

Sub-unit Peptide and Genetic Vaccination with Measles Virus

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Abstract

The development of sub-unit vaccination against measles virus (MV) has been based mainly on N, F and H proteins because the antibodies recognising these proteins have been found to be effective in virus neutralization in animal and rodent models. Four different approaches viz. peptide vaccination, DNA immunization, mimotope mimicking peptide motif and ISCOM complex-based peptide vaccination utilizing MV-F, MV-H and MV-N have been tested in murine, simian and human subjects in the absence and presence of maternal Abs. Mapping of T cell and B cell epitopes by monoclonal antibodies have been undertaken and polyclonal anti-sera (both in human and rodent, mouse or murine) have been analysed. In this article, the results of MV sub-unit vaccination strategies have been reviewed and the questions that remain open despite increasing recent experiences have been summarised.

Introduction

Vaccination with a live attenuated vaccine strain of measles virus (MV) usually confers lifelong protective immunity against the disease. Wild-type (wt) or live attenuated MV induces major antibodies (Abs) against two envelope glycoproteins haemagglutinin (H) and fusion (F) proteins and two minor Abs against nucleocapsid (N) and matrix (M) proteins. The attenuated virus vaccine has now been used for three decades and although the Ab titres induced are lower than in natural infections, specific MV Ab has been detected more than 20 years after vaccination. The failure of inactivated MV vaccines used thus far to protect against measles upon re-exposure to the virus has been associated with an incomplete immune response (Wild, 1999; Pabst *et al.*, 1999; Sniadak *et al.*, 1999). Natural measles virus infection imparts lifelong immunity against

subsequent disease development whereas vaccination with live attenuated measles vaccine does not always assist in enduring protection (Giraudon and Wild, 1985; Malvoism and Wild, 1990). Table 1 summarises the probable roles of antibodies directed against different MV antigens in patients. A number of vaccinees seroconvert but later become susceptible to both infection and disease. Since waning immunity is seen only in the vaccinated population, it seems likely that the immune responses to attenuated virus either differ quantitatively or qualitatively from the responses to wt MV during an acute infection. Protection against measles infection has been observed after the passive transfer of γ -globulin and the specificity of this protection had been confirmed using anti-MV-H and anti-MV-F Mabs in a mouse model (Giraudon and Wild, 1985; Malvoism and Wild, 1990).

The rationale and the demands for the development of inactivated vaccines against measles have recently been formulated by WHO steering committee. The schedule and revised regimes of MV vaccination has been discussed earlier (Wild, 1999; Pabst *et al.*, 1999). The aerosol route (MV spray immunization through naso-pharyngeal route) is considered to be better immunogenic for booster doses than traditional subcutaneous injection (Giraudon and Wild, 1985; Malvoism and Wild, 1990).

MV has a potential to form pseudotypes with vesicular stomatitis virus glycoprotein G and exhibit both cellular and neurotropism (Katz M., 1995; Clements C.J. and Cults F.T., 1995). With the advent of reverse genetics system of the virus, it has been possible to rescue engineered pseudotypes with defined modifications (Radecke and Billeter, 1997). The paradigms of MV vaccination and eradication have been reviewed along with the MV-induced immune responses in humans as well as simian and rodent models in several earlier studies (Horikami S.M. and Moyer S.A., 1995; Wild T.F. and Buckland R. 1995; Rima *et al.*, 1995; Borrow and Oldstone, 1995; Griffin, 1995; Schneider-Schaulis *et al.*, 1995; Van Binnendijk R.S *et al.*, 1995; Liebert and Finke, 1995; Norby, 1995; Radecke and Billeter, 1995; Lliashhenko *et al.*, 1995; Schmidt *et al.*, 1992).

The development and sub-unit vaccination has been targeted to N, F and H proteins of MV because the Abs of these proteins function in virus neutralization in animal and rodent models. Four different approaches viz. peptide vaccination, DNA immunization, mimotope mimicking antigenic peptide motifs and ISCOM complex-based peptide vaccination utilizing MV-F, MV-H and MV-N have been tested in murine, macaque monkey and human subjects in the absence and presence of maternal Abs. Mapping of T cell and B cell epitopes by monoclonal antibody have been carried out and polyclonal anti-sera have been analysed (see Table 4).

Abbreviations

MV= Measles virus; HIV= Human immunodeficiency virus; VV= Vaccinia virus; HLA (DR, DP, DQ) = Human leucocyte antigens with respective designations; Ag= Antigen; Ab = Antibody; Ig (G, A, M and E) = Immunoglobulin of respective subtype; BCE = B cell epitope; TCE = T cell epitope, ThTCE = Helper T cell epitope; CTL = Cytotoxic T lymphocyte; NK = Natural killer cells; PBMC = Peripheral blood mononucleocytes; aa = amino acid; im, sc, CMI = Cell-mediated immunity; ADCC = Antibody-dependent cellular cytotoxicity

Table 1. Role of antibodies directed against different MV proteins in patients

Component	Function
N-Ab (abundant and rapidly produced)	Complement fixation
H-AB	Serum neutralization of virus infectivity
F-Ab	Virus neutralization by preventing fusion of the virus membrane with the cell membrane limiting spread of infection to new cells
Fused F-H Ab	Complement-mediated lysis of infected cells
M-Ab (moderately immunogenic)	Increased synthesis with atypical measles

Epitope Mapping for MV-F

To map the linear immunodominant recognition sites (Ab binding sites) of the F₁, a complete set of 108 overlapping pentadecapeptides were titrated with purified human IgG obtained from donor sera with elevated anti-MV titres (Wiesmuller *et al.*, 1992, Muller *et al.*, 1993). The peptides recognised by the Abs exhibited a binding pattern, defining about 6 or 7 distinctive regions (F31-75, 111-145, 151-165, 191-215, 271-320, 421-440, 481-530) which include the major hydrophobic segment (F111-145) of the inter-subunit region and the C-terminal Cys-cluster region. The binding sites were located in close proximity of the few experimentally defined T cell epitopes (TCEs). This pairing of TCE(s) and BCE(s) was corroborated by computer-assisted epitope prediction. Some of the characteristics of the pattern are:

- I. Regions of enhanced binding were found both on F₁ (113-550) and F₂ (residues 24-112) with a lower binding intensity and a higher density in the smaller F₂ protein.
- II. The epitopes partially included the hydrophobic region residues 489-517, a putative transmembrane segment.
- III. The signal sequence (residues 1-23) did not bind.
- IV. No obvious preferential binding to one of the predicted secondary structures was detectable.
- V. Reactivity was found with putative external portions of the F protein as well as hidden sequences such as putative transmembrane region. Sequences predicted to be exposed did not preferentially react.
- VI. The C-terminal Cys-cluster region reacted with most human sera and were described as major antigenic sites with neutralizing activity in Newcastle disease virus and parainfluenza virus, two other members of paramyxoviridae.
- VII. Binding was 6 amino acids from the N-terminal end of F₂ and involved less regularly the C-terminal end of F₂ (h).

Reactivity was also observed at the hydrophobic N-terminal end of F₁(113-147) *i.e.* peptide M86 (KRFAGVVLAGAALGV) which overlaps by 13 amino acid residues with the sequence FAGVVLAGAALGVATAAQIV which probably mediates cell fusion. The significance of a non-random association of TCE and BCE for processing and presentation has also been observed in MV-H. It is speculated that in long-term immunity against measles (F

protein), B cells of the same sIg specificity play an important role both as APCs and as Ab producing cells. In contrast to human sera from late convalescent donors, mouse and rabbit MV antisera with high neutralizing titres as well as neutralizing MV-F-specific MAbs did not react with the peptides (Wiesmuller *et al.*, 1992). For a list of putative B- and T- cell epitope, please see Table 3.

The peptide F₁240-252 (GDINKVLEKLGYS) bearing a potential Th sequence (TCE) and BCE was based on the presence of a motif of a charged residue followed by two hydrophobic residues and terminating with a polar amino acid and folding as an amphipathic -helix defined by computer algorithm AMPHI (23). Extension of the peptide F₁240-252 by six residues at the C-terminus (GGDLLG) was based on the presence of residues of moderate hydrophobicity and protrusion index along with the presence of a -turn. This could overcome non-responsiveness to the F₁240-252 peptide in BALB/c and SWR mice (Partidos and Steward, 1992), but prevented both cellular and humoral responses of C57BL/6 mice.

A Cys-residue at the C-terminus of each peptide was introduced to increase immunogenicity. The positive and negative effects that the flanking sequence F253-258 exerted on the proliferative responses to the F₁240-252(C) peptide correlated with the Ab responses in mice of different MHC haplotypes. F₁240-252(C) was immunogenic in certain strains of mice capable of recognizing Th TCE determinant of this peptide. But increased immunogenicity was observed with peptide F₁240-258(C) in mice, which were initially non-responders to F₁240-252(C), whereas peptide F₁240-258(C) had negative effects on C57BL/6 mice. Abs raised against the elongated peptide F₁240-258(C) reacted better with F protein and MV than did anti-F₁240-252(C) Abs in SWR and BALB/c mice where both peptides were immunogenic. In SWR mice, peptide F₁240-258(C)-induced Abs failed to react with the virus or the F protein, and none of the anti-peptide Abs mentioned above neutralized or inhibited the MV fusion activity *in vitro*. Peptide F₁240-258(C) also showed no induction of Ab responses in C57BL/6 mice. The peptide emulsified in Freund's complete adjuvant (FCA) were allowed to immunize by intraperitoneal injection of female mice of various haplotypes.

