allele and were immunodominant in several donors. Such ‘promiscuous’ peptides are of great interest to the development of subunit vaccines. By comparison of the proliferation data with data from binding studies using purified class II molecules we identified an epitope common to DR1-positive donors. This epitope was further characterized using T cell lines and peptide substitution analogs. The fine mapping of the interacting amino acid residues suggested that the C- and N-terminal ends of the corresponding peptide are important for T cell recognition.

Methods

Donors

PBL, and sera were obtained from laboratory personnel and volunteer donors after informed consent (22–39 years old). Some donors had had measles, others were immunized during early childhood. Anti-measles antibody titers were determined using a commercial complement fixation kit (Virion, Cham, Switzerland). The neutralizing titers (NT) of the donor sera were determined in Vero cell cultures by plating 100 TCID50 measles virus preincubated with 100 μl/well heat-inactivated diluted (1:40 to 1:10240) human serum for 3 h at 4°C (Table 1). One donor (LK) was negative by complement fixation but had a neutralizing titer of 1:340. Serological typing was kindly performed by Dr Hentges (Luxembourg) and the oligonucleotide typing was done by Dr C. A. Muller (Tübingen) (Table 1).

Proliferation assay

PBL were isolated by density gradient centrifugation on Ficoll-Hypaque gradient (15 min, 800 g) from healthy, HLA-typed adult donors (Table 1). The cells were washed twice in PBS and incubated (triplicates of 2-5×10⁵ cells/well) for 5 days in RPMI 1640 supplemented with penicillin (100 IU/ml), streptomycin (1 μg/ml), 5% autologous serum, 2 mM glutamine and 50 μM 2-mercaptoethanol in the presence of 10 μg/well peptide or culture supernatant from MV-infected or uninfected Vero cells containing 10⁶ p.f.u. of inactivated MV (Edmonston strain, ATCC VR-24). In some experiments, concentrated and purified virus was prepared as described earlier (23), and UV inactivated using a UV-transilluminator (TS-20, 254 nm, nominal intensity 8000 μW/cm²; UVP, Cambridge, UK). The hemagglutinin and fusion protein were derived from recombinant baculovirus kindly made available by Dr C. D. Richardson (Montreal, Canada; 24). On day 4, 0.5 μCi [³H]thymidine was added and the cells were harvested 20 h later. The data are expressed as stimulation indices (SI), which was calculated on the basis of the average of one third of the lowest counts. The background averaged 500–2000 c.p.m. A SI > 3.0 was considered positive. The following mAb were used for blocking: CD4 (OKT4, ATCC CRL8002), CD8 (OKT8, ATCC CRL 8014) and DR (Tü36, kindly given by Dr C. A. Müller).

EBV-transformed cell lines

These cell lines were derived by incubating freshly drawn PBL with supernatant of B95-8 cells in the presence of 1 μg/ml cyclosporin A according to standard protocols.

Peptides

One hundred and eight overlapping 15-mer peptides covering the whole sequence of the MV-F protein (Edmonston strain; 25,26) and selected substitution analogs were prepared for epitope mapping. Nα-Fmoc-protected pentadecapeptides were synthesized on polystyrene–1% divinylbenzene resins with 4-benzyloxybenzyl alcohol anchors using 10 equivalents of F-moc amino acids and disopropylcarbodiimide/1-hydroxybenzotriazole activation. All syntheses were performed with a multiple peptide synthesizer (Gyro, Multisynthech, Bochum, Germany), as previously described (27). The results of gas chromatographic amino acid analysis on the chiral stationary phase Chirasil-Val were as described (27). The results of gas chromatographic amino acid analysis on the chiral stationary phase Chirasil-Val were as described (27). The results of gas chromatographic amino acid analysis on the chiral stationary phase Chirasil-Val were as described (27). The results of gas chromatographic amino acid analysis on the chiral stationary phase Chirasil-Val were as described (27).

