Available online at www.sciencedirect.com
ScienceDirect
Virology 352 (2006) 390–399
VIROLOGY
www.elsevier.com/locate/yviro

Protective immunity provided by HLA-A2 epitopes for fusion and hemagglutinin proteins of measles virus

SangKon Oh [a,b,*], Brian Stegman [a], C. David Pendleton [a], Martin O. Ota [c], Chien-Hsiung Pan [c], Diane E. Griffin [c], Donald S. Burke [b], Jay A. Berzofsky [a,*]

[a] *Vaccine Branch, National Cancer Institute, National Institutes of Health, Bldg. 10-Rm 6B-09, NIH, Bethesda, MD 20892-1578, USA*
[b] *Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD 21205, USA*
[c] *W. Harry Feinstone Department of Molecular Microbiology and Immunology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD 21205, USA*

Received 21 February 2006; returned to author for revision 23 March 2006; accepted 28 April 2006
Available online 15 June 2006

Abstract

Natural infection and vaccination with a live-attenuated measles virus (MV) induce $CD8^+$ T-cell-mediated immune responses that may play a central role in controlling MV infection. In this study, we show that newly identified human HLA-A2 epitopes from MV hemagglutinin (H) and fusion (F) proteins induced protective immunity in HLA-A2 transgenic mice challenged with recombinant vaccinia viruses expressing F or H protein. HLA-A2 epitopes were predicted and synthesized. Five and four peptides from H and F, respectively, bound to HLA-A2 molecules in a T2-binding assay, and four from H and two from F could induce peptide-specific $CD8^+$ T cell responses in HLA-A2 transgenic mice. Further experiments proved that three peptides from H (H9-567, H10-250, and H10-516) and one from F protein (F9-57) were endogenously processed and presented on HLA-A2 molecules. All peptides tested in this study are common to 5 different strains of MV including Edmonston. In both $A2K^b$ and HHD-2 mice, the identified peptide epitopes induced protective immunity against recombinant vaccinia viruses expressing H or F. Because F and H proteins induce neutralizing antibodies, they are major components of new vaccine strategies, and therefore data from this study will contribute to the development of new vaccines against MV infection.
Published by Elsevier Inc.

Keywords: Measles; Vaccines; CD8; Cytotoxic T lymphocytes; Epitopes

Introduction

The live-attenuated measles vaccine has been used for over 30 years and has virtually eliminated measles virus (MV) in many industrialized countries. However, measles control remains a challenge in developing countries due to the low seroconversion rates in young infants. Under the age of 9 months, the live-attenuated vaccine is poorly immunogenic because of both the immaturity of the immune system and the presence of maternal antibody (Gans et al., 1998; Halsey et al., 1985). The decay rates of maternal antibody are not the same in all individuals, resulting in a variable window for the susceptibility to MV before routine immunization. Early vaccination with a high titer vaccine at the age of 4–6 months was attempted but discontinued because of high mortality rates among the female recipients (Garenne et al., 1991; Holt et al., 1993).

To circumvent the limitations of current live-attenuated vaccines, new vaccine strategies are being tested. These strategies include recombinant vaccinia expressing MV antigens (Drillien et al., 1988; Wild et al., 1992, 1993), immune stimulating complex (ISCOM)-based vaccines using subunits or proteins, peptide-conjugated vaccines (Stittelaar et al., 2000; van Binnendijk et al., 1997), plasmid DNA vaccines (Polack et al., 2000; Stittelaar et al., 2002), and alphavirus replicon particle vaccines (Pan et al., in press). Although these strategies have varied in their ability to induce protective B and T cell responses, current vaccine strategies use specific antigens, viral

* Corresponding author. S. Oh is to be contacted at Baylor Institute for Immunology Research, Baylor University Medical Center, 3434 Live Oak, Dallas, TX 75204, USA. Fax: +1 214 820 4813. J.A. Berzofsky, fax: +1 301 402 0549.
E-mail addresses: sangkono@baylorhealth.edu (S. Oh), berzofsk@helix.nih.gov (J.A. Berzofsky).

0042-6822/$ - see front matter. Published by Elsevier Inc.
doi:10.1016/j.virol.2006.04.040

proteins, and immunogenic peptides. This may resolve the problems caused by maternal antibodies and induce more potent immune responses against MV infection.

Although antibody-mediated immune responses are important for the protection against MV, and thus many studies have focused on neutralizing antibodies to hemagglutinin (H) and fusion (F) proteins, many pieces of evidence (Markowitz et al., 1988; Nanan et al., 2000; Permar et al., 2003; van Binnendijk et al., 1990; van Els and Nanan, 2002) indicate that cell-mediated immunity plays an important role in the protection against MV infection. More importantly, T cells can be primed in infants by MV vaccine even in the presence of passively transferred antibody (Gans et al., 1999; Siegrist et al., 1998). Therefore, vaccines eliciting cell-mediated immunity as well as humoral immunity are likely to be the best choice for the eradication of MV. However, cellular immune responses to MV infection and vaccination have not been fully studied, partially because of (Griffin et al., 1994; Rose et al., 1984) limited knowledge of MV epitopes presented by MHC class I and class II molecules to $CD8^+$ and $CD4^+$ T cells (Nanan et al., 1995; van Binnendijk et al., 1992; van Els and Nanan, 2002).

Therefore, characterization of human $CD8^+$ T cell epitopes from MV proteins is important for new vaccine development and will help to evaluate cellular responses induced after vaccination or MV infection. For this purpose, we have initiated a comprehensive plan to identify HLA-A2 epitopes of MV proteins. Previous studies, using prediction and peptide elution, have identified two HLA-A2 epitopes for H, one each in the C, M, and L proteins and 3 in N (Herberts et al., 2001; Nanan et al., 1995; van Els et al., 2000). In the current study, we focused on the H and F proteins. Using HLA-A2 transgenic mice, we report identification of 3 new HLA-A2 epitopes in H and one in F. All four peptides were endogenously processed and presented by HLA-A2 and induced peptide-specific $CD8^+$ T-cell-mediated protective immunity against challenge with recombinant vaccinia virus expressing H or F protein.

Results

HLA-A201 epitope prediction and binding affinity

Forty peptides, twenty 9-mers and twenty 10-mers, were predicted from the H and F proteins of five MV strains, Edmonston (Alkhatib and Briedis, 1986; Richardson et al., 1986), AIK-C (Mori et al., 1993), Halle (Buckland et al., 1987; Gerald et al., 1986), IP-3-Ca (Schmid et al., 1992), and Yamagata (Komase et al., 1990a, 1990b), using the algorithm developed by Parker et al. (1994). Both Edmonston and AIK-C are vaccine strains (Hirayama, 1983). All peptides predicted in this study are commonly expressed in the five strains of MV (data not shown).

Of those forty peptides, six from H and five from F were selected based on the binding scores generated by the algorithm. The binding affinity of each peptide to HLA-A2 molecules was first assessed by the T2-binding assay and the data are summarized in Table 1. Nine (5 for H and 4 for F) out of eleven peptides bound to HLA-A2 molecules, but three of them, H9-117, F9-205 and F10-452, bound more weakly (FI_{50} > 50 μM) than the other six. H10-516 and F9-57 showed the highest binding affinity to the HLA-A2 molecule. The other four peptides, H9-477, H9-567, H10-250, and F9-63, bound relatively strongly to HLA-A2 molecule. However, the affinity of these six peptides, FI_{50} = 5–12 μM, was lower than the affinity of a well-characterized HLA-A2 epitope of the influenza virus matrix protein (FMP: GILGFVFTL), FI_{50} = 0.5 μM.