An immunodominant antigenic region from the F protein (p32; residues 388-402 *viz.*, ANCAGILCKCYTTGT) represents a conserved sequence within the highly conserved Cys-rich domain of F of paramyxoviruses (Atabani *et al.*, 1997). Immunization of two strains of mice (H-2^d and H-2^k) with p32 peptide was shown to induce

anti-peptide Abs that cross-reacted with and neutralized MV infectivity *in vitro*, suggesting that the Th TCE is non-MHC restricted. The anti-p32 Abs were effective in a plaque inhibition assay but did not inhibit MV-induced cell-cell fusion. Some of the shorter versions of p32 peptide containing a Cys-residue at each terminus were less reactive to acute post-MV polyclonal sera. Moreover, the polyclonal sera failed to recognize the substitution peptides with conserved replacements at the site of Cys-residues for the antigenic conformation of the peptide. However, the lack of recognition of all peptides representing the regions within the Cys-rich domain by polyclonal human sera suggests that these peptides (shorter) do not assume the correct conformation on a solid phase support. p32 peptide (F388-402) sequence is highly conserved among Cys-rich regions of several paramyxoviruses (SNV, PF3, NDV and SV5). Epitope mapping of this peptide indicated the necessity of this complete 15-mer sequence for high affinity interaction with anti-MV Abs (24). p32 alone induced Ab-response, indicating the presence of both BCE and Th-TCE within its sequence. Furthermore, a boosting effect observed 3 weeks following priming in the two strains of mice provided further evidence of T cell help in the induction of memory B cells. Mice receiving passively transferred BALB/c or CBA anti-peptide sera were protected against a fatal encephalitis following challenge with neuroadapted MV. Although anti-peptide Ab titre was higher in BALB/c mice, anti-p32 Ab raised in CBA mice, had a higher relative Ab affinity for the homologous peptide. p32 (F388-402) was recognized by all serum samples of MV-infected children but by none of the sera with undetectable anti-MV Abs. Because of p32 sequence convergence among paramyxoviruses, p32 peptide motif could be a useful tool for MV diagnostic purposes (Atabani *et al*, 1997).

p32 peptides (F388-402): Cys residues, substitution at which retained the antigenic conformation of the peptide abolished the recognition by polyclonal sera. The Cys-rich regions of MV-F337-423, comprising 8 Cys residues, interacts specifically with MV-H protein to induce cell-fusion, and a limited region, Cys₃₃₇ to Arg₃₈₁, is all that is required for fusion (Atabani *et al*, 1997).

F288-302 peptide contains both TCE and BCE and is promiscuous in its binding to mouse MHC molecules (Partidos *et al*, 1990; Partidos and Steward, 1990). When this Th TCE was coupled by (a) Gly-Gly spacer to a BCE of F404-414 (INQDPPDKILTY) which is non-immunogenic and (b) by binding either its N- or C-terminus to a neutralizing Ab epitope from the H protein (residues 188-199 CSGPTTIRGQFS(Y)), such chimeric peptides proved to be functional as well as complete immunogens in a range of mouse strains of different H-2 haplotypes and induced the production of Abs which bound to MV and MV-F protein but did not react with MV in either a solid-phase immunoassay or a virus neutralizing assay. Orientation of the TCE with respect to BCE had a significant effect upon the immunogenicity and antigenic specificity of the chimera. The N-terminal orientation of the BCE (F₁404-414) in relation to the TCE (F₁288-302) does not affect the conformation in which the TCE (F₁288-302) binds to the MHC molecules. The N-terminal location of the BCE in relation to the TCE (chimeric peptide H:189/F:288(Y), SGPTTIRGQFSLSEIKGVIVHRLEGV-(Y)) resulted in a

synthetic construct unable to induce a primary Ab response; however, a booster dose induced Ab of H188-199 (CSGPTTIRGQFS(Y)) component. In contrast, F₁404-G₂-288 (Y) (INQDPPDKILTY-GG-LSEIKGVIVHRLEGV(Y)) elicits an anti-F₁404-414 response as a result of having the BCE at the N-terminus of TCE. The presence of two Gly-residues between the two epitopes may allow more conformational freedom for interaction with receptors on immunocompetent cells. But the actual TCE and BCE impose conformational constraints to the synthetic immunogen (Partidos *et al*, 1990). The immunogenicity of F₁288/H188(Y) (LSEIKGVIVHRLEGVCSGPTTIRGQFS(Y)) was not affected by the presence of the BCE (residues H188-199) at the C-terminus of the TCE (residues F₁288-302) and both primary and secondary anti-H188-199(Y) responses were observed in CBA mice (Partidos *et al*, 1990). This peptide F₁288-302 also behaved as a BCE in that immunization with free peptide in adjuvant resulted in anti-peptide Ab production in all mouse strains of different H-2 haplotype (SWR, SJL, CBA, BALB/c and C57BL/6) (Partidos and Steward, 1990). PBMC of 10 human individuals of prior history of measles proliferated in response to culture with MV and PBMC of these 8 individual only responded to this peptide (F₁288-302), indicating the permissive interaction of this antigenic peptide with a wide variety of class II MHC molecules (*e.g.* I-A^d) both in mice and humans (Muller *et al*, 1995). Further modification may be achieved by including BCE for virus neutralizing Ab formation. Lymphocytes from F288-308 peptide-immunized mice from all six H-2 disparate strains (C57BL/I-A^b), SWR/J(I-A^g) and SJL(I-A^e) mounted a proliferative response following *in vitro* culture with the peptide. In addition, lymphocytes from these three strains also proliferated in the presence of a live MV (Partidos and Steward, 1990; Partidos *et al*, 1990). Chimeric peptides containing two copies of TCE induced Abs with higher affinity for BCE than did chimeras containing one copy of the TCE or two copies of BCE. Furthermore, the N-terminal location of the TCE in relation to the BCE in the chimera induced higher affinity anti-B cell Ab than did the reverse orientation (Partidos *et al*, 1992).

MHC class II (H-2^d)-restricted epitopes were identified by co-immunizing BALB/c (H-2^d) mice with 108 pentadecapeptides covering the whole sequence of MV-F protein (Muller *et al*, 1995). F406-420, F91-105 and F256-270 were selected among the most immunogenic peptides. F421-435 and F256-270 obliterated the F91-105-specific immune response after co-immunization. Peptides which did not contain TCEs such as F41-55 or F521-535 did not reduce *in vivo* priming or restimulation *in vitro* by F91-105. F421-435 also eliminated any response to F256-270 after co-immunization. A certain level of antigenicity seemed to be preserved by F256-270 in the presence of lower proportion of F421-435. Thus F421-435 is immunodominant with respect to F 256-270 which in turn is immunodominant over F91-105. These findings indicate that the immune response to a combination of highly immunogenic peptides followed a hierarchy of H-2^d-restricted epitopes, but competition during differential processing at any other level between H-2^d-restricted peptides may affect the efficiency of Ag presentation and account for immunodominance. This is important for the

design of vaccines based on the mixtures of peptides. When lymphocytes from MV-immunized mice were restimulated with the MV-F peptides, the responses to the individual peptides were lower. The MV-primed cells recognized essentially a single immunodominant region, F421-445, which largely corresponded to the peptide F421-435. Thus, the above *in vivo* competition between immunogenic peptides appeared to select the same immunodominant peptide as the mechanism following intramuscular immunization with the whole protein. Some of the peptides covering the region F256-305 were capable of inducing MV-reactive memory T cells even though MV did not induce T cells reacting at a significant level with these peptides. After immunization with the synthetic peptide F256-270, a population of specific T cells is generated which seems to be able to respond to the low level of F256-270-like peptides on MV processing APCs. It is stipulated that naïve T cells and Ag-primed cells respond differently to low levels of MHC-presented immunogenic peptides. It also means that TCEs other than the immunodominant epitope are able to induce an MV-specific T cell response. Several strong T cell epitopes were found including a major cluster of H-2^d-restricted peptides between residues 256 and 305. Some of these peptides including peptide F421-435 and F256-270 induced MV-specific T lymphocytes *in vivo* while other H-2^d-restricted epitopes did not. F256-270 and F261-275 are the only peptides predicted to contain the Sette IE^d motif.

Genetically engineered F proteins and nested sets of synthetic peptides spanning the protein were used to determine sequences of F recognized by a number of F-specific human HLA class I and class II-restricted CTL clones (Van Binnendijk *et al.*, 1993). Combined N- and C-terminal deletions of the respective peptides revealed that human HLA class I and HLA class II-restricted CTL efficiently recognize nonapeptides or decapeptides representing epitopes of F. Three distinct sequences recognized by three different HLA class II-restricted CTL clones appear to cluster between residues 379 and 466 of F, thus defining an important TCE area of F. These are DQw1-restricted peptide ⁴²⁷EVNGVTIQV⁴³⁵, DR4/w53-restricted peptide ⁴⁵⁴PISLERLDVG⁴⁶³ and DR2-restricted peptide ³⁸²FILSQGNLI³⁹⁰. In addition, HLA-DR1 binding motif ²⁸⁸LSEIKGVIVHRLEGV³⁰² as well as HLA-B27-binding peptide motif ⁴³⁸RRYPDAVYL⁴⁴⁶ were identified in F (Van Binnendijk *et al.*, 1993). The sequence consisting of residues F379-463 contains a cluster of epitopes recognized by CD4⁺ and CD8⁺ CTL in association with different class II and class I molecules, respectively as well as three TCEs of class II binders which have common features like hydrophobic residues at positions 1 and/or 2 and at position 9. Deletion analysis revealed that ISLERLDVGT sensitized targets for killing at 10-fold concentration by the CTL clone (29) whereas ISLERLDVG abolished the capacity to be recognized by clone 3-F94. HLA-DR binding motif PISLERLDVG contains hydrophobic residues at positions 2 and 9 and a non-charged residue at position 7. DR-2 binding motif FILSQGNLI contains hydrophobic residues at positions 1, 2, 8 and 9, Gln at position 5 and a small residue at position 3. No binding study with DR2 motif was presented here. HLA-B27 motif RRYPDAVYL contains an Arg anchor at position 2, another

Arg at position 1, a tyrosine at position 3, and a small hydrophobic residue at position 6 (Van Binnendijk *et al.*, 1993).

Sera obtained after immunization with the TTB peptide containing the MV-TCE F421-435 protected mice against a lethal challenge with a neuro-adapted MV-strain (El Kashmi *et al.*, 1999). Intrathecal immunization with the MV-F₁₁₁₃₋₁₃₁ and Rouse sarcoma virus (RSV) TCE (F81-95) chimeric peptide without adjuvant in BALB/c mice was assessed for RSV-specific CTL response and was correlated with reduction in viral load in the lungs (Hsu *et al.*, 1998). The Ab response to MV-F spanning residues F397-420 (BCE) in BALB/c, CBA and C57Bl/6 mice emphasized that the size and mode of peptide presentation (*e.g.*, solid-phase Ag and fluid-phase Ag) affected their antigenicity (Partidos *et al.*, 1999). Results from indirect ELISA using 15-mer peptides (overlapping by one residue) as solid phase Ags have shown that anti-peptide Abs from CBA and C57Bl/6 mice recognized the same BCEs located within the F400-417 region. When the 15-mer peptides were used as fluid-phase Ags in an inhibition ELISA, peptide F405-419 was the most effective inhibitor in all three strains of mice. Analysis of serum samples by SPOTs ELISA has shown that the region F407-417 was predominantly recognized by BALB/c mice, whereas Abs from C57Bl/6 mice recognized the F408-420 region. No reactivity was observed with the serum of CBA mice. The identified boundaries of these BCEs differed irrespective of overlapping sequence by the three methods. The α -glucosidase inhibitor castanospermine (CSP) reduced the detection of the MV-F protein by certain MAbs that appeared to recognize non-linear epitopes, suggesting that CSP induces aberrant folding of peptides that influences their function and antigenicity (Bolt *et al.*, 1999).