MHC binding assay

Peptide binding to MHC was determined in a binding competition assay using a DR1-restricted binder as a fluorescent-labeled indicator peptide (7-amino-4-methylcoumarin-3-acetic acid conjugated to the N-terminus of the influenza matrix peptide 19–31 [AMCA-IM19–31]; 28). HLA-DR1 (DRB1*0101) molecules were isolated and purified from WT100 cells as described by Kropshofer et al. (29). Purified DR1 (0.36–1.2 μg) was incubated for 48 h with 10 μl of test peptide (0.2 mg/ml in 100 mM PBS, 0.05% w/v CHAPS, pH 5.0) in the presence of 20 μl (10 μg/ml) AMCA-IM19–31. After 48 h the mixture was analyzed by HPLC equipped with a dual UV and fluorescence detector. The ratio of UV signal of the reporter peptide corresponded to a strong fluorescence signal of the reporter peptide. A low fluorescence signal of the reporter peptide corresponded to a strong competition of the test peptide (28).
Results

DR1 binding studies of peptides covering the whole sequence of MV-F

The number of epitopes of a virus which mediate the T cell response is usually limited to a few immunodominant T cell determinants. We have recently shown in the mouse that considerably more peptides of the MV-F protein are class II-restricted and that a number of subdominant peptides were able to prime T cells against the virus. Binding to MHC is a necessary but not sufficient property of subdominant peptides which could prime T cells against the virus. To map potential DR1-restricted T cell epitopes, direct binding to purified DRB1*0101 molecules was measured in a competition assay using AMCA-labeled IM19-31 as a reporter peptide (Fig. 1). Eight peptides were strong DR1 binders (>66% competition) and an additional nine were moderate binders (33-66% competition).

Proliferation in response to MV-F peptides

Since the MV interferes with lymphocyte functions (31) the proliferation assays must be carried out with inactivated virus. Under the conditions used, UV inactivation of the virus was completed after 60 s and T cell proliferation was maximal after 50-80 s of UV exposure. In the presence of heat-inactivated virus (30 min, 56°C) the SI was two to three times lower (data not shown). UV-inactivated MV was used throughout this study.

The proliferation of PBL in the presence of UV-inactivated MV was dependent on CD4 and class II (and generally to a lesser extend on CD8). The T cells recognized both MV-H and MV-F protein. MV-free frozen-thawed Vero cells or the supernatant from uninfected Vero cells did not stimulate proliferation (Fig. 2, donor CM). Differences in the efficiency of presentation of MV and the recombinant proteins may be due to the presence of MV antibodies (from the autologous serum) (32; Fournier et al., manuscript in preparation). An isotype-matched irrelevant antibody gave <20% inhibition.

Table 1. MHC class II haplotypes of measles late convalescent donors or MV vaccinated donors and their anti-MV titers determined by complement fixation and MV neutralization (NT)

<table>
<thead>
<tr>
<th>Donor</th>
<th>Complement fixation</th>
<th>NT</th>
<th>MHC class II haplotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>MF</td>
<td>1 256</td>
<td>1 5120</td>
<td>w52</td>
</tr>
<tr>
<td>SA</td>
<td>1 128</td>
<td>1 1240</td>
<td>2 (15), 3</td>
</tr>
<tr>
<td>JF</td>
<td>1 256</td>
<td>1 5120</td>
<td>1,7 [0102, 0701]</td>
</tr>
<tr>
<td>CM</td>
<td>1 64</td>
<td>ND</td>
<td>1,4 [0101, 0403]</td>
</tr>
<tr>
<td>UK</td>
<td>1 64</td>
<td>1 5120</td>
<td>w53</td>
</tr>
<tr>
<td>WS</td>
<td>1 64</td>
<td>1 1240</td>
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<tr>
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<td>1 64</td>
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<td>w52</td>
</tr>
<tr>
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<td>1 32</td>
<td>1 1240</td>
<td>w53, 53</td>
</tr>
<tr>
<td>MS</td>
<td>1 32</td>
<td>1 1240</td>
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</tr>
<tr>
<td>RB</td>
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<td>w52</td>
</tr>
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<td>ND</td>
<td>w52</td>
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<tr>
<td>DK</td>
<td>1 32</td>
<td>1 3400</td>
<td>w52</td>
</tr>
<tr>
<td>AB</td>
<td>1 64</td>
<td>ND</td>
<td>1, 3 [0102, 0301]</td>
</tr>
</tbody>
</table>

*Donors with a confirmed history of measles. Results of the DR oligonucleotide typing (DRB1*) of DR1-positive donors are shown in square brackets.

Proliferation in response to MV-F peptides

To map the antigenic determinants of one of the immunodominant MV proteins the proliferative response to synthetic peptides of the MV-F protein was investigated. The 108 overlapping pentadecapeptides covering the whole sequence of the MV-F protein were added to cultures of freshly isolated human PBL to test their ability to stimulate T cell proliferation. Figure 3 shows the SI values of representative donors.