Table 1
HLA-A201 epitopes for both hemagglutinin and fusion protein and their binding affinity to HLA-A201 molecules

Peptides	Amino acid sequence	FI_{50} in T2-binding assay [a]	Binding scores predicted [b]
A. 9-mers from hemagglutinin			
H9-117	YVLLAVLFV	>50	700
H9-477	KVSPYLFNV	10	264
H9-567	KLWCRHFCV	12.5	11,718
B. 10-mers from hemagglutinin			
HIO-250	SMYRVFEVGV	12.5	610
HIO-516	ILPGQDLQYV	7.5	1495
C. 9-mers from fusion protein			
F9-57	KLMPNITLL	5	635
F9-63	TLLNNCTRV	14	257
F9-205	KLLRYYTEI	>50	202
D. 10-mers from fusion protein			
F10-452	ILLERLDVGT	>50	192

[a] Concentration, μM of peptide that shows geometric fluorescence index is 0.5. FI = (mean fluorescence with peptide − mean fluorescence without peptide) / mean fluorescence without peptide. Background fluorescence without 887.2 was subtracted for each individual value.

[b] Binding scores were predicted by the algorithm developed by Bioinformatics and Molecular analysis section at National Institute of Health (Parker KC 1994 JI 152:163).

Immunogenicity of the peptides in HLA-A201-transgenic mice

To test whether those peptides could induce peptide-specific $CD8^+$ T cells, we immunized animals with either the peptide-adjuvant mixture or DC pulsed with the peptides. In both experiments, animals were boosted once, and then $CD8^+$ T cells from the immunized mice were restimulated with syngeneic splenocytes pulsed with the immunized peptides for 1 week.

Data in Fig. 1 show the lytic activity of $CD8^+$ T cells against target cells pulsed with individual peptides from H. Four peptides, H9-477, H9-567, H10-250, and H10-516, induced measurable levels of peptide-specific $CD8^+$ T cell responses. These $CD8^+$ T cells lysed target cells pulsed with 10 or 0.1 μM of the corresponding peptides. In terms of the magnitude of lytic activity, $CD8^+$ CTL induced with H9-567 and H10-516 were slightly higher than $CD8^+$ CTL induced with other peptides when target cells were pulsed with a 10 μM concentration of the peptides. In this assay, Jurkat-A2K^b cells, expressing α1 and α2 of HLA-A2 and α3 of K^b, but no murine MHC molecules, were used as target cells. Therefore, the CTL are specific for those peptides and are restricted by HLA-A2, not murine MHC molecules.

Two out of four peptides from F protein could also induce peptide-specific $CD8^+$ T cell responses when animals were

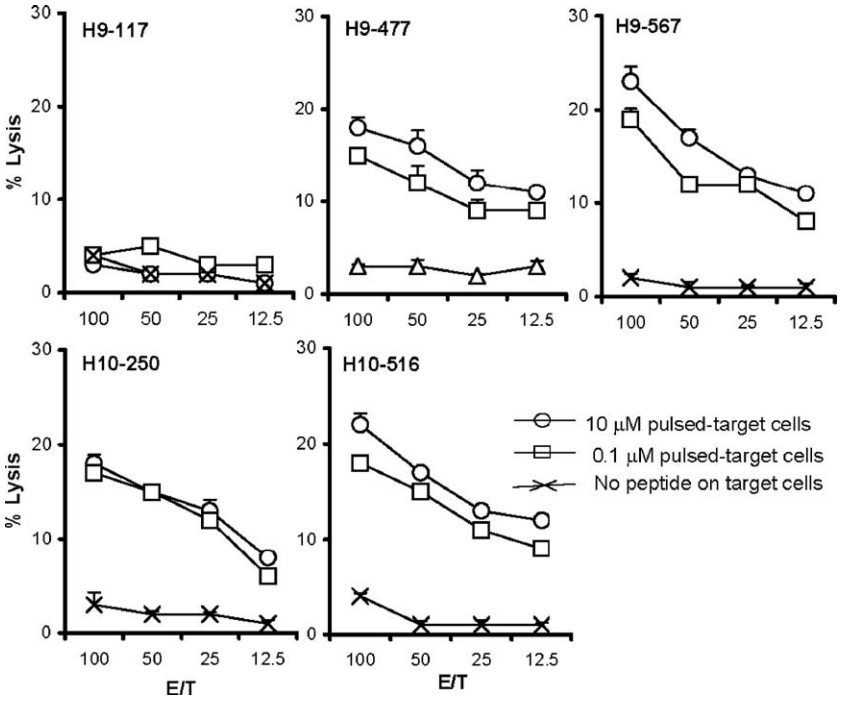

Fig. 1. Immunization with peptides from measles hemagglutinin induces peptide-specific $CD8^+$ T cell responses in $A2K^b$ transgenic mice. For each peptide, 3–4 mice were immunized s.c. in the base of the tail with 100 μl of an emulsion containing 1:1 IFA and PBS with antigens and cytokines (50 nmol CTL epitope, 50 nmol HBV core 128–140 helper epitope, 3 μg of IL-12, and 5 μg of granulocyte macrophage colony stimulating factor (GM-CSF). Mice were boosted s.c. with the emulsion on 3–4 weeks after the primary. Three to four weeks after the boost, pooled-spleen $CD8^+$ T cells were restimulated with a 10 or 0.1 μM concentration of the peptides used for immunization for 1 week. In a 5-h ^{51}Cr release assay, Jurkat-$A2K^b$ cells were used as target cells. Target cells were pulsed with 10 or 0.1 μM peptides. Control cells were not pulsed with peptides. Data from two repeated experiments were consistent and error bars represent mean ± SE of a triplicate assay.

immunized with the peptide-adjuvant mixtures (Fig. 2). F9-57 and F9-63 showed higher binding affinity to HLA-A2 molecules than the other two peptides from F, indicating that binding affinity of peptide to the MHC molecules could impact peptide-specific $CD8^+$ T cell responses. Consistently, F9-57 induced a higher frequency of peptide-specific CD8 CTL than F9-63. It is also interesting that $CD8^+$ T cell responses induced with the peptides from F were slightly weaker, albeit very reproducible, than the responses induced with the peptides from H in our repeated experiments even though there was no dramatic difference in their binding affinities.

To confirm these data, we immunized animals with peptide-pulsed DC, and we measured the lytic activities of the peptide-specific $CD8^+$ CTL (Fig. 3A). Consistent with the data in Fig. 1, H9-567 induced a higher frequency of peptide-specific $CD8^+$ T cells than three other peptides from the same protein. In addition to the increased frequency of $CD8^+$ T cells, CTL induced with either 0.1 or 10 μM H9-567 produced a similar magnitude of lytic activity against target cells pulsed with 10 μM peptide. Although DC immunization did not increase the numbers of $CD8^+$ CTL dramatically compared to peptide-adjuvant mixtures, we confirmed that all four peptides could induce peptide-specific $CD8^+$ T cells and those $CD8^+$ T cells were able to recognize target cells in an antigen-specific manner. For the peptides from F protein, we selected F9-57 and F9-63 from the data in Fig. 2. The data in Fig. 3A indicated that the number of peptide-specific $CD8^+$ CTL induced by F9-57 was slightly higher than $CD8^+$ CTL induced by F9-63 as observed in Fig. 2.

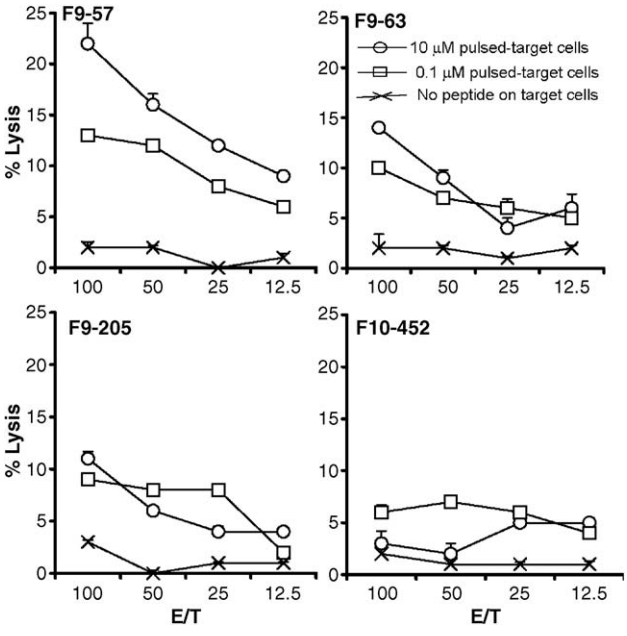

Fig. 2. Immunization with peptides from measles fusion protein induces peptide-specific $CD8^+$ T cell responses in $A2K^b$ transgenic mice. For each peptide, 3–4 mice were primed and boosted as described in Fig. 1. Three to four weeks after the boost, pooled-spleen $CD8^+$ T cells were restimulated with a 10 or 0.1 μM concentration of the immunized peptides for 1 week. For the 5-h ^{51}Cr release assay, Jurkat-$A2K^b$ cells pulsed with the peptides (10 or 0.1 μM) were target cells. Control cells were not pulsed with peptides. Data are representative of two repeated experiments showing consistent results and error bars represent mean ± SE of a triplicate assay.