When administered to mice without adjuvant, a chimeric peptide consisting of the fusion peptide F(FAGVVLAGAALGVAAAAQI) from measles protein linked at the C-terminus of a cytotoxic T cell epitope from the respiratory syncytial virus (RSV) M2 protein (81ESYIGSINNITKQSA95) efficiently primes for an MHC-class I-restricted CTL response. By microspectrofluometry, the fusion peptide moiety was shown to bind to the plasma membrane of living cells. When the fusion peptide was linked to the C-terminus of the CTL epitope, the chimeric peptide M2-F (ESYIGSINNITKQSAFAGVVLAGAALGVAAAAQI) adopted a marked β -sheet conformation. In contrast, when the fusion peptide adopted an α -helical conformation in the presence of trifluoroethanol. The immunogenicity of the two chimeric peptides to class I-restricted CTL, was also significantly different, the one adopting the α -helical conformation being more immunogenic. Probably due to its obvious conversion to an α -helical conformation, the F-M2 peptide could have a higher propensity to insert into membranes, as shown by microspectrofluometry, with a resultant better immunogenicity than the M2-F peptide (D Lelivre *et al.*, 1997, Partidos *et al.*, 1997, Yang *et al.*, 1997).

ISCOM-Based Vaccination with MV-F

F incorporated in immune-stimulating complexes (ISCOMS) is able to sensitize targets for class II as well as class I-restricted killing by CTL (Fournier *et al.*, 1997). ISCOMS containing viral glycoproteins induce virus-neutralizing response and protection, and can prime for class I-restricted CTL. This shows the potential of an ISCOM-based vaccine containing F in addition to other structural proteins of MV (Cardoso *et al.*, 1996, Torres *et al.*, 1999). ISCOMS of MV also stimulate the formation of Ab to MV-F, which is considered to be poorly immunogenic in tested animals (Norriby *et al.*, 1975, Merz *et al.*, 1980). More recently, while comparing the efficacy of VV-F (MV) or VV-H(MV) vaccination to that of ISCOM-based vaccination in macaques with passively transferred virus neutralizing macaques Ab, the potency of ISCOM-based MV vaccine has been emphasized (Osterhaus *et al.*, 1999). These sub-unit vaccines induced both IgG and IgM in serum including MV-neutralizing Ab in the presence of maternal Ab. Although early life immunization with live attenuated measles virus led to preferential Th2 polarization of T cells compared with adult primed animals, it allowed the induction of CTL responses which had not been observed following immunization with a live recombinant canary pox vector. Thus, conversely to a non-replicating canary pox recombinant vaccine expressing MV-HA, viral vaccines with limited but persistent replication capacity appear capable of activating neonatal APCs to trigger Th1 and CTL responses, as recently observed for DNA vaccines (Siegrist *et al.*, 1999) in mice immunized by i.m. and s.c. injections. The liposomal technology for preparing ISCOM has been helpful in obtaining a complex measles preparation consisting of the structural proteins of MV in the bilayer phospholipid membrane. Immunization with the resultant preparation, induced anti-MV Abs of anti-haemagglutinins and anti-hemolysins, whose biological activities were confirmed in the neutralization test (Mamaeva *et al.*, 1998). MV-sub-unit-ISCOM preparation has a potential for use as a vaccine vehicle but has not yet been claimed to be successful (Hsu *et al.*, 1996, deVries *et al.*, 1988, van Binnendijk *et al.*, 1992). ISCOM-based MV-H preparation was also tested in mice (Varsanely *et al.*, 1987).

Epitope Mapping for MV-H

The sequence H379-410 of MV-H protein forms a surface-exposed loop, contains three Cys-residues (Cys-381, Cys-386 and Cys-394) which are conserved among all MV isolates and comprises the minimal sequential BCE H386-400 HNE (haemagglutinin noose epitope) of the neutralizing and protective MAb BH6 that neutralizes all viruses tested (El Kashmi *et al.*, 2000). Peptides containing one or two copies of TCEs and BCEs of different lengths (H386-400, B(CC); H379-400, B(CCC)), in different combinations and orientations were produced and optimized for inducing neutralizing Abs. Peptides with shorter BCE induced sera that cross-reacted with MV but did not neutralize. The longer BCE containing the three cysteins (B(CCC)) and two homologous TCEs induced sera that neutralized wt strains of different clades and geographic origins. Neutralizing serum was also obtained after immunization

with human promiscuous TCEs that mimic HNE loop. Furthermore, B(CCC)-based peptides were fully immunogenic even in the presence of pre-existing MV-specific Abs. The neutralizing and protective MAb BH47 defines the sequential epitope H236-255 NE (neutralizing epitope) of MV-H (El Kashmi *et al.*, 1999). This study design included peptides combining BCE and TCE. Most TTB peptides based on the 15-mer BCE H236-250 induced MV-cross-reactive Abs, but only certain TCE induced virus neutralizing Abs. The shortest BCE required for MV-reactivity and MV-neutralizing activity was the 8-mer H243-250 containing residue R243 implicated in downregulation of CD46. Sera obtained after immunization with the TTB peptide containing the MV-derived TCE F421-435 protected mice against a lethal challenge with a neuro-adapted MV strain and is fully immunogenic, even in the presence of protective levels of pre-existing MV-specific Abs, suggesting that sub-unit vaccines based on such peptides could potentially be used to immunize infants in the presence of maternal Abs. It is interesting that neutralizing Abs were also obtained with a human promiscuous TCE (H830). These results emphasize the need to test sera induced with epitope-based vaccines against different virus strains, in particular if the epitope is not fully conserved. After immunization with MV, several MAbs reacting with peptides MV-H361-410 were obtained (Zeigler *et al.*, 1996). Three of these Mabs (BH6, BH21 and BH216) inhibited haemagglutination, neutralized MV *in vitro* and protected animals from a lethal challenge of rodent-adapted neurotropic MV. These MAbs reacted with the 15-mer peptides H381-386 defining their overlapping region H386-395 as a sequential neutralizing and protective epitope, which can be imitated by a short peptide H381-386 sharing Cys-residues (C³⁸⁶KGKIQALC³⁹⁴ENPEWA). For optimal MAb binding peptide (or MV) disulphide bonds were required in addition to a linear C-terminal extension. Other MAbs bound to peptides C-terminal (BH147, BH195) and N-terminal (BH168, BH171) adjacent to the loop but did not neutralize or protect. When sera from MV patients or from women of child-bearing age were tested with these peptides corresponding to this haemagglutinating and neutralizing epitope(HNE), none of the sera recognized the 15-mer peptides of this region, while some reactivity was found to 30-mer homologous to different wt mutants. Its lack of recognition by maternal Abs and its high degree of conservation would make the HNE loop an attractive candidate sub-unit vaccine for administration in early childhood, independent of immune status (Zeigler *et al.*, 1996). The putative H-epitopes have been implicated in mouse model (Hu *et al.*, 1993; Hu and Norby, 1994; Obeid *et al.*, 1994; El Kashmi *et al.*, 1998).

The induction of MV-cross-reactive antibodies did not correlate with the antigenicity of the peptides (H236-256). The best MV-cross-reactive antibodies were obtained with TB oriented constructs containing TCEs of the MV fusion(F) protein and BCE (H236-250)(TB/15-mer) or H236-255(TB/20-mer). *In vitro*-virus neutralizing sera were obtained solely with the latter construct. A glycine scan showed that binding to depended on a defined pattern of contact residues compatible with the putative alpha helical nature of this epitope (see Tables 3 and 4).

The contact residues of the neutralizing

Table 2. DNA immunization for Ab production

DNA	Vector	Model for Ab production	References
H21-1874 (also H)	pJW4303(CMV promoter)	ANT+ in BALB/c mice and NZW rabbit	Yang <i>et al.</i> , 1997
F(570-2222)	pJW4303(CMV promoter)	ANT+ in BALB/c mice and NZW rabbit	Yang <i>et al.</i> , 1997
MV-H (also sH) and MV-NP	pVIJ	ANT+ and CTL (class I-restricted)- responses in P815(H-2 ^d)	Cardoso <i>et al.</i> , 1996
MV-H, MV-N, N281-289 (Tyr-Pro-Ala-Leu-Gly-Leu-His-Glu-Phe)	Vaccinia virus	ANT+ and CTL in H-2 ^d and H-2 ^k mice	Krstenansky <i>et al.</i> , 1989
Mv-H, MV-F	Vaccinia virus	ANT+ in BALB/c mice, CD4+ CTL for F	Wild <i>et al.</i> , 1993
MV-N, MV-M	Vaccinia virus	PMACC	Wild <i>et al.</i> , 1993
MV-N	Vaccinia virus	PMALC, CD4+ CTL	Wild <i>et al.</i> , 1993
h. MV-N, MV-H, MV-F	Vaccinia virus	CTL responses (H-2 ^k >H-2 ^b >H-2 ^d)	Niewiesk <i>et al.</i> , 1993

Ab, Antibody response; CTL, cytotoxic T lymphocytes; ANT, virus-neutralizing Antibody; PMALC, protection of mice against lethal challenge; PMACC, protection of mice against CDV challenge

serum(S244EL-QL249) differed from those of the non-neutralizing serum(S244EL246) but no unique differences in the immunoglobulin subclasses were detected. Surface plasmon resonance measurements detected a higher for the neutralizing serum compared to the TB/15-mer serum. These results emphasize the need of an optimal design of immunogenic peptides which cannot always be guided by the antigenicity of the peptide constructs. Thus the neutralizing antibodies can be generated with peptides mimicking the helical epitope, provided that the critical contact residues are recognized with high affinity and underlines the potential of the epitope as an element reactivity was tested in BT and TB polarity with two different TCEs, reactivity against the homologous peptide was similar, but the BCE and MV-reactivity was better in the TB constructs but not for all. The desparate binding of the BT induced sera to the two BCE reporter peptides H236-250 and H241-255 suggests that the immune response is directed preferably towards the free peptide in the BT constructs (Table 3). This also explains the loss of MV-specific immunogenicity by C-terminal elongation, but not the reactivity of the 20-mer BCE. The immunogenicity of the C- and N-termini of proteins has been well documented (in influenza virus, coxsackievirus) and preferential reactivity of antisera to the C-terminal part of chimeric peptides is evident from studies with synthetic peptides (El Kashmi *et al.*, 1998).