Diverging proliferation patterns were found with PBL from 13 HLA-typed adult donors (Fig. 4). As expected, the SI values generally were quite low and only occasionally exceeded a value of 6 after peptide stimulation. Most donors recognized between four and 15 peptides (even though donor SK recognized 21 peptides) or between three and nine regions (with the exception of SK) containing partially overlapping peptides. The number of peptides may be somewhat overestimated by our attempt to set the cut-off uniformly at SI > 3, but increasing this value would have missed peptides which were consistently 'positive' when the background noise was low. On the 113 positive reactions shown in Fig. 4, about two-thirds were directed against partially overlapping peptides which may co-stimulate T cells of the same (or a different) specificity since they have 5 or 10 of their 15 amino acids in common. About 75% of the peptides reacted with the PBL of a single donor or none. Several peptides were recognized by three (F61, F181, F206, F231 and F236) or more donors (F51, F56, F121, F196, F211 and F261). Three major immunodominant regions (F121-135), (F51-70) and (F211-225) that were recognized by about half of the donors emerged. These regions are likely to contain peptides which bind to MHC encoded by more than one haplotype.

The donors JF, CM, SA and DM are DR1-positive. The
peptides F51 and F211 which induced a significant proliferation in DR1-positive PBL were also strong DR1 binders (cf. Fig. 1). Peptide F56 and the regions of F286 and F291 were moderate or weak DR1 binders. Other strong (F126, F181, F221, F276, F331, F466 and F481) and moderate (F11, F256, F271, F316, F346, F376 and F501) binders did not correspond to sequences which stimulated more than one DR1 donor.

**DR1 restriction of F51 and F56**

The comparison of the reactivities of Fig. 4 with the class II haplotypes of the donors (Table 1) indicated that the region of F51–70 may be DR1 restricted. The donors JF, CM, SA and DM (and AB) share the haplotypes DR1 and DQw1. All of these donors, including AB (cf. Fig. 6), reacted with F51 and F56, and in a less consistent way also with F211 (cf. Fig. 4). The addition of anti-DR antibody (Tu36) to the proliferation
T cell interaction with a DR1-restricted peptide

Fig. 2. Proliferation of peripheral blood lymphocytes (donor CM) in response to partially purified, UV-inactivated MV (1-0.001 μg/well) grown on Vero cells, frozen-thawed Vero cell debris (1-0.001 μg/well) and recombinant MV-F (1-0.01 μg/well) and MV-H (1-0.01 μg/well) protein. Anti-CD4, anti-CD8 and anti-MHC II (mAb IVA12) were used for blocking the response to 1 μg/well MV. Data are shown as cpm. ± SD of triplicates.

assay inhibited the proliferation of PBL to F51 or F56, while the proliferation in the presence of F26, another stimulating peptide, or F51-A10, an unreactive Ala substitution analog of F51 (cf. Fig 5B), or the unsupplemented medium were unaffected by the antibody (data not shown).

Fine mapping of DR1-binding F51 residues (Fig. 5A)
The above competition binding assay was used to identify the F51 contact residues with DR1. According to most authors the first anchor residue (position i) of a DR1-binding peptide is the most important. In the relative position i hydrophobic residues have consistently been found while charged or polar residues are not permitted (33-37). In peptide F51, L4, V5, L6 or L8 were the only candidate residues which could serve as the first anchor.

Polar substitutions of L8 (Ser, Arg) did not influence binding of the analogs. Triple substitution of L4, V5 and L6 showed that the first anchor must be one of these residues. Double and single substitutions of L4, V5 and L6 with aspartic acid clearly identified L6 as the first and most important anchor residue. The position i + 1 is not usually occupied by hydrophobic residues but rather by polar or charged residues (35). The substitutions permitted for K7 (E, D, R, H but not Y or F) correspond to the spectrum of residues found in position i + 1 (35). The high affinity of the R7 and H7 substitution analogs corresponds to the preference of polar amino acids in position i + 1 as shown by Hammer et al. (38). This further confirms L6 as the first anchor.

