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Induction of measles virus-specific cytotoxic T-cell responses after intranasal immunization with synthetic peptides

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SUMMARY

We have investigated the structural requirements for the induction of cytotoxic T-cell responses (CTL) *in vivo* after intranasal immunization with an immunodominant CTL epitope from the nucleoprotein of measles virus (MV). For the induction of CTL responses, covalent linkage of the CTL epitope to a helper T-cell epitope was required and the orientation of the epitopes influenced the immunogenicity of the CTL epitope. The presence of two copies as compared with one copy of a T-helper epitope, rendered the CTL epitope more immunogenic and resulted in the *in vivo* induction of MV-specific CTLs without the need for an adjuvant. The role of CTL responses to this epitope in protection after intranasal administration was evaluated in a mouse model against challenge with a neuroadapted strain of MV. Although a decreased mortality in the peptide immunized compared with that in unimmunized mice was observed, the protection achieved was not significant. These findings highlight the importance of the rational design of synthetic immunogens for the induction of CTL responses and the potential of the intranasal route for immunization.

INTRODUCTION

Measles virus (MV), a member of the Morbillivirus genus of the Paramyxoviridae family causes an acute infection in early childhood and despite the existence of an effective vaccine, the virus still remains a major cause of infant morbidity and mortality in developing countries. This may be attributed to: (1) the inability to immunize efficiently before the age of 9 months in the presence of maternal antibodies that block the replication of vaccine virus *in vivo*, and (2) the necessity for an effective cold chain for storing the vaccine. There is an urgent need for the development of new stable vaccines that can be effective even in the presence of maternal antibodies. The use of synthetic peptides as candidate vaccines has given promising results in an animal model of MV infection[1] and recent studies have highlighted the potential of peptides for immunization in humans.[2] Compared with conventional vaccines, peptide vaccines have the advantage of being inexpensive, easy to manufacture, and stable. Although antibodies have been shown to confer long-lasting immunity to MV, patients with depressed T-cell responses suffer from severe complications.[3] Thus, for a future measles synthetic peptide vaccine, epitopes stimulating antibody production (B-cell epitopes) and epitopes stimulating cellular immunity (T-helper and cytotoxic T-cell epitopes) will be required. The importance of cytotoxic T-lymphocytes (CTLs) in controlling MV infection is still unclear. However, several studies have suggested the role of $CD8^+$ T cells in recovery from measles infection[4,5] and the possibility exists that they may function as memory cells in maintaining lifelong immunity against measles.[6] Such CTLs kill virally infected cells when their T-cell receptor recognizes viral peptides associated with major histocompatibility complex (MHC) class I glycoproteins.[7] For the experimental induction of CTL responses, replicating vectors have generally been used but it is likely that their use in humans will be problematic and thus synthetic peptide vaccines may be more appropriate for use in man. Since MV initiates infection in the upper respiratory tract, the intranasal route may be most appropriate for immunization. This route offers the advantage that it has the potential to stimulate both mucosal and systemic immunity, is simple, safe, and can be used for immunization of large population groups. Studies in animals and in humans have shown protection against a variety of viral and bacterial pathogens following intranasal immunization. In particular, intranasal immunization with live influenza vaccines has ben tested successfully in children as well as in adults,[8,9] and infants with and without maternal antibody have been successfully immunized intranasally with an aerosolized measles vaccine.[10]

In the present study the potential of the intranasal route for immunization was tested using a synthetic peptide representing a CTL epitope from MV nucleoprotein (NP). The structural and adjuvant requirements for the induction of CTL responses were examined and the protective efficacy of the CTL epitope

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was evaluated in a mouse model after intracerebral challenge with a neuroadapted strain of MV.