Mimotopes of H

More vaccine candidacy of MV-H protein has been described under DNA immunization.

H236-255 propeller-like epitope corresponds to the neutralizing and protective MAb BH129 and includes Arg243, implicated in CD46-downregulation (Bartz *et al.*, 1996; Lecouturier *et al.*, 1996) and Arg253 (putative

enzymatic site)(Langedijk *et al.*, 1997). Fine mapping by truncation, elongation, Gly- and Ala-substitution analogues defined EL-QI as the critical residues of the minimal epitope S₂₄₄ELSQL₂₄₉. CD spectra of peptides, comparison of 3D structure of homologous sequences, and prediction algorithms suggested a helical structure with the contact residues E₂₄₅L-QL₂₄₉ located on the protein surface) in a variety of different molecular environments. Mimotopes obtained with a 6-mer phage display library contained a consensus Pro (important for binding) instead of Ser247 of the wild-type sequence (irrelevant for binding). The kink induced by Pro appeared to be essential to bring the 4 contact-residues in the mimotopes and in the corresponding short peptides together. The non-helical conformations of the phage insert and of the peptides may favorably mimic the antigenic helical turns of the wt-type sequence, resulting in an upto 135 times higher antigenicity of the Mab towards the mimotope peptides (Deroo *et al.*, 1998). The wt sequence SKRSELSQLSMY was confirmed as the epitope of BH129. The contact residues EL-QL defined by Ala-or Gly-scans is to be located on the same side (*i.e.* within less than 18 degrees) of two consecutive helical turns. Since these residues interact with Ab, these must be accessible on the protein surface, while Ser247 faces the protein-core and is, therefore directly involved in Mab binding. According to this model, 3 serines (in positions 244, 247 and 250) are masked in the protein. If not attached by complementary residues or water molecules, these would be strongly destabilizing. The interactions with the Ab binding site can promote helix folding of the shorter peptides (Leder *et al.*, 1995; Verdaguer *et al.*, 1996). A list of DNA immunizations tested is presented in Table 2.

However, in the absence of Ab, a small peptide is unlikely to display -helix (Krstenansky *et al.*, 1989) and other configurations may dominate the immune response against such a peptide and lower Ab cross-reactivity with

the protein. By stabilizing the helix can overcome this problem (Yang *et al.*, 1997). Alternatively, mimotopes may efficiently mimic complex conformations. None of the peptide analogues corresponding to a known mutation in this epitope competed better than the wt sequence. Pro is a helix breaker in position *i+3* and favorably replaces Ser247, inducing an intrinsic turn that bends Gln248-Leu249 back towards the Ab binding site. In contrast to wt-type Ser247, Pro is implicated to be necessary for Ab binding to the mimotopes (LYMPQLS, SEMPQLP) with Alascan. Stabilization by Pro in the *i+2* position may favor the mimotope sequence to form a single loop of helix or a -turn, although Gln residue was selected in all phages including LYMPQLS9 flanked by viral sequences with -propensity. The region MV-H236-256 (K₂₅₆PNLSSKRSELSQLSMYRVFE₂₅₆) corresponds to a loop connecting strand 2 with strand 3 on loop 23 of -sheet 1 which protrudes towards the centre of symmetry of the homotetramer (Fournier *et al.*, 1997). Abs directed against this region could therefore destabilize this tetramer, which in paramyxoviruses may be relevant for fusion (Beauverger *et al.*, 1997). It is flanked by the amino acid Arg243 critical for CD46 downregulation (Bartz *et al.*, 1996; Lecouturier *et al.*, 1996) and by Arg253. If the helix extends to this residue, it would be indeed exposed on the accessible face of the helix, whereas the inner face will cover all the conserved Ser residues, although the precise role of the helix has not been defined.

Sub-Unit Vaccine Candidacy of MV-N

Recombinant MV-N and synthetic peptides spanning the length of the N-protein-coding region were used with Ab-binding and a proliferation assay to identify human TCEs in vaccinated and naturally infected adults (Hickman *et al.*, 1997). A number of epitopes were mapped to specific regions of MV-N. To assess baculovirus-expressed MV-N-specific T cells in HLA-typed PBMC, the proliferative response of two donors (donors A and E) was mediated by CD4⁺ class II-restricted T cells in association with HLA DR Ags. Over 70% of all donors tested responded to peptides representing N271-290, N367-386, N400-420, and N483-502, suggesting that these peptides may be broadly recognized within an HLA diverse population. 8 HLA DR and 5 HLA DQ alleles as well as a skewed representation of DR13 in the vaccinated group could be interpreted. The most frequently recognized TCEs (recognized by a variety of MHC class II molecules) in both naturally infected and vaccinated donors were located in the genetically heterogeneous C-terminal of the MV-N. Analysis of patterns of peptide reactivity among vaccinated and naturally infected subjects identified several regions of potential difference between these two groups. Peptides N221-240 and N237-257 were recognized among 100% of naturally infected donors but among only 37.5% of vaccinees and therefore may be of further interest in studies to investigate induction of lifelong versus transient immunity to measles. Six of eight vaccinees with no single MHC class II Ag in common responded to peptide N271-290 while five of eight recently vaccinated donors with no single MHC class II Ag in common responded to peptides N1-17, N51-70, N400-420, N417-436 and N509-525, suggesting

a broad recognition within an HLA diverse population. Most reactive epitopes lie in the C-terminal half of the N-protein molecule. In a proliferative response assay with reference to MV-N peptides, six naturally infected donors responded to peptides N221-241, N237-256, N367-388, N400-420, and N483-502, while five of six donors responded to peptides N202-220, N271-290, N287-303, N335-354, and N433-452. None of the naturally infected donors had proliferative responses to peptide N1-17, whereas five of eight vaccinated donors had significant response. 100% of naturally infected donors had responses to peptides N221-240 and N237-256 while 37.5% of vaccinated donors had responses to these peptides. In donor A, peptides N83-102, N271-290 and N509-525 were specifically inhibited by Ab specific for HLA DR, suggesting HLA DR binding motif in these peptides are presentable to T cells of donor A. No specific inhibition was seen with Ab to HLA DQ, HLA A, B, or C. About 20% specific inhibition was seen with Ab to HLA DR, DP and DQ, presumably due to Ab directed against HLA DR. In donor E, peptides N51-70 and N400-420 were specifically inhibited by Ab to HLA DR, suggesting DR molecule-mediated presentation of these peptides. Presentation of peptide 167-184 is not clear. Immunosurveillance of HIV variants was explained by escaping recognition by human CTL for evasion of immune response (Phillips *et al.* 1991). Similarly, lack of immune recognition of critical wt MV TCEs in vaccinees may help explaining the susceptibility of some vaccinee individuals to reinfection with MV and, indeed, the greatest degree of amino acid variability among wt MV variants was shown to be within the C-terminal 125 amino acid residues of N spanning the most immunogenic T cell determinants. Three CTL epitope HLA-A2.1 binding motifs were identified in the peptides N210-218, N226-234 and N340-348 as being capable of inducing MV-specific CTL in PBMC from measles seropositive individuals (Nannan *et al.*, 1995).

These peptides were strong proliferative epitopes among naturally infected donors than vaccinees (Hickman *et al.*, 1997). In murine model, aa sequence N67-98 exhibited a major proliferative epitope in both H-2^d and H-2^k mice, whereas aa sequence N457-525 proved to be another epitope in H-2^d mice (Giraudon *et al.*, 1991). Both of these regions were recognized by a number of vaccinated and naturally infected donors (Hickman *et al.*, 1997). Two CTL epitopes, N52-59 and N81-88 in H-2^k mice (Beauverger *et al.*, 1993) as well as another CTL epitope N281-289 in H-2^k mice (which was shown to sensitize P815 cells to CTL lysis to the same level as cells transfected with measles N alone) were found to be stimulatory in another study by utilizing the peptides corresponding to the similar regions N83-102 and N 271-290 (Hickman *et al.*, 1997).

Three BCEs viz. N-terminal N122-150 as well as C-terminal N457-476 and N519-525 were identified (Giraudon *et al.*, 1988, 1991; Buckland *et al.*, 1989). The reactivity of the Abs defining these sites varies with the virus isolate. Recognition of several peptides sharing overlapping epitopes sequences by individuals with no single MHC class II Ag in common suggest binding by disparate MHC class II molecules which were described in other systems (cf. Hickman *et al.*, 1994).