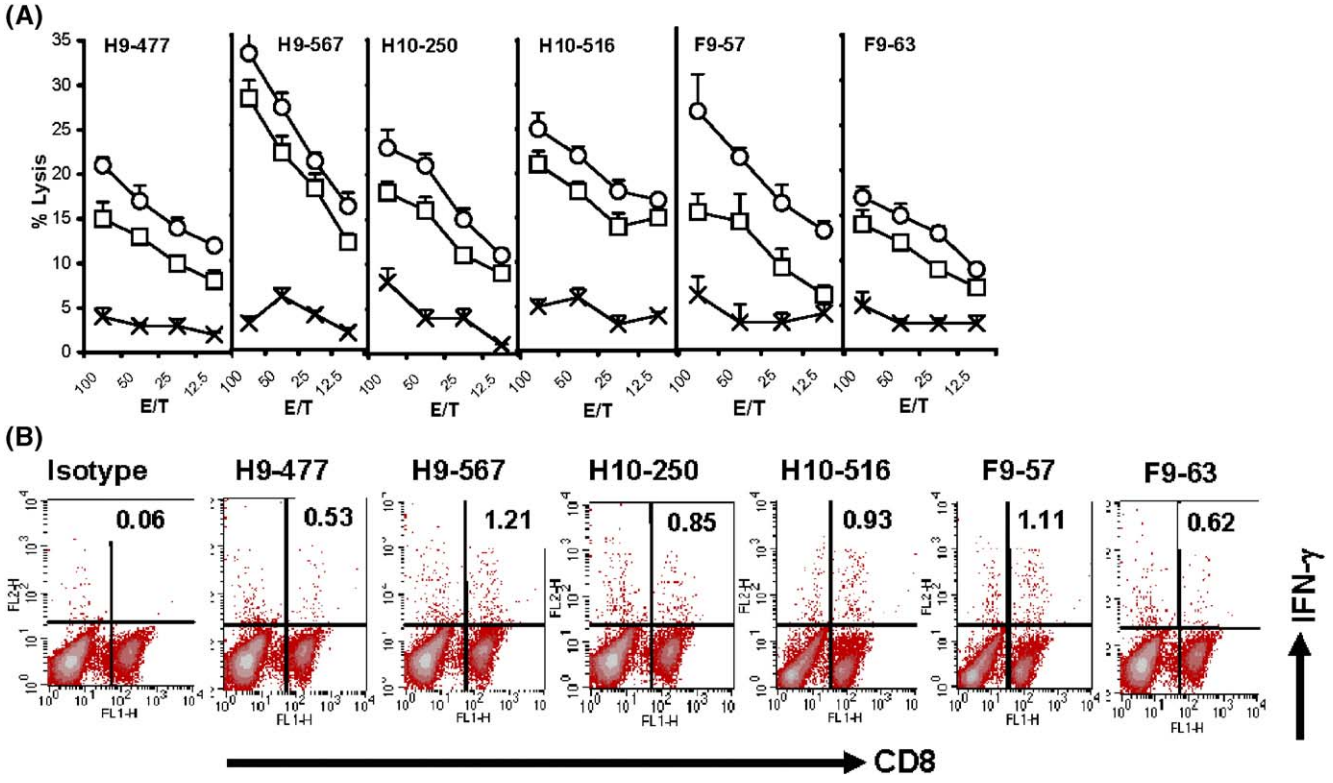

Fig. 3. Peptide-pulsed dendritic cells (DC) induced peptide-specific $CD8^+$ T cell responses. Bone marrow-derived DC were prepared as described in Materials and methods. On day 7 of the culture, DC were pulsed with 10 μM peptide in serum-free RPMI for 2 h at 37 °C, and mice were immunized s.c. with $1–3 \times 10^5$ DC/mouse without washing. Three weeks after priming, mice were boosted with the same peptide-pulsed DC. Three to four weeks after the boost, pooled-spleen $CD8^+$ T cells were restimulated with 10 μM (circles) or 0.1 μM (squares) concentrations of the peptides for 1 week. For the 5-h ^{51}Cr release assay, Jurkat-$A2K^b$ cells pulsed with 10 μM peptides were used as target cells. In each experiment, 3–4 mice/group were used and data from two repeated experiments were consistent. Error bars represent mean ± SE of a triplicate assay. For intracellular IFN-γ staining, cells were stimulated with 10 μM peptides overnight and the data are representative of two repeated experiments showing consistent results. The number in upper quadrant is % of IFN-γ producing cells in total $CD8^+$ T cells.

In addition to measuring functional activity of $CD8^+$ CTL, the frequency of peptide-specific $CD8^+$ T cells was measured by counting the number of γ-IFN-producing $CD8^+$ T cells when $CD8^+$ T cells were restimulated with 10 μM peptides ex vivo (Fig. 3B). All peptides tested induced significant numbers of γ-IFN-producing $CD8^+$ T cells. Moreover, H9-567 and F9-57 induced slightly higher frequencies of γ-IFN-producing $CD8^+$ T cells than the other peptides when CTL were stimulated with 10 μM concentrations of the peptides. The differences in the magnitude of functional activity could be the result of in vitro expansion of $CD8^+$ CTL before measuring CTL activity (Fig. 3A).

Peptide epitopes of H and F proteins are endogenously processed and presented on HLA-A2

Four peptides from H and two from F were immunogenic in HLA-A2 transgenic mice and $CD8^+$ CTL induced with these peptides recognized the peptide-pulsed target cells (Figs. 1–3). We next tested whether these peptides were endogenously processed and presented on HLA-A2 molecules. For this purpose, animals were immunized with plasmid DNA containing cDNA of H or F of the Edmonston strain. Data in Fig. 4 show that DNA plasmids expressing H and F induced measurable levels of peptide-specific $CD8^+$ CTL, indicating that these peptides were endogenously processed and presented on HLA-A2 molecules followed by the induction of peptide-specific $CD8^+$ T cells. However, the plasmid DNAs injected did not induce significant levels of H9-477 or F9-63-specific $CD8^+$ T cell responses. Both H9-477 and F9-63 induced peptide-specific $CD8^+$ T cell responses when they were administered as the peptide-adjuvant mixture (Figs. 1 and 2) and peptide-pulsed DC (Fig. 3), suggesting that these two peptides may not be processed or presented efficiently on HLA-A2. Although F9-57 showed the highest binding affinity of all peptides tested and could induce measurable levels of peptide-specific $CD8^+$ T cell responses when animals were immunized with either peptide-adjuvant mixture or peptide-pulsed DC, the magnitude of the F9-57-specific $CD8^+$ T cell response induced by plasmid DNA was marginal. In this study, mice were also immunized with recombinant vaccinia viruses expressing H or F protein, but no significant $CD8^+$ CTL responses for the H9-477 and F9-63 were observed (data not shown).