MATERIALS AND METHODS

Selection of peptides

Residues 288–302 of the fusion (F) protein of MV represent a promiscuous T-helper (Th) epitope in mice[11] that provides help for antibody production to B-cell epitopes for the same protein or from other proteins.[12] Residues 51–59 and 81–88 of the NP of MV represent CTL epitopes identified after immunization of CBA mice with vaccinia virus expressing MV NP.[13]

Peptide synthesis

Peptides representing residues 51–59, 81–88 and 288–302 were synthesized by solid phase synthesis using Fmoc-chemistry. In addition, chimeric peptides were produced in which the CTL epitope 51–59 was co-linearly synthesized at either the amino or carboxyl-terminus of one or two copies of the T-helper epitope (Table 1). Fmoc-protected amino acids were converted to the hydroxybenzotriazole-activated esters by treatment with hydroxybenzotriazole and N,N′-diisopropylcarbodiimide in dimethylformamide (DMF). The subsequent coupling reactions were performed in DMF and the Fmoc groups were removed with 20% piperidine in DMF followed by a series of washes in DMF. After synthesis, side-chain-protecting groups were removed and the peptide was cleaved in trifluoroacetic acid in the presence of scavengers. After cleavage, peptides were precipitated by diethylether, and their purity was assessed by analytical high-performance liquid chromatography (HPLC) and mass spectroscopy.

Mice

CBA (H-2^k) mice were 6–8-weeks old at the start of the experiments and were kept in the Biological Services Unit at the London School of Hygiene and Tropical Medicine (London, UK). Three-week-old CBA mice were used for the protection study.

Virus

The rodent neuroadapted strain of MV (CAM/RBH strain; kindly provided by Dr U. Liebert, Institute fur Virologie und Immunbiologie, Wurzburg, Germany) was used. Stocks of virus were prepared by passage in suckling-mouse brains. Mouse brain homogenates (25%) from infected suckling mice were used as a source of virus and were stored at −70°.

Immunization and virus challenge

Groups of three CBA mice were immunized intranasally with 50 μg/dose of the peptides in 30 μl phosphate-buffered saline (PBS) on three consecutive days with or without cholera toxin-B subunit (CTB) as an adjuvant (10 μg/dose, Sigma, Dorset, UK). After 3 weeks, the mice received a booster intranasal administration of the peptides (50 μg/dose) with or without CTB (10 μg/dose). In experiments where the adjuvant effect of CTB was tested, a recombinant preparation of CTB was used (kind gift from Professor J. Holmgren, Dept of Medical Microbiology and Immunology, University of Goteborg, Sweden). Two weeks later mice were killed and spleens were removed. In the virus-protection study, mice (10 per group) were immunized intranasally with 50 μg/dose of chimeric peptide TT-NP6 with 10 μg/dose CTB. Control mice received PBS. Mice were initially immunized three times on consecutive days and 2 weeks later were boosted with 50 μg/dose of chimeric peptide with 10 μg/dose CTB and challenged as described previously[1] by intracerebral injection of a neuroadapted strain of MV [10^4 plaque-forming units (PFU) in 25 μl volume]. The health and survival of the mice was monitored for a period of 30 days post-challenge.

Cell lines and culture media

The C1300 neuroblastoma, clone NS20Y of the A/J mouse strain (H-2^a) and the clone of NS20Y persistently infected with MV, termed NS20Y/MS[14] were kind gifts from Professor B. R. Zisman (Dept of Microbiology and Immunology, Ben Gurion, University of the Negev, Israel). The persistently infected cell line NS20Y/MS expresses augmented levels of both H-$2K^k$ and H-$2D^d$ MHC class I glycoproteins.[15] L929 is a fibroblast cell line of the H-2^k haplotype. All cell lines were maintained in RPMI-1640 medium supplemented with 10% heat-inactivated foetal calf serum (FCS) (Gibco, Paisley, UK) 2 mM L-glutamine, and antibiotics (10 U/ml penicillin, 10 μg/ml streptomycin). For

Table 1. Notation and sequences of peptides used in this study

Free peptides	Sequences
Th epitope (T)	
F: (288–302)	LSEIKGVIVHRLEGV
CTL epitope (NP6)	
N: (52–59)	LDRLVRLIG
CTL epitope (NP9)	
N: (81–88)	VESPGQLI