Table 3. Putative BCE and TCE of MV

S. No.	Sequence and T-cell restriction	Epitope	Specification of Ab	Reference
1.	F240-258 (GDINKVLEKLGYSGLLG)	BCE/TCE(Th)	IA ⁺ in H-2 ^d and H-2 ^k mice; cross-reactive CTL in H-2 ^k mice	Partidos and Steward, 1992
2.	F113-127 (KRFAGVVLGAALGV)	BCE	BI ⁺	Muler <i>et al.</i> , 1993
3.	F115-134 (FAGVVLGAALGVATAAQIV)	BCE	BI ⁺ , AHLI ⁺	Muler <i>et al.</i> , 1993
4.	F388-402 (ANCAGILCKCYTTGGT)	BCE	ANT ⁺⁺ in H-2 ^d and H-2 ^k mice	Atabani <i>et al.</i> , 1997
5.	F288-302 (LSEIKGVIVHRLEGV)	HLA-DR1(Th TCE)	IA ⁺ in H-2 ^d and H-2 ^k mice; recognised by Lymphocytes from H-2 disparate haplotype (different IA molecules)	Partidos <i>et al.</i> , 1990; 1992; Van Binnendijk <i>et al.</i> , 1993
6.	F427-435(EVNGVTIQV)	HLA-DQw1	MHC-class II-restricted (human)	Van Binnendijk <i>et al.</i> , 1993; El Kashmi <i>et al.</i> , 1999
7.	F454-463(PISLERLDVGT)	HLA-DR4/w53	MHC-class II-restricted (human)	Van Binnendijk <i>et al.</i> , 1993
8.	F382-390(FILSQGNLI)	HLA-DR2	MHC-class I-restricted (human)	Van Binnendijk <i>et al.</i> , 1993
9.	F438-446(RRYPDAVYL)	HLA-B27	MHC-class II-restricted, H-2 ^d (human)	Partidos <i>et al.</i> , 1990
10.	F404-414(INQDPDKILTY)	BCE	IA ⁺ in H-2 ^d mice	Muller <i>et al.</i> , 1995
11.	F406-420	ND	MHC-class II-restricted, H-2 ^d	Muller <i>et al.</i> , 1995
12.	F91-105	ND	MHC-class II-restricted, H-2 ^d	Muller <i>et al.</i> , 1995
13.	F256-270 (LLGILESRIKARIT) and F261-276	Sette IEd motif and DR1(TCE)	MHC-class II-restricted, H-2 ^d	Muller <i>et al.</i> , 1995
14.	F400-417 (yTTGIINQDPKILTYI) F407-417(NQDPKILTYI)	BCE	IA ⁺ in BALB/c mice	Partidos <i>et al.</i> , 1999
15.	F397-420 (CYTTGTINQDPDKI LTYIAAD HC) and F400-414	TCE		Muller <i>et al.</i> , 1995
16.	F408-420(QDPKILTYIAaD)	TCE	C57BL/6(H-2 ^d)	Partidos <i>et al.</i> , 1999
17.	NIIRTKKQ(cf.F153-184)	Mimotope	ANT ⁺⁺ in H-2 ^d and H-2 ^k mice	Partidos <i>et al.</i> , 1999
18.	H188-199(CSGPTTIRGQFSY)	BCE	ANT ⁺	Partidos <i>et al.</i> , 1999
19.	H386-400 (CKGKIQCENPEWA)	BCE	ANT ⁺	Partidos <i>et al.</i> , 1990
20.	H236-255(NE)KPNLSSKRSE LSQLSMYRVFE)	BCE	ANT ⁺	El Kashmi <i>et al.</i> , 1999
21.	N122-150 (SRfCWFenKEIS DIEVDGPEGfmMILGTI)	BCE	IA ⁺	El Kashmi <i>et al.</i> , 1999
22.	N457-476 (ipTgtptdICTasEssq DpGSSrRSAdaLL)	BCE	IA ⁺	Giraudon <i>et al.</i> , 1991; Buckland <i>et al.</i> , 1989
23.	N519-525	BCE	IA ⁺	Giraudon <i>et al.</i> , 1991; Buckland <i>et al.</i> , 1989
24.	N281-289(NLYQQMGet)	Ld-restricted TCE	CD8 ⁺ CTL in H-2 ^k and H-2 ^k mice	Giraudon <i>et al.</i> , 1991; Buckland <i>et al.</i> , 1989
25.	N52-59(LDRLYRII), N81-88(VESPGQtl)	TCE	CTL in H-2 ^k mice	Beauverger <i>et al.</i> , 1993
26.	N210-218(RRFMYALIL) N226-234(NKPRIAEMI) N340-348(AYFrLGQEM)	HLA-A2.1	ICM ⁺ (human)	Beauverger <i>et al.</i> , 1993
27.	N83-102 (PGQLIQRITDDPDVSI RLL) N271-290 (GELSTLES LM NLYQQMGetA) N509-525, N51-70 (LLDRLYRIICnPDvS GPKLT) N400-420(inGQsENE LPrIGgKEDrRvK)	HLA-DR	human	Hickman <i>et al.</i> , 1997; Nannan <i>et al.</i> , 1995; Giraudon <i>et al.</i> , 1991
28.	N335-344(YAMGVGVELE)	TCE	H-2 ^d	Steward <i>et al.</i> , 1995
29.	N381-391(ASELGITAEDA)	TCE	H-2 ^d	Steward <i>et al.</i> , 1995
30.	N367-386 (TAEDARLVSEIA MNItEDrI) N483-502 (GISEEQSDtprVYNQrDL)	TCE	Recognised by CD4 ⁺ T cells (human diverse HLA population)	Hickman <i>et al.</i> , 1997
31.	N67-98	ND	H-2 ^d , H-2 ^k Proliferative Response in murine system	Hickman <i>et al.</i> , 1997; Giraudon <i>et al.</i> , 1991; Buckland <i>et al.</i> , 1989
32.	N457-525	ND	H-2 ^d , Proliferative response in murine SYSTEM	Hickman <i>et al.</i> , 1997; Giraudon <i>et al.</i> , 1991
33.	N221-241, N237256, N367-388, N400-420, N202-220, N271-290, N287-303, N335-420	TCE	Proliferative response in human, bind to multiple MHC-class II molecules	Hickman <i>et al.</i> , 1997

IA, induce Ab; BI, bind to Ab; AHLI, haemagglutination positive Ab; AHLI, haemolysin positive Ab; ANT, neutralizing Ab; ND, not defined; I, determined by lymphocyte proliferative assay; ICM, induce CTL (MV-specific) in human PBMC.

Table 4. Functional characteristics of Mabs

S. No.	Epitope	MAb	Functions	References
1.	H381-400	(h)BH6	NT+, HI-, HLI-	Fournier <i>et al.</i> , 1997
2.	H381-400	(h)BH21	NT+, HI-, HLI-	Fournier <i>et al.</i> , 1997; Niewiesk <i>et al.</i> , 1993
3.	H381-400(HNE)	(h)BH216	NT+, HI+, HLI+	Fournier <i>et al.</i> , 1997; Niewiesk <i>et al.</i> , 1993
4.	H244-250 (NE)	(h)BH47	NT+, HI-, HLI+	Fournier <i>et al.</i> , 1997; Niewiesk <i>et al.</i> , 1993
5.	H244-250	(h)BH59	NT+, HI-, HLI+	Fournier <i>et al.</i> , 1997; Niewiesk <i>et al.</i> , 1993
6.	H244-250	(h)BH129	NT+, HI-, HLI+	Fournier <i>et al.</i> , 1997; Niewiesk <i>et al.</i> , 1993
7.	H244-250	(h)BH103	NT+, HI-, HLI+	Fournier <i>et al.</i> , 1997; Niewiesk <i>et al.</i> , 1993
8.	H200-370	(h)I-41	NT+, HI-, HLI-	Niewiesk <i>et al.</i> , 1993; Sheshbaradaran and Payne
9.	H200-370	(h)7-AG11	NT+, HI-, HLI-	Niewiesk <i>et al.</i> , 1993; Sheshbaradaran and Payne
10.	H300-318	(h)I-29	NT+(313/314), HI-, HLI+	Niewiesk <i>et al.</i> , 1993; Sheshbaradaran and Payne
11.	H200-370	(h)I-44	NT+, HI-, HLI+	Niewiesk <i>et al.</i> , 1993; Sheshbaradaran and Payne
12.	H200-370	(h)79-XV-V17	NT+(243)	Tamin <i>et al.</i> , 1994
13.	H233-240	16DE6	NT+(211)	Tamin <i>et al.</i> , 1994; Rota <i>et al.</i> , 1992
14.	H200-291	(h)BH1	NT ^a	Tamin <i>et al.</i> , 1994; Niewiesk <i>et al.</i> , 1993
15.	F(Epitope1)	16-CD-11	NT ^a	Liebert <i>et al.</i> , 1994
16.	F(Epitope1)	(m)M186B19 ^B	NT ^a	Hummert and Bellini, 1995
17.	F(Epitope1)	(m)186A	NT ^a	Hummert and Bellini, 1995
18.	F(Epitope1)	(m)M186B22 ^B	NT ^a	Hummert and Bellini, 1995
19.	F(Epitope1)	(m)M263-5 ^B	NT ^a	Hummert and Bellini, 1995
20.	F(Epitope1)	(m)M263-18 ^B	NT ^a	Hummert and Bellini, 1995
21.	F(Epitope1)	(m)M77-4 ^B	NT ^a	Hummert and Bellini, 1995
22.	F(Epitope1)	(m)27 ^B	NT ^a	Hummert and Bellini, 1995

^a, *in vivo/in vitro*; ^b, passively protect mice

DNA Immunization for Candidate Sub-Unit Vaccine

The isotype of the DNA-raised Abs depended on both the delivery and the form of the expressed Ag. Following intramuscular injections (i.m.), DNAs expressing membrane-bound forms of the MV-H raised predominantly IgG2a, whereas DNAs expressing the secreted form of the MV-H raised IgG1 antibodies. By contrast, gene gun delivery resulted in predominantly IgG1. Ab responses for both secreted and membrane-bound forms of MV-H were present in oligomeric forms that appear in Western blotting under reducing and non-reducing conditions (Torres *et al.*, 1999).

Plasmids (pV1J driven by CMV promoter) encoding either the MV-H or NP proteins inoculated i.m. into BALB/c mice (H-2d) induced both humoral and CTL class I-restricted responses in P815 cells (H-2^d) which expresses only class I MHC molecules. Ab responses were not increased by multiple inoculations. The major Ab isotype induced by both the membrane-bound MV-H and MV-NP was IgG2a, consistent with a Th1 response. In contrast, immunization with a plasmid which directed the synthesis of a particular form of MV-H (pv1J-sol-sH) induced mainly IgG1 Ab typical of Th2 response. When the amount of DNA was reduced for the H-coding plasmid (1 or 10g/ animal), although the Ab was not induced, a CTL response was observed. Even during a 6-week period, there was no correlation between CTL and Ab level for individual animals. A single inoculation-induced Abs persist more than 8 months. When lower quantities of DNA were inoculated, only CTL responses (which did not cross-react between MV-H and MV-N) were specific in P815 cells and did not lyse NK-sensitive YAC targets. Compared to IgG1, IgG2a has the advantage to activate both the class Ical and the alternative pathways of complement fixation. Thus, the Ag presentation may differ substantially to alter the type of

immune response. Similarly, vaccinia virus (VV) vector-expressing MV-H and MV-NP (VV-H and VV-NP) in BALB/c mice induced both humoral and CMI (Langedijk *et al.*, 1997). The DNAs raised titres of neutralizing Ab comparable to those raised by natural infection, and better than those raised by live-attenuated measles vaccines (Leder *et al.*, 1995; Verdaguer *et al.*, 1996; Deroo *et al.*, 1998). Soluble proteins are relatively easy to produce using current recombinant DNA and cell culture technologies. The raising of an IgG1 or IgG2a isotypic predominant response was independent of dose of DNA consistent with the previous study (Felquate *et al.*, 1997).

DNA-mediated immunization has been used to raise neutralizing Abs for MV. Single inoculations of plasmids (5.1Kb pJW4303 driven by CMV promoter for expression) expressing MV-H (H21-1874 and sH189-1874) cDNA and complete MV-F cDNA (comprising coding sequence F570-2222 including 5' non coding sequence) raised neutralizing Abs in BALB/c mice. Neutralization assays were conducted on Vero cells with Edmonston Chicago-1 (Ed Chi1) strain for reduction of plaque formation by 50% or 90% (Englehard, 1994). Plasmids expressing both secreted and membrane-bound forms of MV-H raised neutralizing Abs that persisted for 1 year in mice by two immunizations at 4 week intervals. Sera from pJW4303/H-inoculated mice showed neutralizing titres of 1000-10000 whereas sera from pJW4303/sH-inoculated mice developed lower titres of 100-3000. Both titre values are above the normal value >120 for 50% plaque reduction sufficient for protection (Englehard, 1994). Although single inoculations of the H, F and a combination of F and H expressing DNAs raised Abs, lower neutralizing Abs induced by F also showed poorer persistence than the neutralizing Ab to H. Inoculations with both H and F DNA (which used one half of the H or F DNA used for immunization with a single plasmid) achieved an intermediate titre of neutralizing Ab.