Peptide-specific $CD8^+$ T cells protect animals challenged with recombinant vaccinia viruses

The data from Figs. 1–4 indicate that three peptides from H and one from F protein can be endogenously processed and presented on HLA-A2 molecules and this results in the induction of peptide-specific $CD8^+$ T cell responses. To test

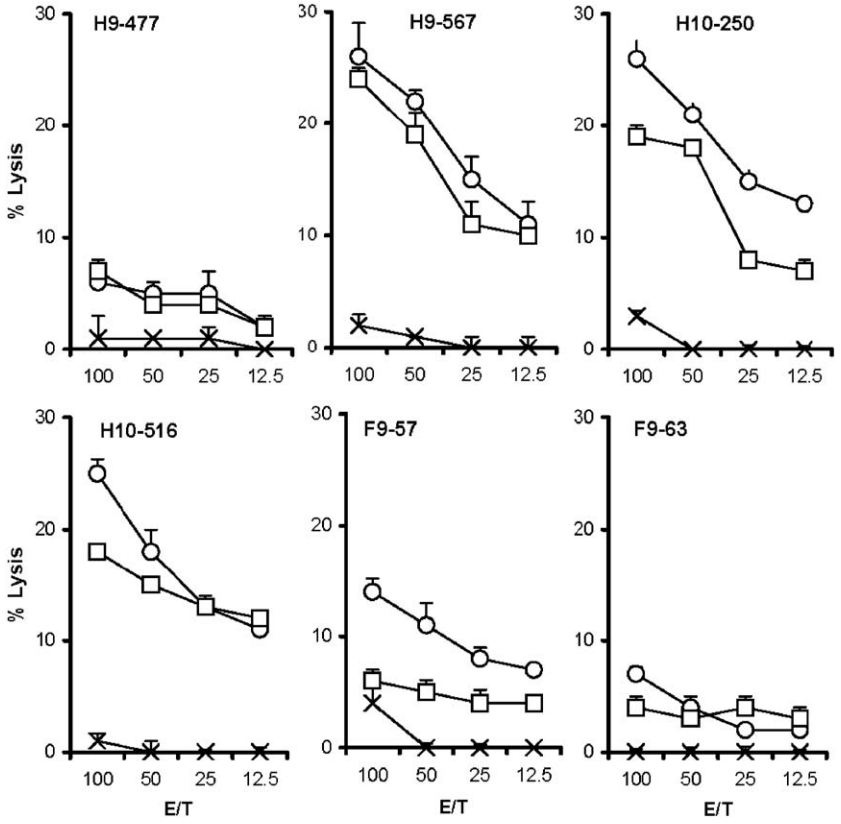

Fig. 4. Immunization with DNA plasmids expressing measles hemagglutinin or fusion protein induces peptide-specific $CD8^+$ T cell responses. Three to four $A2K^b$ mice in each group were immunized intramuscularly with 100 μg of the plasmids, SINCP/H, or SINCP/F. At 3 week-intervals, mice were boosted twice. Three to four weeks after the final immunization, pooled-spleen $CD8^+$ T cells were restimulated with 10 μM (circles) and 0.1 μM (squares) peptides for 1 week. For the 5-h ^{51}Cr release assay, Jurkat-$A2K^b$ cells pulsed with peptides, 10 μM were used as target cells. Data are representative of three-repeated experiments and error bars represent mean ± SE of a triplicate assay.

the protective efficacy of those CTL against viral infection, animals were immunized with each peptide, and then challenged with recombinant vaccinia virus expressing H or F.

Data in Fig. 5A show that both H9-567 ($P = 0.0009$) and H10-250 ($P = 0.0034$) induced $CD8^+$ CTL that result in the significant protection of animals against recombinant vaccinia virus expressing H protein. The average vaccinia titer in the animals immunized with H10-516 ($P = 0.0506$) was slightly lower than the titers in the control. Consistent with the data in Figs. 1 and 4, neither H9-117 nor H9-477 induced any protection against the virus challenge. Although $CD8^+$ CTL induced with H9-567 and H10-250 are restricted by HLA-A2 (Figs. 1–4), it is possible that $CD8^+$ CTL specific for the mouse $H\text{-}2K^b$ or D^b could also contribute to the protection against the vaccinia virus challenge. However, the same experiment was performed in wild-type C57BL/6 animals, and none of the peptides induced protection against the recombinant virus challenge (data not shown), suggesting that the protection observed here was not due to the contribution of $CD8^+$ CTL specific for the mouse $H\text{-}2K^b$ or D^b. Furthermore, we performed the same experiment using HHD-2 mice that express only human HLA-A2 molecules. Because of the limitation in the number of mice available, we tested three mice in each group. Nevertheless, the data in Fig. 5B clearly show that all three peptides, H9-567, H10-250, and H10-516, induced statistically significant levels of protective immunity against the recombinant vaccinia virus challenge. The differences in the vaccinia titers between control and experimental groups were greater in HHD-2 mice than $A2K^b$ mice, probably due to the increased expression of HLA-A2 molecules in HHD-2 mice (data not shown).

The animals immunized with the peptides from F protein were challenged with recombinant vaccinia expressing F (Fig. 6). In $A2K^b$ transgenic mice, only F9-57 ($P = 0.0442$) showed significant protection against the virus challenge. This peptide also induced protective immunity in HHD-2 mice ($P = 0.031$), which express only the human HLA-A2 molecule, and this protection was even greater than in $A2K^b$ transgenic mice. HHD-2 mice immunized with F9-63 were not protected to a significant extent.

Discussion

The type of immune responses to viral infections and the response conferring protection are dependent on the nature of the infection. Neutralizing antibody is an important immune arm for preventing MV infection. However, cellular immunity is also critical for controlling MV infection. Moreover, significant levels of cell-mediated immunity are induced in both naturally infected and vaccinated individuals (Bautista-Lopez et al.,

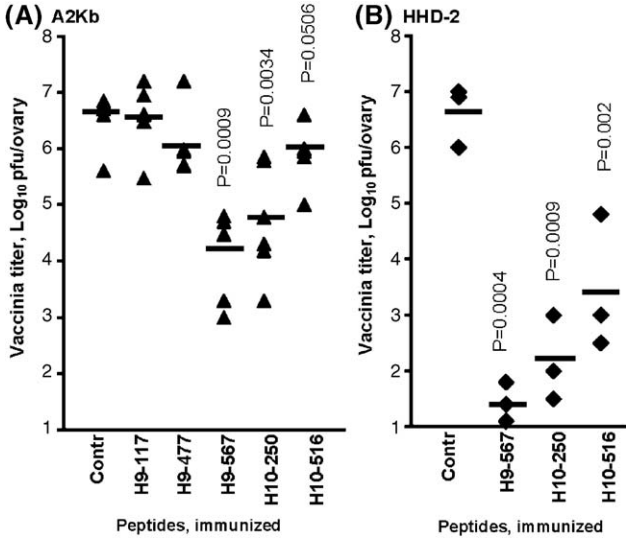

Fig. 5. Protection against recombinant vaccinia virus expressing measles hemagglutinin (H) can be provided by immunization with the peptide epitopes from measles H protein. (A) Female A2K^b and (B) HHD-2 mice were immunized with the peptide-adjuvant mixtures and boosted once as described in Fig. 1. For the control, animals were immunized with the adjuvant mixture containing cytokines and CD4 helper epitope. Ten days after the boost, mice were challenged i.p. with 5–6 × 10^6 PFU of vaccinia virus expressing measles H. On day 5, virus titers in the ovaries were determined. Five mice in panel A and three mice in panel B were tested and the statistical significance was tested by Student's *t* test.

2000; Gans et al., 1999; Jaye et al., 1998a, 1998b; Nanan et al., 1995, 2000; Ovsyannikova et al., 2003; Siegrist et al., 1998; van Binnendijk et al., 1990; van Els and Nanan, 2002; Ward et al., 1995). Impaired T cell immunity results in more severe disease and delayed recovery from measles (Bautista-Lopez et al., 2000; Gans et al., 1999; Jaye et al., 1998a, 1998b; Nanan et al., 1995, 2000; Ovsyannikova et al., 2003; Siegrist et al., 1998; van Binnendijk et al., 1990; van Els and Nanan, 2002; Ward et al., 1995) and MV-specific long-term memory CD8$^+$ T cells are also generated after MV infection (Jaye et al., 1998a, 1998b; Nanan et al., 1995; Ovsyannikova et al., 2003). Therefore, new vaccines against MV should include components that induce cellular immunity against MV infection and finding new epitopes for CD8$^+$ T cells will be necessary for development and evaluation of new MV vaccines.