According to the DR1 motif methionine in position i + 3 (M9) could serve as the second anchor. Bulky, charged or polar residues are normally not well tolerated in position i + 3 (35,21), but when M9 was replaced by Tyr, Arg or Gln binding was essentially unchanged (77-79%). When P10 was replaced by Ala, Trp or Phe binding was reduced to 33, 12 or 18% respectively (Fig. 5A and B). The substitution of P10 by Ala could influence binding in different ways: (i) by eliminating proline as an anchor, (ii) by changing the conformation of the analog in the groove due to the removal of the proline kink or (iii) a combination of both. The kink induces a ‘contraction’ of the peptide so that P10 (in position i + 4 instead of i + 3) could become the second anchor.

A third and a fourth anchor is often found in position i + 5 and i + 8. In particular, the latter anchor is quite degenerated (34-36) and was not observed in the earlier studies (39). Different unfavorable substitutions of N11, I12, T13, L14 and L15 were unable to clearly identify additional residues essential for MHC binding.

When adjacent peptides of F51 were analyzed, the N-terminal adjacent peptide F46 had no activity, while the C-terminal peptide F56 was still a moderate binder (Fig. 1). In light of observations that a decrease in peptide length is generally accompanied by a corresponding decrease in potency (40), this result also confirms the importance of L6 as a first anchor amino acid of the epitope.

DR1-binding of Ala substitution analogs of F51 (Fig. 5B)
The above substitutions have permitted us to position the peptide in the MHC binding groove and to identify residues that were potentially interacting with DR1. In those experiments substitution analogs were used which were active both by the residues removed as well as by the residues introduced. In order to keep the influence of the residue introduced minimal, each amino acid of F51 was sequentially replaced by an Ala residue (Ala-scan). The results showed that the binding was sensitive only to the replacement of P10 by Ala. A minor reduction in MHC binding was associated with the M9 (75%) and L6 (81%) Ala analogs. This result was not surprising because the most important anchor residues for binding to DR1 (relative positions 1, 4, 6 and 9) are known to be permissive for Ala (35,41).

MV-induced T cell lines and F51 Ala substitution analogs (Fig. 6A and B)
Since most of the above Ala replacements were introduced without loss of MHC binding, these peptide analogs were useful tools to map residues which would interact with the
TCR of specific T cells. For this purpose short-term T cell lines were derived by re-stimulating and expanding fresh DR1-positive PBL in the presence of MV or F51 and IL-2/PHA as described in Methods. After 6–8 weeks the cell lines induced against the MV were highly responsive to MV and to F51 (10 µg/ml; cell line AB2-MV, Fig. 6A and B). When the cells were reacted with the Ala analogs most amino acids could be individually replaced by Ala without loss of stimulation. However, replacing H1, L4, M9 and L15, and to a lesser extend P10 and T13, by Ala resulted in a loss of reactivity. In light of the above substitution data P10 was involved in MHC binding. Thus we conclude that H1, L4, M9, T13 and L15 were recognized by the T cell (Fig. 6B).

**F51-induced T cell lines and Ala substitution analogs (Fig. 6C and D)**

Fresh PBL underwent several rounds of re-stimulation and expansion in the presence of purified peptide F51. The resulting T cell lines cross-reacted with MV. The F51-specific line (AB2-F51) exhibited a similar pattern of reactivity with the above Ala substitution analogs (Fig. 6C). H1, L4, M9, N11, T13 and L15, and to a lesser extend Q2 and K7, were recognized by the TCR. In addition to its MHC interaction, 16 seems to react also with the peptide induced T cells (Fig. 6D).

**Reactivity of DRB1*0102-positive PBL with F51 substitution analogs (Fig. 7)**

The donors SA and DM shared only the DRB1*0102 allele and the DQw1 allele but no alloreactivity of DM donor cells was observed in the presence of EBV-SA. We took advantage of this unresponsiveness to use EBV-SA cells pulsed with selected peptides of the region F(41–80) or with UV-inactivated MV as APC to stimulate PBL from DM. With only these restriction elements capable of interacting with the donor cells, the reaction to the MV is as expected to be lower than in the standard proliferation assay. Under these conditions...
We have attempted to map T cell epitopes of the MV-F protein in a small panel of donors. As expected, the proliferation of different residues of the same peptide are involved in amino acids, and by the strong reduction of T cell recognition the substitution analogs differed from the DRB1*0101 donors.