In the singly inoculated animals, Ab responses rose over the first 8 weeks, with the titres of Ab at 8 weeks being 4-10 times higher than the Ab titres at 4 weeks. Interestingly, the boost (individual plasmid or a combination of plasmids) at 4 weeks interval increased the plateau Ab titres achieved by single inoculations. For both forms of H, the effectiveness of the raised Ab (ratio of neutralizing activity to ELISA activity) was similar. A series of inoculations at one month intervals were used to raise polyclonal anti-H and anti-F sera in NZW rabbits. MV-H-induced Ab was rapid and higher than F-induced Ab in mice after 3 immunizations. The neutralizing Ab titre for H fell with time, while the pattern for F-induced antibody titre is not certain.

Vaccinia virus (VV) as a vector for MV-F, MV-N and MV-H demonstrated protection against MV infection in rodents. In a murine model (BALB/c), immunization with either MV-H or MV-F could protect animals against a fatal challenge (Drillen *et al.*, 1988; Wild *et al.*, 1992; Beauverger *et al.*, 1993). The sera from mice immunized with recombinants containing either HA, HA/F, HA/NP, or HA/NP/F had similar levels of virus neutralizing Abs which remained constant throughout 7 month period. Analysis of sera confirmed the presence of specific Ab to each of the Ags. Vaccination of BALB/c (H-2^d) mice with H and F, but not N, recombinants completely protected the animals against a lethal measles challenge. In contrast, although the VV-H recombinant protected CBA (H-2^k) mice, the VV-F recombinant did so poorly. Both N and F expression was more effective in protection in CBA mice. The combination of all Ags in a single recombinant is able to overcome host-related restriction of the immune response to particular Ags.

In murine model, challenging the mice with another morbillivirus, the closely related canine distemper virus (CDV), immunization with vaccinia virus (VV) recombinants expressing the F, N or M (VV-F, VV-N and VV-M) partially protected the animals. In the case of NP and M proteins, Ab was not involved (Beauverger *et al.*, 1993; Englehard, 1994; Fournier *et al.* 1997). Thus, successful vaccination against this type of virus probably involves not only the induction of Abs, but also cell-mediated immunity (CMI). Rats immunized with VV-NP induced class II-specific CTLs which protected against an intracerebral challenge by a rat-adapted MV. In BALB/c mice (H-2^d haplotype), both VV-HA and VV-NP induced CTLs were effective against target cells expressing these Ags in animals infected by CDV in an in-vitro assay (Beauverger *et al.*, 1993; Fournier *et al.* 1997; Yang *et al.*, 1997). Unlike VV-H-specific CTLs, VV-NP-specific ones lysed target cells. In C3H(H-2^k) mice, only the VV-NP-induced CTLs and as in the case of BALB/c mice, there was a cross reactivity with CDV. The predictive motif for the H-2 L^d is a nonamer containing XProXXXXXXPhe (or Met or Leu). In the MV-NP sequence, there are six such motifs of which four appear in the same position in CDV-NP and only two (N281-289 and N329-337) have an identified sequence. Peptide 4 corresponding to N281-289 (Tyr-Pro-Ala-Leu-Gly-His-Glu-Phe) sensitized immune cells. MV proteins include at least one cross-reactive CD8+ epitope (N281-289) and one serotype-specific (HA) epitope in BALB/c mice, whereas the NP induces a cross-reactive NP-specific CD8+ epitope in C3H mice. In C3H mice only the VV-NP can induce a strong CTL response, whereas in BALB/c mice both the NP and

HA do so. The presentation by the MHC molecules of both Ags in the BALB/c mice is known to be L^d-restricted, whereas the N-specific CTL activity in C3H mice is associated with the K^k class I molecule. Partial protection of BALB/c mice against CDV could be obtained after vaccination with VV-recombinant expressing the F, N or M proteins of MV, but not HA. No cross-reacting Abs for any of the MV proteins were observed. Furthermore, the same protection was observed when the animals were challenged 9 days after vaccination suggesting that CMI response may be involved. Eliciting CTL response only avoiding the humoral response(HR) can be an alternative strategy for vaccination. Only a limited number of viral Ags may induce CTL activity and that the majority of the epitopes involved induced cross-reactive CTLs (Beauverger *et al.*, 1993).

BALB/c (H-2^d) mice immunized with VV-recombinants containing either H, H/F, H/N, H/F/N had similar levels of MV neutralizing Abs (specific Ab to each of the Ags where appropriate) which remained constant throughout a 7 month period, and protected mice. Vaccination of BALB/c (H-2^d) mice with H and F as well as CBA mice (H-2^k) with H (and F very poorly) protected mice after lethal challenge, whereas vaccination of both F and N together in CBA mice (H-2^k) did the same. The association of all three components in a single vaccinia virus recombinant allowed to overcome host-related restriction of the immune response to particular Ags. Recombinant vaccinia with MV- H and F (VV-MV-H and VV-MV-F) lysed only weekly VV-MV-H and VV-MV-F-infected target cells and did not kill MV-infected cells at all while VV-MV-M and VV-MV-P did not induce an MV-specific CTL response in mice (Wild *et al.*, 1992). Several vectors were used for vaccination: vaccinia virus(VV), avipox (AV), Bacille Calmette Guerin(BCG), baculovirus containing -galactosidase, fowl po1, canine distemper virus and pox virus(Horikami and Moyer, 1995; Wild and Buckland, 1995; Rima *et al.*, 1995; Borrow and Oldstone, 1995; Griffith, 1995; Schneider-Schaulis *et al.*, 1995; Van Binnendijk *et al.*, 1995; Liebert and Finke, 1995; Norby, 1995; Radecke and Billeter, 1995; Lliashhenko *et al.*, 1999; Schmidt *et al.*, 1992; Wild *et al.*, 1990, 1993; Taylor *et al.*, 1991, 1992).

Discussion

Sub-unit vaccination emphasized on the presentation of the MV-component peptide sequences in an immunogenic form in inactivated vaccine candidate to elicit the recognition and presentation of receptors. Because, CD4+ T lymphocytes which assist in the activation of B lymphocytes and in their differentiation to Ab-producing cells recognize processed fragments of endocytosed protein Ag presented by class II molecules of MHC complex at the surface of APCs. CD8+ CTLs eliminate virus-infected cells, since they express at their cell surface processed fragments of endogenously synthesized viral proteins which associate with class I MHC complex molecules. This aspect has been recently reviewed (Corradin and Demotz, 1997). Ag degradation and defined sequential peptide loading (including MV proteins) to major MHC class I and class II molecules assembled following multiple pathways have been discussed in this review with special emphasizes on

“non-cannonical” pathways, which must be a help in designing vaccine candidate for therapeutic and medicinal purposes. For example, DR1-restricted determinants derived from component F are processed following distinctive pathways, one of them relying on mature DR1 for peptide binding (Demotz and Pelliriaux, 1996). Sequence analysis of peptides eluted from human and murine MHC class II molecules has revealed that such naturally processed peptides range from 13 to 17 aa in length (Partidos and Steward, 1992; Van Binnendijk *et al.*, 1993). In addition, there is a computer-assisted algorithm to identify BCE, TCE and MHC molecular motifs which are discussed above and also discussed in the context of peptide epitope mapping. The evidence so far suggests a preferential association (adjacent or overlapping or pairing in different orientation) of T and B cell epitopes requires a mechanism which allows preferential processing and presentation of TCEs adjacent to the Ab-inducing site (Muller *et al.*, 1995; Wild *et al.*, 1992).

Through differential binding to epitopes of the same Ag, B cells could define the BCE and can direct processing and selection of the peptide fragment-mediated T cell help. B cells are directly involved as APCs, particularly when memory cells have already been established. The persistent memory is dependent on long-term conservation of Ag. Memory exists in adult donors with persisting immunity to the MV. Late convalescent donors are therefore well suited to study pairing of TCE and BCE on a clonal level (Wiesmuller *et al.*, 1992; Muller *et al.*, 1993; Hickman *et al.*, 1997).

In contrast to peptides presented by class I molecules, those binding to class II molecules are very heterogeneous in length (Partidos and Steward, 1990; Partidos *et al.*, 1992). A sequence of a charged amino acid or a glycine followed by two or three hydrophobic residues and a charged or polar residue or more conventionally a cluster of a positively charged aa and three hydrophobic residues in relative positions 1, 4, 5 and 8 corresponds to the postulated motif for Th TCE. In an amphithallic -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 (class I and class II molecules have the same binding sites), and the opposite face would bind to the TCR. Furthermore, it appears that a central core within the sequence is necessary for binding to the MHC whereas the flanking residues mainly make important contacts with the MHC protein or contribute to the ability of the peptide to fold into an appropriate conformation for optimal binding. So, there appears to be a minimal length requirement for T helper cell determinants which can be defined in two ways: the shortest sequence which gives maximum responses (11 to 12 residues) or the sequence which generates any response (7 to 8 residues). For synthetic peptide to act as an effective immunogen, this needs to contain two distinct sites, one BCE to promote B cell interaction and a TCE to induce cognate T helper (Th) cell activity (Wiesmuller *et al.*, 1992; Partidos and Steward, 1990, 1992; Van Binnendijk *et al.*, 1993; Hickman *et al.*, 1997; Wild *et al.*, 1992). The use of TCE from unrelated proteins could also be appropriate but would not provide memory T cell response (Muller *et al.*, 1993). Peptides

eluted from different class II molecules were found to be 13-24 (DR1) or 13-17 (IA^b) residues long. IA^b-peptides contain 16-18 residues comprising 6 residue-motif for IA^b or IE^b-eluted peptides which- when present- were variably located on IA^b-eluted peptides (Partidos and Steward, 1990; Muller *et al.*, 1995; Beauverger *et al.*, 1993). I-A^d and I-A^k molecules bind to very similar structures.