HLA-A2 is common in most populations, and therefore we have comprehensively examined HLA-A2 epitopes from the H and F proteins in this study. We have identified 3 new HLA-A2-restricted epitopes in H and the first HLA-A2-restricted epitope in F. Furthermore, we have shown that these CD8$^+$ T cell epitopes were endogenously processed and presented on HLA-A2 molecules, and they induced protective immunity in animal models. Because most new vaccine strategies against MV focus on H and F for inducing neutralizing antibodies and little is known about human T cell epitopes of both proteins, new CD8 T cell epitopes for both F and H will be useful in future.

The expression levels of both H and F are lower than MV proteins NP and M during viral infections (van Binnendijk et al., 1992). Both H and F are glycosylated surface viral proteins that are cotranslationally inserted into the ER. Maturation and translocation to the cell membrane take place via the Golgi network, limiting opportunities for processing by the classical MHC class I pathway. Even without using the classical pathway, however, peptide could be presented by alternative pathways, such as proteosomal and TAP-independent pathways (Watts and Powis, 1999) and by back trafficking of proteins into the cytosol (Wiertz et al., 1996). For the H protein, 3 out of 6 peptides tested were presented by HLA-A2. Although we did not show all peptides predicted in this study, higher numbers of HLA-A2 epitopes showing higher binding scores were predicted from H compared to F protein even though F is more abundant than H in MV-infected cells (Herberts et al., 2001; van Binnendijk et al., 1992). Two overlapping A2-restricted H epitopes were previously identified through use of a predictive algorithm (H29-37) (Nanan et al., 1995) and elution of peptides from MV-infected B cells (H30-38) (van Els et al., 2000). These peptides were both identified in our predictions, but their predicted binding scores were lower than those of the peptides tested in this study.

All peptides predicted and tested, except for H10-250, in this study lack amino acid residues associated with poor binding to HLA-A2, such as Asp, Glu, and Pro at position 1; Asp and Glu at position 3; Arg, Lys, His, and Ala at position 4; Pro at position 5; Arg, Lys, and His at position 7; Asp, Glu, Arg, Lys, and His at position 8; and Arg, Lys, and His at position 9 (Rammensee et al., 1995; Ruppert et al., 1993). H10-250 possesses Arg at position 4, known to be associated with poor HLA-A2.1 binding at the secondary anchor positions. Although there were discrepancies in rank order between the peptide-binding scores

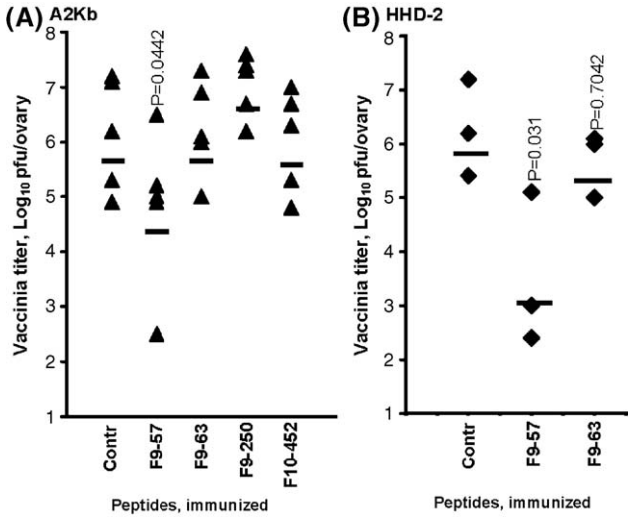

Fig. 6. Protection against recombinant vaccinia virus expressing measles fusion (F) protein in the animals immunized with the peptide epitopes from measles F. (A) Female A2K^b and (B) HHD-2 mice were immunized with the peptide-adjuvant mixtures and boosted once as described in Fig. 1. Control animals were immunized with the adjuvant mixture containing cytokines and CD4 helper epitope. Ten days after the boost, mice were challenged i.p. with 5–6 × 10^6 PFU of vaccinia virus expressing measles fusion protein. On day 5, virus titers in the ovaries were determined. Five mice in panel A and three mice in panel B were tested and the statistical significance was tested by Student's *t* test.

predicted by the algorithm and the actual binding affinities by T2-binding assay, the peptides predicted by this algorithm were relatively good binders to HLA-A2 molecules. Binding affinity of peptide epitopes may correlate with the strength of CD8 T-cell-mediated immunity. However, for $CD8^+$ T cell responses to the peptides from H, such a correlation was not apparent. This difficulty in seeing a correlation is probably because their FI_{50} values were in a similar range, 7.5–12.5 μM. Moreover, $CD8^+$ T cells specific for each peptide may use different TCR repertoires.

In this study, we used HLA-A2 transgenic mice to test the immunogenicity of predicted peptides. This animal model has been widely used for determining human HLA-A2 epitopes and has been proven to be a good predictor of human $CD8^+$ T cell epitopes (Oh et al., 2004; Shirai et al., 1995; Tishon et al., 2000). One concern is that the peptide/HLA-A2 complex recognized by murine T cells may differ from that recognized by human T cells. However, MV CTL responses in transgenic mice resembling those in humans have been demonstrated (Tishon et al., 2000) and $A2K^b$ transgenic mice could generate anti-viral CTL in the absence of human β2 m and human CD8. Possible cross-reactions with mouse K^b were eliminated by using HHD-2 mice that express only human HLA-A2. Moreover, none of the peptides tested in this study protected wild-type animals. The better protection against vaccinia virus challenge (Figs. 5 and 6) in HHD-2 mice could be due to the fact that HHD-2 mice express only one class I molecule, human HLA-A2. Indeed, a greater number of peptide-specific $CD8^+$ CTL were observed in HHD-2 mice compared to $A2K^b$ transgenic mice in our other studies (data not shown). Data from this study are limited to HLA-A2 and were tested in a transgenic animal model. However, studies in progress will reveal the peptide epitopes presented on human cells. Furthermore, it is important to map epitopes for other HLA types to broaden the coverage of the human population. Nearly half of most populations express HLA-A2, and it has been estimated that only 5 class I HLA alleles are sufficient to cover 90% of almost any given population (Gulukota and DeLisi, 1996).

In conclusion, identification and evaluation of $CD8^+$ T cell epitopes are necessary for selecting vaccine components of new MV vaccines as well as for evaluating cellular immune responses. In this study, we have identified and characterized four endogenously processed and presented HLA-A2-restricted epitopes, one from F and three from H. These peptides also induced protective immunity in animals challenged with recombinant vaccinia viruses expressing MV proteins. These studies may contribute to the development and evaluation of new vaccines against measles.

Materials and methods

Animals

$A2K^b$ transgenic mice express a chimeric HLA-A2.1 transgene with the α1 and α2 domains from HLA-A2.1 and the α3 domain from $H\text{-}2K^b$ to allow binding to mouse CD8 (Vitiello et al., 1991). HHD-2 (Firat et al., 2001; Harrer et al., 1996; Pascolo et al., 1997) mice have the murine β2-microglobulin and $H\text{-}2D^b$ genes knocked out and are transgenic for a chimeric human HLA-A2.1 expressing the α1 and α2 domains of HLA-A2.1 and a murine D^b-derived α3 domain to allow interaction with mouse CD8 and also have a covalently linked human β2-microglobulin to compensate for lack of free β2-microglobulin. As a result of this lack of any free β2-microglobulin, even though the $H\text{-}2K^b$ gene is not knocked out, the only class I MHC molecule expressed is the chimeric human HLA-A2.1 with the covalent human β2-microglobulin.

These mice were bred and housed in appropriate animal care facilities. All procedures with animals were conducted in accordance with institutionally approved protocols.