**Discussion**

We have attempted to map T cell epitopes of the MV-F protein in a small panel of donors. As expected, the proliferation of bulk PBL in response to the MV-F peptides was low and of similar magnitude than in other systems when peptides which were not optimized for maximal stimulation were used (19). These results probably reflect the low frequency of T cells specific for a given peptide in late convalescent donors. The heterogeneity of T cell epitopes found here in the MV-F protein is comparable to the results of similar studies (18,42).

Donor MF (DR2(15),3 DRw52, DQw1) displays a cluster of stimulating peptides between F396 and F431. van Binnendijk et al. (4) have described a cluster of T cell epitopes restricted by DR2, DRw53 and DQw1 in this region. Two donors were found to react with peptide F511, which includes the cysteine cluster region. Cysteine cluster regions have been found to represent T cell epitopes in several animal studies with other
viruses (43–45) or parasites (46,47) and in target structures for human autoantibodies (48). The measles T cell epitope (amino acids 288–302) described by Partidos and Steward (13) corresponds to our peptides F281–F291. These authors found the sequence 288–302 to generate a SI > 2.0 in eight of 10 adult donors. In our study five of 11 donors gave an SI > 2, but only two donors passed our criteria for positivity. One of the above immunodominant peptides is located at the N-terminal end of the F1 protein (amino acids 121–135, F121) and represents the core region of the MV-F "fusion peptide", i.e., the sequence which is involved in the cell fusion process of different morbilliviruses (49). Lymphocytes reacting with this highly conserved sequence may also have been induced in response to another paramyxovirus and/or cross-react with related viruses.

Three major immunodominant regions, i.e., F121–135, F51–70, and F206–225, recognized by about half of the donors emerged. These regions are likely to contain peptides which bind to more than one haplotype-encoded restriction element. Such promiscuous regions are of interest for being incorporated into a subunit vaccine.

In a recent study, we have shown in mice that the number of MHC-restricted T cell epitopes in the MV-F sequence is far greater than the number of immunodominant T cell epitopes "used" after immunization with whole virus. Some of the subdominant T cell epitopes were capable of priming T cells against the MV (30). In the mouse, these results were obtained by immunizing with peptides and re-stimulating in vitro with the same peptides or with MV. Obviously a different strategy was required in the human. Therefore, to identify potential human T cell epitopes, overlapping peptides covering the whole sequence of the MV-F protein were tested for their ability to bind to purified DR1 molecules. This assay identified a limited number of strong DR1 binders. The region F51–70 included two adjacent peptides which were strong and intermediate binders, and which in the functional assays reacted with all four DR1-positive donors. Also, peptide F211 reacted with three of the four DR1 donors and was found to be a strong DR1 binder. In analogy to our mouse studies (30), we expect that in addition to the latter two immunodominant epitopes, additional DR1 binders will be able to prime against the MV. Such (potentially) subdominant epitopes are of interest.
for the development of subunit vaccines since they increase the number of possible epitopes from which to choose.

The DR1-restricted peptide F51 was chosen for further characterization of the interactions within the DR1-TCR-peptide ternary complex. The binding motif of DR1-restricted peptides has been extensively investigated, sometimes with diverging results. However, the importance of the first anchor position and its range of residues are well defined (21,33). Additional features such as hydrophobic residues in position $i + 3$, small residues in position $i + 5$ or a hydrophobic residue
in position i + 8 were found with decreasing consistency (33–37,50–52). Using selected substitution analogs based on favorable or unfavorable residues at positions predicted by the consensus motif, we have identified I6 as the first anchor and IKLMPN as the peptide core (for review see 40). The definition of the anchor positions was complicated by the fact that four hydrophobic residues (L4, V5, I6 and L8) could potentially serve as a first anchor. The second anchor was defined at position i + 4 which is compatible with the ‘condensation’ of the peptide as a result of the proline kink in position 10. The structural constraint imposed by proline also seemed to have blurred additional anchors.