Thus class II binding motifs can be shared by overlapping peptides but their position on the peptides may be favorable enough for primed cells but not for naïve cells to respond (Muller *et al.*, 1995; Hickman *et al.*, 1997). Because immune responses tend to be haplotype-specific, MHC restriction is a potentially limiting factor in vaccine strategies. Ideally, candidate peptide vaccines could consist of BCEs combined with multiple TCEs (helper and cytotoxic) that are recognised by a broad range of MHC allotypes. Furthermore, because vaccine virus is delivered intramuscularly but natural diseases results from infection of the respiratory mucosa, differences in peptide response patterns are attributable to alternate processing of peptides by APC present at the site of inoculation (macrophage vs dendritic cells) (Demotz and Pelliriaux, 1996). Nonetheless, peptides recognized by naturally infected individuals but not by vaccinated donors can become a starting point for investigations into the induction of lifelong versus transient immunity to measles (Muller *et al.*, 1993, 1995; Hickman *et al.*, 1997). Antiviral Abs have been considered to be directed toward conformational epitopes, with the role played by linear BCEs being debatable (Atabani *et al.*, 1997). MAbs (ex. mouse) could function as a tool for identification of peptide mimics (mimotopes mimicking wt peptide motif with or without TCE) of conformational BCEs from screening of a solid-phase and phage-display peptide library. Identified CTLs can be used for TCE mapping of the representative peptides, whereas MAbs to MHC molecules can be used for MHC specificity and mapping. Isolation of B-lymphoblastoid cell lines (BLCL or B cell APC) and CTL clones further specify the TCE in proliferative response. The presence of clonable CD8+ CTL recognizing MV Ag in association with MHC class I molecules in PBMC of healthy adult MV-seropositive individuals (Partidos *et al.*, 1992; Van Binnendijk *et al.*, 1993; Beauverger *et al.*, 1993; van Binnendijk *et al.*, 1990) led to the isolation of clonable memory CD8+ class I MHC-restricted CTL from the stimulation of PBMC of children recovering from acute measles in bulk cultures with B-LCL (APC) infected with MV. In contrast, CD4+ MV-specific T cells were primarily expanded in bulk cultures of PBMC when using UV-irradiated MV (UV-MV) as Ag. Chloroquine, a widely used inhibitor of class II MHC-restricted processing, does not prevent presentation of MV-derived antigens by infected B cells (van Binnendijk, 1989, 1990). It has been proposed that Ags have an access to alternative processing and presentation pathways.

Analysis of processing requirements for Ag presentation to CTL, studied by the effects of chloroquine in proliferative assays, showed that both CD8+ class I MHC-restricted and, in agreement with the results of others, CD4+ class II-MHC-restricted MV-specific CTL recognize viral Ag processed via the endogenous/cytoplasmic pathway in the elimination of MV infection. However, in conclusion, short synthetic peptides representing BCEs and

Th TCEs coupled to a carrier protein has a potential to induce Ab but may not be relevant to vaccine production because of the failure to induce priming of a T cell memory as well as carrier-induced suppression (Muller *et al.*, 1993; Partidos and Steward, 1992; Partidos and Steward, 1990; Partidos *et al.*, 1992; Van Binnendijk *et al.*, 1993; Fournier *et al.*, 1997; Torres *et al.*, 1999; Cardoso *et al.*, 1996; Siegrist *et al.*, 1999; Beauverger *et al.*, 1993; Wild *et al.*, 1992; Steward *et al.*, 1995; Partidos *et al.*, 1992; Obeid *et al.*, 1995). Short synthetic peptides indeed did induce both antipeptide and neutralizing antiviral Abs *in vivo*.

Anti-F MABs prepared by using a vaccine-MV-F recombinant (Vidalin *et al.*, 2000) were not only neutralizing MV-infectivity and inhibited fusion *in vitro* and *in vivo*, but also passively protected mice. These MABs recognize a conformational epitope. In contrast to human sera (Hickman *et al.*, 1997), which did react with N-peptides despite elevated neutralizing Ab titres (Cardoso *et al.*, 1996). This suggests that the neutralizing Abs recognize conformational epitopes of MV-F protein which are not imitated by peptides and that most of the human Abs identified by MV-F short peptides (Muller *et al.*, 1993) are not neutralized. In contrast, Mabs (Bh6, B21 and BH216) which reacted with peptides corresponding to H361-410 regions, inhibited haemagglutination, neutralized MV *in vitro* and protected animals from a lethal challenge, while human sera from MV patients or women of child-bearing age did not react with peptides but did react weakly with 30-mer peptide homologs of this region (Zeigler *et al.*, 1996). The Abs from the convalescent donors may well represent a different level of maturation associated with long-lasting memory. The inability of the MV to elicit proliferative response in mice or rabbits would be another difference between the human and the animal systems which could result in functionally different sets of Abs. Mouse Mabs and sera reacted specifically with MV-F protein and exhibited strong neutralizing activities, but did not bind to any of MV-F short peptides in Western blots suggesting that these MABs recognise the conformational epitopes. The rabbit anti-MV-F sera had a neutralizing titre but essentially no peptide binding was found (Muller *et al.*, 1993). Sera from late convalescent donors were used to compare the Ab-binding pattern of 108 peptides covering the whole MV-F sequences of MV-neutralizing human IgG fractions with known and predicted TCEs. The use of fractionated human IgG permitted only a narrow range of BCEs as found with MV-H protein (Atabani *et al.*, 1997). The influence of MHC class II phenotype on the Ab-binding pattern was also examined. When human sera were reacted with MV-F peptides, the regions of Ab-binding were less well defined (Lliahshenko *et al.*, 1999; Schmidt *et al.*, 1992; Wiesmuller *et al.*, 1992). This only became apparent when fractionated IgG was used in peptide ELISA (Muller *et al.*, 1993; Wiesmuller *et al.*, 1992; Atabani *et al.*, 1997).

Evidence that TCE and BCE may be paired in a random fashion was also found in the MV-H (Muller *et al.*, 1993). Ab-binding of all donors, irrespective of HLA type, was clustered in some defined, narrow regions as would be expected of functionally active Abs. For most DR haplotypes reactivity was clustered in the same dominant regions with, however, detectable differences between disparate haplotypes, e.g. peptides M48 and M97 (specific

for MV-F) were recognized only by both homozygous and heterozygous DR3-positive donors but not by DR3-negative donors. On the other hand, M59 and M47 of MV-F were recognized by several haplotypes but not by DR3 donors. Differences between other haplotypes also appeared to the small numbers of donors. Thus, if the BCEs are adjacent to TCEs, it is expected that the B cell specificity is also MHC class II-restricted or -dependant in a more or less degenerate way (Muller *et al.*, 1993; Wiesmuller *et al.*, 1992; Atabani *et al.*, 1997). When samples of sera raised to each chimeric peptide (F₁404-G₂-288(Y), F₁288/H188, H189/F₁288(Y)) in mice, reactivity was observed only with anti-F₁288/H188(Y) Abs, which had no demonstrable virus-neutralizing activity, whereas peptide F₁404-G₂-288 (Y) containing a Th determinant produced both primary and secondary anti-F₁404-414 without Th determinant peptide response in all tested strains of mice (Partidos *et al.*, 1990). MV-infection to dendritic cells induces TRAIL-mRNA and protein expression, allowing the cells to be cytotoxic via the TRAIL pathway, causing immunosuppression (Vidalin *et al.*, 2000). The sex-associated differences *i.e.* the Ab-dependent cellular cytotoxicity (ADCC) may contribute to the lower survival rate observed in females receiving high titres of measles vaccination (Atabani *et al.*, 2000). Reading frame shift mutation arising from G deletion at 8205 and restoration of original frame by insertion of C at 8235 of MV-H was apparently driven by immune selection (Barren *et al.*, 2000). Most MABs with haemolysis inhibiting activity (HLI+) are F-specific. Human late convalescent sera did not react with peptides representing H200-291 and reactivity of mouse and rabbit hyperimmune sera was variable (cf. Hu and Norby, 1994), whereas MAB 16-CD-11 reacted with H200-291 peptides. Mab BH1 reacted with H233-240 peptides but not with the MV-infected EBV-transformed cells or a eukaryotically expressed H protein. Neutralizing Abs directed against MV-H and MV-F (to a lesser extent) as well as CD4+ T lymphocytes directed against MV-N and MV-F were reported to be protective against MV (cf. Wild *et al.*, 1993).

The viruses considerably differed in terms of their sensitivity to neutralization by MABs (BH30, BH125 and BH129), but 3 selected Abs were sufficient to distinguish between African strains with the exception of B3 cluster 2 viruses (Truong *et al.*, 1999). B3 cluster 1 and 2 MVs differed by 3 aa at positions 240, 283 and 471. The mutations at position 471 (E471>A471 or E471>S471) may abrogate the binding of BH30 since it was specific to B3 cluster 2 viruses only. BH125 neutralized all African MVs except for D4 isolates. The D4 viruses differed from genotype A, B3 and D2 MV by the following mutations T174>A174, T176>A176, L296>F296, G302>R302, S305>A305, I473>V473. Residues R302 has been proposed (Jin *et al.*, 1988) as the binding site of MAb CV5 for the UK genotype III strains. BH129 exclusively neutralized virus isolates from clade A. BH129 has been mapped to a linear epitope H236-255 with E₂₄₅L-QL₂₄₉ being the main contact residues (Favolla *et al.*, 1999). Irrespective of whether viruses of genotypes B3, D2 and D4 had mutations in these regions or not, these could not be neutralized by this Mab. However, one mutation at position 276 reliably distinguished clade A (L276) from all other African wt viruses (F276). The combination of these

3 MAbs allowed for antigenic differentiation between 4 African genotypes and even between different clusters within one genotype. BH30 neutralized genotypes A, B3 (I), D2 and D4, whereas BH125 neutralized genotypes A, B3(I), B3(II) and D2 while BH129 neutralized only genotype A (Table 4).

All wt-type MV strains from the three genotypes found in the UK showed antigenic heterogeneity compared to the vaccine strain, Mor-v (Table B) and sequence variation at several positions between 4 and 577 of MV-H (Jin *et al.*, 1998). Three of the MAbs derived against Chi1/U889 (genotype III related strain), failed to react with strain Mor-v, whereas all MAbs derived from Ed-w and LEC strains reacted well with the vaccine strain. Mab CV2 was specific for the UK genotype III, suggesting that the epitope of Mab CV5 might be located at Arg302 (a charged, hydrophobic aa) in these strains. CV4 and CV7 reacted with epitopes shared between the three UK genotypes. The antigenic sites of MAbs 129 and 144 were identified as Ser313 or Gly 314 and Ser189 respectively, and the reactivities shown by all the strains tested reflected the identical aas at these positions in these strains. Vaccine and wt-type strains were distinguished by Mab V17 with the exception of strain NJ/US894 (UK genotype I). The reactivity patterns of MAbs suggested that each genotype of the current wt-type viruses comprises of some epitopes that were not present on the vaccine strains. However, epitope mappings were not defined in this study. The MV-H-specific MAbs raised against Ed-w (B2 and V17), LEC (129,144 and 112) and Chi1/US89(CV2,CV3,CV5 and CV11) showed mixed reactivity pattern against these MV strains. Passively transformed Abs protect both in humans and animal models against MV infection, even in the absence of a T cell response, and most functional Abs recognize conformational epitopes (Fournier *et al.*, 1997).