Chimeric peptides	Sequences
T-NP6	LSEIKGVIVHRLEGVLDRLVRLIG
TT-NP6	LSEIKGVIVHRLEGVLSEIKGVIVHRLEGVLDRLVRLIG
NP6-T	LDRLVRLIGLSEIKGVIVHRLEGV
NP6-TT	LDRLVRLIGLSEIKGVIVHRLEGVLSEIKGVIVHRLEGV

CTL preparations, complete medium was used containing RPMI-1640 supplemented with 10% heat-inactivated FCS, 2 mM L-glutamine, 10 mM HEPES, 50 μM 2-mercaptoethanol and antibiotics (10 U/ml penicillin, 10 μg/ml streptomycin).

In vitro *expansion of CTLs*

Spleens were removed, teased with single-cell suspensions and cultured for 7 days *in vitro* with 5 μg of NP6 peptide/ml of medium. On day 3, 10% (v./v.) rat Con A supernatant was added as a source of IL-2 to the cultures. Con A supernatants were generated by culturing 5×10^6 spleen cells/ml for two days with 5 μg/ml Con A in DMEM medium supplemented with 5% FCS, 2 mM L-glutamine, and antibiotics (10 U/ml penicillin, 10 μg/ml streptomycin).

Cytotoxicity assay

10^7 target cells in 1 ml of medium were labelled with 200 μCi of sodium [^{51}Cr] chromate (Amersham, Aylesbury, UK) for 60 min at 37°, after which the cells were washed three times with medium. 10^6 cells were resuspended in 100 μl of medium and incubated for a further 1 hr with 10 μM of NP6 or NP9 peptide. 10^4 target cells loaded with peptide in 100 μl were added to varying numbers of effector cells in 100 μl volumes, which had been washed twice prior to the assay, in 96-well flat-bottomed plates (Nunc; Life Technologies, Paisley, UK) and incubated for 5 hr at 37°, in a 5% CO_2 atmosphere. 100 μl supernatant was collected and the percentage of specific ^{51}Cr release was calculated by the following formula:

$$\% \text{ specific release} = \frac{\text{experimental} - \text{spontaneous}}{\text{maximum} - \text{spontaneous}}$$

Spontaneous release was between 10–20%. Maximum release was determined by lysis of targets by detergent (5% Triton X-100, Sigma). All assays were performed in triplicate. The standard deviation (SD) of triplicate wells was consistently less than 10% of the mean.

RESULTS

Requirement for covalent linkage between Th and CTL epitopes for the *in vivo* induction of CTLs

Effector cells from mice immunized with NP6 peptide alone or mixed with the Th epitope exhibited only marginal CTL activity (Figs 1a and b). On the other hand, effector cells from mice immunized with chimeric peptides T-NP6 and NP6-T were able to kill L929 (Figs 1c and d) and NS20Y (Figs 2a and b) targets pulsed with the NP6 peptide but not with the unrelated NP9 peptide or non-pulsed cells. Furthermore, these effector cells could also lyse NS20Y/MS target cells persistently infected with MV (Figs 2a and b respectively).

Potentiation of the immunogenicity of NP6 CTL epitope

Given that T-cell help was required for the *in vivo* induction of CTL responses, we tested whether peptides comprised of two copies of the Th epitope covalently linked to either the amino or carboxyl terminus of the CTL epitope would prime mice more effectively for a CTL response than peptides containing one copy of the Th epitope. Mice were immunized intranasally with