When Ala substitutions were introduced in the different positions the binding of the analogs was hardly affected, as was expected from other studies (41). These analogs therefore were used to study peptide–TCR interactions. When MV- or F51-induced T cell lines were reacted with the Ala substitution analogs it became apparent that the T cells reacted in a much more sensitive fashion to Ala substitutions (Fig. 6). Our analysis of the MHC–TCR interacting residues of peptide F51 revealed several noteworthy features: (i) Two residues were identified to be essential for the MHC binding. (ii) All residues involved in the interaction with the MV-induced T cell line (H1, L4, M9, T13 and L15) were also important for the interaction with the peptide-specific T cell line. In addition, Q2, I6, K7 and N11 were important for stimulation of the F51-induced line. One possible interpretation of this is that the MV-specific T cells are more heterogeneous than the F51-specific line with regard to their reactivity to the latter residues, but that most clones react with the H1, L4, M9, T13 and L15. (iii) The peptide-induced T cell line recognized almost twice as many residues than the MV-induced T cell line but both cell lines recognize MV and the peptide. (iv) In both cell lines an identical subset of residues did not interact with the MHC or TCR. (v) I6 was identified as a major anchor residue for MHC interaction. Ala substitution of I6 reduced MHC binding reproducibly, but the reaction of the peptide induced T cell line was impaired to a greater extent. This may be a reflection of the oligoclonality of the T cells and/or of the interaction of I6 with both the TCR and the MHC. Residues along the upper edge of the binding groove have been shown to interact with both TCR and MHC (50,53 and references therein). (vi) Another interesting finding of this substitution study is that three or four of the five most important residues for T cell recognition are located outside of the peptide core (H1, L4, L15 and perhaps T13): the C- and N-terminal amino acids (H1 and L15) did not influence MHC binding but surprisingly they were critical for T cell stimulation. This demonstrates that not only core residues but also residues of the ragged tail which are five amino acids away from the core are involved in T cell recognition.

Since these studies were done with uncloned lines it cannot be concluded from our data whether a same T cell clone interacts with both the N- and C-terminal end. Also, substitutions, in principle, do not distinguish between direct effects on MHC–TCR contacts and indirect effects resulting from stabilization of the MHC, conformational changes in the peptide (56) or from interactions with co-stimulatory molecules. Since our binding assays measure primarily on-rates, while in the functional assays off-rates may be more important the comparison of the two assays may not be fully valid. However, this approach is widely used to map peptide contact residues in MHC–TCR complexes (40) and the results were recently validated by X-ray crystallography studies of the DR1 molecule (50,53).

In other studies, the length of class II restricted peptides varied from 12 to 25 residues but for T cell recognition the length of the peptide did not seem to be important as long as the core was maintained (54,55). In the mouse, a role in T cell recognition of sequences flanking the peptide core has been shown after immunization with hen egg lysozyme (57,58).

The AB2-MV cell line was derived from a late convalescent donor >25 years after measles virus infection and our findings may be typical for the end-point of a mature immune response. In this cell line the critical residues for TCR interaction are mainly outside of the peptide core. Increasing the number of interactions with the ragged tail could potentially limit the number of participating T cell clones to an economic size. We speculate that the involvement of the ragged tail of nested sets of antigenic peptides in T cell recognition is not a random phenomena but a process related to the maturation of antigen presentation. General mechanisms which may account for the different lengths of the peptides generated during processing include: (i) variations in protease composition in different APC, (ii) temporal maturation or activation of the endocytic compartments of APC (59,60) and (iii) the transition from the macrophage or dendritic cells to the B cell as the main APC during the maturation of the immune response (59). Additional factors may play a role with respect to specific pathogens. In the case of the MV both endogenous (61,62) and exogenous virus (32) can be presented via class II molecules. This involves two different pathways (the latter which is chloroquine-sensitive, the former which is not) which are likely do generate peptides of different lengths since the processing of endogenous proteins usually results in (MHC class I-presented) shorter peptides, while class II-presented peptides are constitutively longer peptides. Thus, trimming class II-presented peptides to different lengths would permit the APC to control the T cell clones involved in the recognition of the antigen once the initial immune response has taken its course and only memory cells are required.

Even though a role in T cell recognition of sequences flanking the peptide core has been shown in an experimental model (57,58), this is one of the first studies in which the MHC–TCR contact residues of a peptide defining an epitope of a natural infection have been mapped and where residues of the ragged tail have been shown to be involved in T cell stimulation. Since the T cells were obtained from late convalescent donors, our findings may correspond to the final stage of maturation of antigen presentation of the MV.

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**Abbreviations**

- APC: antigen-presenting cell
- EBV: Epstein-Barr virus
- MV-F: measles virus fusion protein
- MV-H: measles virus hemagglutinin
- NT: neutralizing titer
- PBL: peripheral blood lymphocyte

**References**


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