Cells

The Jurkat-$A2K^b$ cell line, a gift from Dr. L. Sherman (Scripps Research Institute, La Jolla, CA), is transfected with the HLA chimeric molecule containing the α1 and α2 domains from human HLA-A2.1 and α3 from mouse $H\text{-}2K^b$. The T2 cell line is deficient in TAP1 and TAP2 transporter proteins and expresses low levels of HLA-A2 (Nijman et al., 1993; Oh et al., 2004). BSC-1 cells, monkey kidney cells, were acquired from ATCC. Cells were maintained in 10% FCS-RPMI containing 1 mM sodium pyruvate, nonessential amino acids (Biofluids, Rockville, MD), 4 mM glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 50 μM 2-ME.

Synthetic peptides

Peptides were prepared in an automated multiple peptide synthesizer (Symphony; Protein Technologies, Tucson, AZ) using fluorenylmethoxycarbonyl chemistry. They were purified by reverse phase HPLC, and sequences were confirmed where necessary on an automated sequencer (477A; Applied Biosystems, Foster City, CA).

Peptide-binding assay

Peptide binding to HLA-A2 molecules was measured by using the T2 cell line (Nijman et al., 1993; Oh et al., 2004). T2 cells (3×10^5/well) were incubated overnight in 96-well plates with culture medium (a 1:1 mixture of RPMI 1640-Eagle-Hank's amino acid (EHAA) media containing 2.5% FCS, 100 U/ml penicillin, and 100 μg/ml streptomycin) with 10 μg/ml human β2-microglobulin (Sigma-Aldrich, St. Louis, MO) and peptides. Cells were washed twice with cold PBS containing 2% FCS and then incubated for 30 min at 4 °C with anti-HLA-A2.1 BB7.2 mAb (1/100 dilution of hybridoma supernatant). After washing, cells were stained with 5 μg/ml FITC-labeled goat anti-mouse Ig (BD PharMingen, San Diego, CA) and expression levels of HLA-A2.1 were measured by flow cytometry (FACSCaliber; BD Biosciences, Mountain View, CA). HLA-A2.1 expression was quantified as fluorescence index (FI) according to the formula: FI = [(mean fluorescence with peptide − mean fluorescence without peptide) / mean fluorescence without peptide]. Background fluorescence

without BB7.2 was subtracted for each individual value. To compare the different peptides, FI_{50}, the peptide concentration, μM, that increases HLA-A2.1 expression by 50% over no peptide control background, was calculated from the titration curve for each peptide.

Immunization

Mice were immunized with syngeneic peptide-loaded dendritic cells (DC), plasmid DNAs expressing measles H or F protein, or the mixture of peptide and cytokine in incomplete Freund's adjuvant (IFA). DC were prepared from bone marrow as previously described (Celluzzi et al., 1996). On day 7 of the culture, DC were pulsed with 10 μM peptide in serum-free RPMI for 2 h at 37 °C, and then mice were immunized s.c. with 1–3 × 10^5 peptide-pulsed DC. Three weeks after the primary immunization, mice were boosted with the same peptide-pulsed DC. Alternatively, mice were immunized intramuscularly with 100 μg plasmid DNA, SINCP/H, or SINCP/F (Polo et al., 2000; Song et al., 2005). Mice were boosted with 100 μg plasmids 3 weeks after the primary immunization. Mice were immunized s.c. in the base of the tail with 100 μl of an emulsion containing 1:1 IFA and PBS with peptide antigens and cytokines (50 nmol CTL epitope, 50 nmol HBV core 128–140 helper epitope, 3 μg of IL-12, and 5 μg of granulocyte macrophage colony stimulating factor; GM-CSF). Mice were boosted with the emulsion 3–4 weeks after the primary immunization. IFA and cytokines were purchased from Sigma and Peprotech (Rocky Hill, NJ), respectively.

CTL assay

$CD8^+$ T cells from the immunized mice were restimulated with peptide-loaded splenocytes for 1 week as previously described (Oh et al., 2003) and then tested in 5 h ^{51}Cr release assays. Target cells were labeled with ^{51}Cr and washed twice. Cells were then pulsed with peptides for 2 h and used as target cells without further washing. Control cells were not pulsed with peptides. The mean of triplicate samples was calculated, and the percentage of specific lysis was determined by using the following formula: Percentage of specific lysis = 100 × (experimental ^{51}Cr release − spontaneous ^{51}Cr release) / (maximum ^{51}Cr release − spontaneous ^{51}Cr release). The maximum release refers to counts from targets in 2.5% Triton X-100.

Viral challenge and protection assay

Female mice, $A2K^b$ and HHD-2, were immunized with the peptide-adjuvant mixture as described above, boosted 3–4 weeks after the primary immunization. Ten days after the boost, animals were challenged i.p. with recombinant vaccinia virus (6 × 10^6 PFU/mouse) expressing measles H or F protein (Tamin et al., 1994; Zhu et al., 2000), kindly provided by Dr. Paul Rota (Center for Disease Control, Atlanta GA). On day 5, virus titers in the ovaries of individual mice were determined on BSC-1 cells as previously described (Ahlers et al., 2001).

Antibodies and flow cytometry

FITC-labeled anti-mouse CD8 (53-6.7), CD11c, CD80 (B7-1), and CD54 (ICAM-1) were used for staining of cell surface molecules. Intracellular IFN-γ staining followed the manufacture's protocol (Pharmingen). All Abs and reagents were purchased from Pharmingen. For flow cytometric analysis of cell surface antigens, 5 × 10^5 cells were washed and resuspended in PBS containing 0.2% BSA and 0.1% sodium azide. Cells were incubated on ice with the appropriate antibody for 30 min and then washed. Samples were analyzed on a FACSCaliber (BD Biosciences, Mountain View, CA). Background staining was assessed by using isotype control antibodies.

Acknowledgments

We thank Dr. Linda Sherman (Scripps) and Dr. François Lemonnier (Institut Pasteur for kind gifts of breeding pairs of the A2Kb and HHD-2 mouse strains to be bred in our facility). This work was supported in part by a grant from the Bill and Melinda Gates Foundation and by intramural research funds of the CCR, NCI, and NIH.

References

Ahlers, J.D., Belyakov, I.M., Matsui, S., Berzofsky, J.A., 2001. Mechanisms of cytokine synergy essential for vaccine protection against viral challenge. Int. Immunol. 13 (7), 897–908.

Alkhatib, G., Briedis, D.J., 1986. The predicted primary structure of the measles virus hemagglutinin. Virology 150 (2), 479–490.

Bautista-Lopez, N., Ward, B.J., Mills, E., McCormick, D., Martel, N., Ratnam, S., 2000. Development and durability of measles antigen-specific lymphoproliferative response after MMR vaccination. Vaccine 18 (14), 1393–1401.

Buckland, R., Gerald, C., Barker, R., Wild, T.F., 1987. Fusion glycoprotein of measles virus: nucleotide sequence of the gene and comparison with other paramyxoviruses. J. Gen. Virol. 68 (Pt. 6), 1695–1703.

Celluzzi, C.M., Mayordomo, J.I., Storkus, W.J., Lotze, M.T., Falo Jr., L.D., 1996. Peptide-pulsed dendritic cells induce antigen-specific, CTL-mediated protective tumor immunity. J. Exp. Med. 183, 283–287.

Drillien, R., Spehner, D., Kirn, A., Giraudon, P., Buckland, R., Wild, F., Lecocq, J.-P., 1988. Protection of mice from fatal measles encephalitis by vaccination with vaccinia virus recombinants encoding either the hemagglutinin or the fusion protein. Proc. Natl. Acad. Sci. U.S.A. 85, 1252–1256.

Firat, H., Tourdot, S., Ureta-Vidal, A., Scardino, A., Suhrbier, A., Buseyne, F., Riviere, Y., Danos, O., Michel, M.L., Kosmatopoulos, K., Lemonnier, F.A., 2001. Design of a polyepitope construct for the induction of HLA-A0201-restricted HIV 1-specific CTL responses using HLA-A*0201 transgenic, H-2 class I KO mice. Eur. J. Immunol. 31 (10), 3064–3074.

Gans, H.A., Arvin, A.M., Galinus, J., Logan, L., DeHovitz, R., Maldonado, Y., 1998. Deficiency of the humoral immune response to measles vaccine in infants immunized at age 6 months. JAMA 280 (6), 527–532.