Lys-1, an MV virus isolate having 7 aa changes in DNA sequence did not react with anti-F Mab and a single aa change at F73 (RW) abolished the reactivity to anti-F Mab (Fayolla *et al.*, 1999).

In several negative strand viruses, the MV-N gene is the major Ag for CTL response (cf Niewiesk *et al.*, 1993). For rabies and influenza viruses, N-specific T helper cells boosts the Ab response to the other viral Ags (cf. Niewiesk *et al.*, 1993). VV recombinants expressing N or adaptive transfer of the recombinant-induced N-specific CD4+ T lymphocytes also protected rats against MV challenge (Bankamp *et al.*, 1991). The resistance of inbred strains of mice to MV-induced encephalitis correlated with the MHC haplotype and that only resistant mouse strains mounted an effective CTL response to MV (Niewiesk *et al.* 1993). Mice with low susceptibility to MV infection, such as the BALB/c strain (H-2^d), generated CTL, whereas the highly susceptible mice, C3H (H-2^k), CBA (H-2^k) and C57BL/6(H-2^b), revealed very poor CTL responses. MV-induced CTL were usually CD8+, and the generation of these cells was independent of the route of infection or the time of post-infection. CD4+ T cells were generally only weakly lytic. The N protein was the major target Ag for CTL in BALB/c mice, although H was recognized poorly. CTL from C3H and C57BL/6 mice did not lyse MV-infected target cells. However, targets infected with VV recombinants expressing the N (H-2^k) or H (H-2^b) were lysed, but levels of cytotoxicity

were still low. Target cells transfected with single MHC class I genes suggested inefficient Ag presentation of MV proteins (rather than Ag processing) by the MHC molecules of the H-2^k and H-2^b haplotypes. An alternative explanation for haplotype restriction or block is the inability to produce Abs against the appropriate epitopes (Wild *et al.*, 1992), although a booster immunization was reported to augment MV-neutralizing Abs. Mice strains DBA/2(H-2^d), BALB/c (H-2^d), B10D2/N(H-2^d), C57BL/6(H-2^b), BALB/c(H-2^k), C3H(H-2^k), CBA(H-2^k) and BALB/k (H-2^k) were studied for MV-induced encephalitis (Niewiesk *et al.*, 1993). The binding and presentation of a certain peptide epitope is thought to be entirely dependent on the MHC class I molecules which choose the appropriate nonameric peptide from a mixture of peptides of different sizes independently of cell type and species (Cardoso *et al.*, 1990; Mason *et al.*, 1991; Atabani *et al.*, 2000; Barrero *et al.*, 2000). For example, the ability of single L^d molecule transfected into L cells (H-2^k) to present Ag to BALB/c CTL and the non-recognition of P185-D^b cells by C57BL/6 CTL clearly argue against a defective Ag processing or transport of MV proteins. The P185-D^b cells infected with VV-MV-H were lysed by MV-CTL, where MV was not recognized. Lysis was restricted to the D^b molecule, since P185-K^b cells were not recognized. MV-CTL generated from F₁ offspring of BALB/c and C3H crossbreeding mice lysed only weekly MV-infected P815 (H-2^d) cells, not L^d 29(L-2^k) cells, whereas F₁ fibroblasts were lysed by BALB/c (H-2^d) CTL but not by C3H(H-2^k) CTL. These observations suggest the impaired Ag presentation rather than defective Ag processing to be the cause of inefficient generation of CTL especially in H-2^k and H-2^b haplotype-specific mice. However, the susceptibility of inbred strains of mice to MV-induced encephalitis is shown to be correlated strongly with MHC haplotype (H-2^k > H-2^b > H-2^d). The positive effect of increasing length on peptide antigenicity is well documented in H-2^s, H-2^k and H-2^d mice (Partodos and Steward, 1992). H-2^d mice are generally L^d-restricted.

Ag processing can be mimicked chemically by proteolysis (Sheshbaradaran H and Payne, 1988) or enzymatically with proteases (van Noort *et al.*, 1989). The putative peptide transporters (TAP, DM) seem to be able to transport a wide variety of antigenically unrelated peptides into the endoplasmic reticulum (Spies *et al.*, 1990). After transfection of certain MHC class I molecules into mouse cell lines of different inbred strains, the appropriate peptide was generated in each cell lines. Also, mice transgenic for a human MHC class I molecule produce and transport, after influenza virus infection, the same peptide as human cells. Differential Ag processing of MV-specific determinants in the context of MHC class II processing event might have an implication in CTL response.

Further, a haplotype restriction on the immune response of MV-F protein can be overcome by the incorporation of MV-N gene in the recombinant. Since F protein accumulation at the membrane leads to cell-to-cell fusion aiding the transmission of virus in the late stages of infection, anti-F Abs was targeted for sub-unit vaccination because of atypical measles resulting from formaldehyde-inactivated MV. In contrast, sera from naturally infected children contain anti-F Abs with the desired properties (Sato *et al.*, 1991). Murine MAbs with similar properties, *i.e.*

neutralizing and anti-fusion activity, could be obtained by vaccinating mice with the VV-recombinant encoding the F-protein, but not with the purified MV, suggesting the suitable form of the Ag presentation in inducing the correct immune response. VV-recombinants expressing Japanese encephalitis virus proteins assembled into extracellular particles had the ability to induce high levels of neutralizing Abs (Mason *et al.*, 1991). Therefore, a recombinant construct that can form extracellular particles of MV can evoke a possibility in order to boost the neutralizing Ab level. VV-MV-N induced only a CTL response to MV. Co-immunization of mimotopes (NIIRTKKQ, and TRRAGPMQ) mimic MV-F153-181 and MV-F 257-277(Th TCE) and in BALB/c (H-2^d) and SWR (H-2^k) induced Abs which had highest affinities for their homologous peptides, but only anti-mimotopes (NIIRTKKQ) Abs reacted with MV and had MV plaque inhibition reactivity. This MV-F153-181 is 91% identical with CDVF145-210 (Steward *et al.*, 1995). Co-immunization of this mimotope (NIIRTKKQ) with Th TCE (F257-277) in mice conferred protection against fatal encephalitis induced following challenges with MV and with the structurally related CDV. As compared to the mimotope-induced polyclonal Ab capable of virus neutralization, the original detecting Ab was devoid of virus neutralization. Protection against morbillivirus-induced encephalitis was obtained by immunization with a rationally designed synthetic peptide vaccine containing B- and T cell epitopes from the MV-F (Obeid *et al.*, 1995).

Epitopes (TCE/BCE) inducing Ab in mice does not necessarily imply the ability of this epitope to induce neutralizing Ab production in humans, but a closer match could be effective because of haplotype restriction or block. Immunization with mimotopes of conformational epitopes has a potential to induce protective Ab response, which has been verified in mouse model. Mimotopes of structurally related epitopes may not necessarily be the wt-type sequence but consist of some wt-type core sequences which attribute to the correct conformation and can be recognized by the immune system in the same way as the original epitope. Mimotopes can be generated from solid phase combinatorial peptide library or phage-display library and then verified by binding to Mab raised against the conformational epitope (usually a wt sequence). Combinatorial synthetic peptide libraries, in which peptides are expressed on either the surface of filamentous bacteriophages or an insoluble solid phase, have been used to generate vast numbers of different peptides for biological screening both *in vivo* and *in vitro* for diagnostic, therapeutic and new vaccine strategies. This offers several advantages over the conventional vaccine strategies in terms of safety, cost, stability, and relative ease of production. In addition, peptides can be designed to include BCE and TCE in correct orientation to stimulate desired immune responses (*e.g.*, Th cells, B cells and T cells) while avoiding structures that stimulate unwanted responses (*e.g.* immune suppression). A significant proportion of humoral Abs are directed towards the conformational epitopes (rather than linear antigenic epitopes). Thus, the identification of peptide structures mimicking linear epitopes from the primary aa sequence of a particular protein should be relatively straight forward. The verification of mimotopes can be performed for *in-vitro* analysis by sera of late

convalescent MV donors (both seropositive and seronegative) as well as vaccinated and naturally infected donors, and the *in-vivo/ex-vivo* experimental set-up preferably with a choice for animal model (transgenic for MHC molecule) or cell system (transfected with MHC molecule). Sometimes, mimotopes as an immunogen can induce more efficient virus neutralizing Ab (or polyclonal sera) than the original wt-type sequence (Steward *et al.*, 1995; Obeid *et al.*, 1995; Fayolla *et al.*, 1999).

MV infection causes depletion of activated T cells from PBMC. MV infection interferes with lymphocyte trafficking and reallocation. Disruption of recirculation and random homing of lymphocytes might contribute to the immunosuppression (Nanan *et al.*, 1999). Natural MV infection as well as vaccination with attenuated MV induce immunosuppression, which is responsible for part of the morbidity and mortality associated with measles. T cell activation stimulated with MV Ags and HI Ab titres persisted for almost 30 years in the vaccinated group, implicating that CMI persists for long periods after vaccination and is not influenced by antigenic drift (Toyoda *et al.*, 1999). Skewing of the T cell receptor repertoire of CD8+ T cells has been shown in some persistent infections with viruses (*e.g.* HIV, SIV and EBV) as well as non-persistent infection with viruses (*e.g.* MV). The expanded lymphocyte populations were monoclonal or oligoclonal and lysed target cells infected with recombinant VV-MV protein (Mongkolsapaya *et al.*, 1999). Recent investigation has focussed on cotton rats (*Sigmodon hispidus*) as an alternate animal model to study the pathogenesis of MV infection (Niewiesk, 1999; Wyde *et al.*, 1999; Mevioner *et al.*, 2000). The role of N-linked oligosaccharide chains and 5'-flanking sequences along with leucine zipper region have been investigated in MV reduction (Wild and Buckland, 1997; Hu *et al.*, 1994; Klagge and Schneide-Schaulis, 2000). The molecular determinants of lasting immunity, persistent infection and immunosuppression continue to be areas of interest for further investigation.

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