Gans, H.A., Maldonado, Y., Yasukawa, L.L., Beeler, J., Audet, S., Rinki, M.M., DeHovitz, R., Arvin, A.M., 1999. IL-12, IFN-gamma, and T cell proliferation to measles in immunized infants. J. Immunol. 162 (9), 5569–5575.

Garenne, M., Leroy, O., Beau, J.P., Sene, I., 1991. Child mortality after high-titre measles vaccines: prospective study in Senegal. Lancet 338 (8772), 903–907.

Gerald, C., Buckland, R., Barker, R., Freeman, G., Wild, T.F., 1986. Measles virus haemagglutinin gene: cloning, complete nucleotide sequence analysis and expression in COS cells. J. Gen. Virol. 67 (Pt. 12), 2695–2703.

Griffin, D.E., Ward, B.J., Esolen, L.M., 1994. Pathogenesis of measles virus infection: an hypothesis for altered immune responses. J. Infect. Dis. 170 (Suppl. 1), S24–S31.

Gulukota, K., DeLisi, C., 1996. HLA allele selection for designing peptide vaccines. Genet. Anal. 13, 81–86.

Halsey, N.A., Boulos, R., Mode, F., Andre, J., Bowman, L., Yaeger, R.G., Toureau, S., Rohde, J., Boulos, C., 1985. Response to measles vaccine in Haitian infants 6 to 12 months old. Influence of maternal antibodies, malnutrition, and concurrent illnesses. N. Engl. J. Med. 313 (9), 544–549.

Harrer, E., Harrer, T., Barbosa, P., Feinberg, M., Johnson, R.P., Buchbinder, S., Walker, B.D., 1996. Recognition of the highly conserved YMDD region in the human immunodeficiency virus type 1 reverse transcriptase by HLA-A2-restricted cytotoxic T lymphocytes from an asymptomatic long-term nonprogressor. J. Infect. Dis. 173, 476–479.

Herberts, C.A., Stittelaar, K.J., van der Heeft, E., van Gaans-Van den Brink, J., Poelen, M.C., Roholl, P.J., van Alphen, L.J., Melief, C.J., de Jong, A.P., van Els, C.A., 2001. A measles virus glycoprotein-derived human CTL epitope is abundantly presented via the proteasomal-dependent MHC class I processing pathway. J. Gen. Virol. 82 (Pt. 9), 2131–2142.

Hirayama, M., 1983. Measles vaccines used in Japan. Rev. Infect. Dis. 5 (3), 495–503.

Holt, E.A., Moulton, L.H., Siberry, G.K., Halsey, N.A., 1993. Differential mortality by measles vaccine titer and sex. J. Infect. Dis. 168 (5), 1087–1096.

Jaye, A., Magnusen, A.F., Sadiq, A.D., Corrah, T., Whittle, H.C., 1998a. Ex vivo analysis of cytotoxic T lymphocytes to measles antigens during infection and after vaccination in Gambian children. J. Clin. Invest. 102 (11), 1969–1977.

Jaye, A., Magnusen, A.F., Whittle, H.C., 1998b. Human leukocyte antigen class I- and class II-restricted cytotoxic T lymphocyte responses to measles antigens in immune adults. J. Infect. Dis. 177 (5), 1282–1289.

Komase, K., Haga, T., Yoshikawa, Y., Sato, T.A., Yamanouchi, K., 1990a. Molecular analysis of structural protein genes of the Yamagata-1 strain of defective subacute sclerosing panencephalitis virus: III. Nucleotide sequence of the hemagglutinin gene. Virus Genes 4 (2), 163–172.

Komase, K., Haga, T., Yoshikawa, Y., Sato, T.A., Yamanouchi, K., 1990b. Molecular analysis of structural protein genes of the Yamagata-1 strain of defective subacute sclerosing panencephalitis virus: IV. Nucleotide sequence of the fusion gene. Virus Genes 4 (2), 173–181.

Markowitz, L.E., Chandler, F.W., Roldan, E.O., Saldana, M.J., Roach, K.C., Hutchins, S.S., Preblud, S.R., Mitchell, C.D., Scott, G.B., 1988. Fatal measles pneumonia without rash in a child with AIDS. J. Infect. Dis. 158 (2fs), 480–483.

Mori, T., Sasaki, K., Hashimoto, H., Makino, S., 1993. Molecular cloning and complete nucleotide sequence of genomic RNA of the AIK-C strain of attenuated measles virus. Virus Genes 7 (1), 67–81.

Nanan, R., Carstens, C., Kreth, H.W., 1995. Demonstration of virus-specific $CD8^+$ memory T cells in measles-seropositive individuals by in vitro peptide stimulation. Clin. Exp. Immunol. 102 (1), 40–45.

Nanan, R., Rauch, A., Kampgen, E., Niewiesk, S., Kreth, H.W., 2000. A novel sensitive approach for frequency analysis of measles virus-specific memory T-lymphocytes in healthy adults with a childhood history of natural measles. J. Gen. Virol. 81 (Pt. 5), 1313–1319.

Nijman, H.W., Houbiers, J.G.A., Vierboom, M.P.M., van der Burg, S.H., Drijfhout, J.W., D'Amaro, J., Kenemans, P., Melief, C.J.M., Kast, W.M., 1993. Identification of peptide sequences that potentially trigger HLA-A2.1-restricted cytotoxic T lymphocytes. Eur. J. Immunol. 23, 1215–1219.

Oh, S., Hodge, J.W., Ahlers, J.D., Burke, D.S., Schlom, J., Berzofsky, J.A., 2003. Selective induction of high avidity CTL by altering the balance of signals from antigen presenting cells. J. Immunol. 170, 2523–2530.

Oh, S., Terabe, M., Pendleton, C.D., Bhattacharyy, A., Bera, T.K., Epel, M., Reiter, Y., Phillips, J., Linehan, W.M., Kasten-Sportes, C., Pastan, I., Berzofsky, J.A., 2004. Human CTL to wild type and enhanced epitopes of a novel prostate and breast tumor-associated protein, TARP, lyse human breast cancer cells. Cancer Res. 64, 2610–2618.

Ovsyannikova, I.G., Dhiman, N., Jacobson, R.M., Vierkant, R.A., Poland, G.A., 2003. Frequency of measles virus-specific CD4+ and CD8+ T cells in subjects seronegative or highly seropositive for measles vaccine. Clin. Diagn. Lab. Immunol. 10 (3), 411–416.

Pan, C.-H., Valsamakis, A., Colella, T., Nair, N., Adams, R.J., Polack, F.P., Greer, C.E., Perri, S., Polo, J.M., and Griffin, D.E., in press. Modulation of disease, T cell responses, and measles virus clearance in monkeys vaccinated with H-encoding alphavirus replicon particles. Proc. Natl. Acad. Sci.

Parker, K.C., Bednarek, M.A., Coligan, J.E., 1994. Scheme for ranking potential HLA-A2 binding peptides based on independent binding of individual peptide side-chains. J. Immunol. 152 (1), 163–175.

Pascolo, S., Bervas, N., Ure, J.M., Smith, A.G., Lemonnier, F.A., Perarnau, B., 1997. HLA-A2.1-restricted education and cytolytic activity of CD8+ T lymphocytes from B2 microglobulin (B2 m) HLA-A2.1 monochain transgenic H-2Db B2 m double knockout mice. J. Exp. Med. 185, 2043–2051.

Permar, S.R., Klumpp, S.A., Mansfield, K.G., Kim, W.K., Gorgone, D.A., Lifton, M.A., Williams, K.C., Schmitz, J.E., Reimann, K.A., Axthelm, M.K., Polack, F.P., Griffin, D.E., Letvin, N.L., 2003. Role of CD8(+) lymphocytes in control and clearance of measles virus infection of rhesus monkeys. J. Virol. 77 (7), 4396–4400.

Polack, F.P., Lee, S.H., Permar, S., Manyara, E., Nousari, H.G., Jeng, Y., Mustafa, F., Valsamakis, A., Adams, R.J., Robinson, H.L., Griffin, D.E., 2000. Successful DNA immunization against measles: neutralizing antibody against either the hemagglutinin or fusion glycoprotein protects rhesus macaques without evidence of atypical measles. Nat. Med. 6 (7), 776–781.

Polo, J.M., Gardner, J.P., Ji, Y., Belli, B.A., Driver, D.A., Sherrill, S., Perri, S., Liu, M.A., Dubensky Jr., T.W., 2000. Alphavirus DNA and particle replicons for vaccines and gene therapy. Dev. Biol. (Basel) 104, 181–185.

Rammensee, H.-G., Friede, T., Stevanovíc, S., 1995. MHC ligands and peptide motifs: first listing. Immunogenetics 41, 178–228.

Richardson, C., Hull, D., Greer, P., Hasel, K., Berkovich, A., Englund, G., Bellini, W., Rima, B., Lazzarini, R., 1986. The nucleotide sequence of the mRNA encoding the fusion protein of measles virus (Edmonston strain): a comparison of fusion proteins from several different paramyxoviruses. Virology 155 (2), 508–523.

Rose, J.W., Bellini, W.J., McFarlin, D.E., McFarland, H.F., 1984. Human cellular immune response to measles virus polypeptides. J. Virol. 49 (3), 988–991.

Ruppert, J., Sidney, J., Celis, E., Kubo, R.T., Grey, H.M., Sette, A., 1993. Prominent role of secondary anchor residues in peptide binding to HLA-A2.1 molecules. Cell 74, 929–937.

Schmid, A., Spielhofer, P., Cattaneo, R., Baczko, K., ter Meulen, V., Billeter, M.A., 1992. Subacute sclerosing panencephalitis is typically characterized by alterations in the fusion protein cytoplasmic domain of the persisting measles virus. Virology 188 (2), 910–915.

Shirai, M., Arichi, T., Nishioka, M., Nomura, T., Ikeda, K., Kawanishi, K., Engelhard, V.H., Feinstone, S.M., Berzofsky, J.A., 1995. CTL responses of HLA-A2.1-transgenic mice specific for hepatitis C viral peptides predict epitopes for CTL of humans carrying HLA-A2.1. J. Immunol. 154, 2733–2742.

Siegrist, C.A., Barrios, C., Martinez, X., Brandt, C., Berney, M., Cordova, M., Kovarik, J., Lambert, P.H., 1998. Influence of maternal antibodies on vaccine responses: inhibition of antibody but not T cell responses allows successful early prime-boost strategies in mice. Eur. J. Immunol. 28 (12), 4138–4148.

Song, M.K., Vindurampulle, C.J., Capozzo, A.V., Ulmer, J., Polo, J.M., Pasetti, M.F., Barry, E.M., Levine, M.M., 2005. Characterization of immune responses induced by intramuscular vaccination with DNA vaccines encoding measles virus hemagglutinin and/or fusion proteins. J. Virol. 79 (15), 9854–9861.

Stittelaar, K.J., Boes, J., Kersten, G.F., Spiekstra, A., Mulder, P.G., de Vries, P., Roholl, P.J., Dalsgaard, K., van den Dobbelsteen, G., van Alphen, L., Osterhaus, A.D., 2000. In vivo antibody response and in vitro CTL activation induced by selected measles vaccine candidates, prepared with purified Quil A components. Vaccine 18 (23), 2482–2493.

Stittelaar, K.J., deSwart, R.L., Vos, H.W., vanAmerongen, G., Sixt, N., Wild, T.F., Osterhaus, A.D., 2002. Priming of measles virus-specific humoral-

and cellular-immune responses in macaques by DNA vaccination. Vaccine 20 (16), 2022–2026.

Tamin, A., Rota, P.A., Wang, Z.D., Heath, J.L., Anderson, L.J., Bellini, W.J., 1994. Antigenic analysis of current wild type and vaccine strains of measles virus. J. Infect. Dis. 170 (4), 795–801.

Tishon, A., LaFace, D.M., Lewicki, H., van Binnendijk, R.S., Osterhaus, A., Oldstone, M.B., 2000. Transgenic mice expressing human HLA and CD8 molecules generate HLA-restricted measles virus cytotoxic T lymphocytes of the same specificity as humans with natural measles virus infection. Virology 275 (2), 286–293.

van Binnendijk, R.S., Poelen, M.C., Kuijpers, K.C., Osterhaus, A.D., Uytdehaag, F.G., 1990. The predominance of CD8+ T cells after infection with measles virus suggests a role for CD8+ class I MHC-restricted cytotoxic T lymphocytes (CTL) in recovery from measles. Clonal analyses of human CD8+ class I MHC-restricted CTL. J. Immunol. 144 (6), 2394–2399.

van Binnendijk, R.S., van Baalen, C.A., Poelen, M.C.M., De Vries, P., Boes, J., Cerundolo, V., Osterhaus, A.D.M.E., Uytdehaag, F.G.C.M., 1992. Measles virus transmembrane fusion protein synthesized de novo or presented in immunostimulating complexes is endogenously processed for HLA class I- and class II-restricted cytotoxic T cell recognition. J. Exp. Med. 176, 119–128.

van Binnendijk, R.S., Poelen, M.C., van Amerongen, G., de Vries, P., Osterhaus, A.D., 1997. Protective immunity in macaques vaccinated with live attenuated, recombinant, and subunit measles vaccines in the presence of passively acquired antibodies. J. Infect. Dis. 175 (3), 524–532.

van Els, C.A., Nanan, R., 2002. T cell responses in acute measles. Viral. Immunol. 15 (3), 435–450.

van Els, C.A., Herberts, C.A., van der Heeft, E., Poelen, M.C., van Gaans-van den Brink, J.A., van der Kooi, A., Hoogerhout, P., Jan ten Hove, G., Meiring, H.D., de Jong, A.P., 2000. A single naturally processed measles virus peptide fully dominates the HLA-A*0201-associated peptide display and is mutated at its anchor position in persistent viral strains. Eur. J. Immunol. 30 (4), 1172–1181.

Vitiello, A., Marchesini, D., Furze, J., Sherman, L.A., Chesnut, R.W., 1991. Analysis of the HLA-restricted influenza-specific cytotoxic T lymphocyte response in transgenic mice carrying a chimeric human-mouse class I major histocompatibility complex. J. Exp. Med. 173, 1007–1015.

Ward, B.J., Boulianne, N., Ratnam, S., Guiot, M.C., Couillard, M., De Serres, G., 1995. Cellular immunity in measles vaccine failure: demonstration of measles antigen-specific lymphoproliferative responses despite limited serum antibody production after revaccination. J. Infect. Dis. 172 (6), 1591–1595.

Watts, C., Powis, S., 1999. Pathways of antigen processing and presentation. Rev. Immunogenet. 1 (1), 60–74.

Wiertz, E.J., Jones, T.R., Sun, L., Bogyo, M., Geuze, H.J., Ploegh, H.L., 1996. The human cytomegalovirus US11 gene product dislocates MHC class I heavy chains from the endoplasmic reticulum to the cytosol. Cell 84 (5), 769–779.

Wild, T.F., Bernard, A., Spehner, D., Drillien, R., 1992. Construction of vaccinia virus recombinants expressing several measles virus proteins and analysis of their efficacy in vaccination of mice. J. Gen. Virol. 73 (Pt. 2), 359–367.

Wild, T.F., Bernard, A., Spehner, D., Villeval, D., Drillien, R., 1993. Vaccination of mice against canine distemper virus-induced encephalitis with vaccinia virus recombinants encoding measles or canine distemper virus antigens. Vaccine 11 (4), 438–444.

Zhu, Y., Rota, P., Wyatt, L., Tamin, A., Rozenblatt, S., Lerche, N., Moss, B., Bellini, W., McChesney, M., 2000. Evaluation of recombinant vaccinia virus-measles vaccines in infant rhesus macaques with preexisting measles antibody. Virology 276 (1), 202–213.