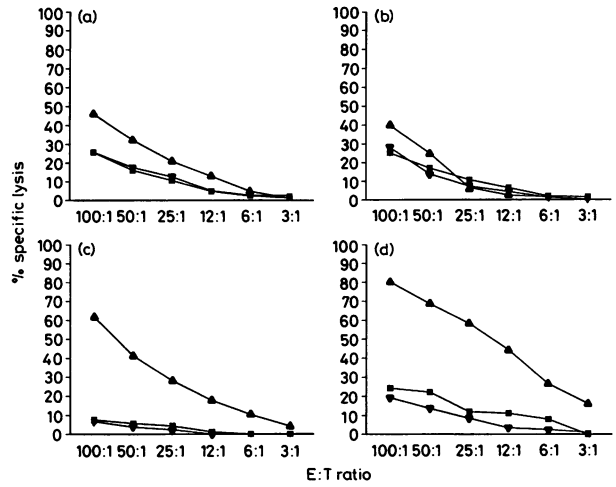

Figure 1. Induction of NP6-specific CTL responses after intranasal immunization of CBA mice with NP6 (a), mixture of NP6 and T (b), T-NP6 (c) and NP6-T (d) peptides coadministered with CTB. After *in vitro* restimulation of splenocytes with NP6 peptide, effector cells were co-cultured with L929 target cells pulsed with the NP6 peptide (▲), NP9 unrelated peptide (■) and non-pulsed targets (▼) at the indicated E : T ratios.

the TT-NP6 or NP6-TT chimeric peptides using CTB as an adjuvant. Following *in vitro* restimulation with NP6 peptide and IL-2, immune spleen cells were tested for cytolytic activity. As shown in Fig. 3(a), effector cells from mice immunized with the TT-NP6 peptide more efficiently lysed L929 target cells pulsed with NP6 peptide than cells from mice immunized with the NP6-TT chimeric peptide (Fig. 3b). No lysis was observed in non-pulsed target cells. When the responses of effector cells from mice immunized with T-NP6 and TT-NP6 were compared, TT-NP6 effector cells were shown to lyse L929 target cells pulsed with NP6 peptide more efficiently even at 3 : 1 E : T ratio (Table 2). Similarly, NS20Y cells pulsed with the NP6 peptide or persistently infected by the MV (NS20/MS cells) were more efficiently lysed by the TT-NP6 immune effector cells than by T-NP6 immune effector cells (Table 2). Neither T-NP6 nor TT-NP6 could prime *in vivo* for primary CTL responses (data not shown).

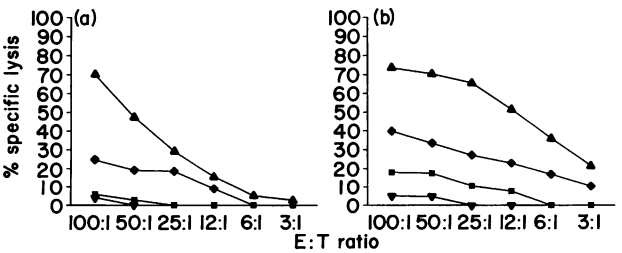

Figure 2. CTL induced after intranasal immunization of CBA mice with T-NP6 (a) or NP6-T (b) chimeric peptides coadministered with CTB. After *in vitro* restimulation of splenocytes with NP6 peptide, effector cells were co-cultured with NS20Y target cells pulsed with the NP6 peptide (▲), NP9 unrelated peptide (■), non-pulsed targets (▼), or persistently infected with MV NS20Y/MS cells (◆) at the indicated E : T ratios.

Table 2. Effect of the number of copies of T-helper epitopes on the immunogenicity of NP6 peptide. CBA mice were immunized intranasally with T-NP6 or TT-NP6 chimeric peptides using CTB as an adjuvant. After *in vitro* restimulation of splenocytes with NP6 peptide, effector cells were co-cultured with L929 or NS20Y target cells pulsed with the NP6 peptide or persistently infected with MV NS20Y/MS cells at the indicated E : T ratios. Background lysis using unpulsed L929 or NS20Y cells was less than 12% (data not shown). Data are presented as the percentage net specific lysis by subtracting the background lysis

E : T ratio	% net specific lysis					
	L929 + NP6		NS20Y + NP6		NS20Y/MS	
	T-NP6	TT-NP6	T-NP6	TT-NP6	T-NP6	TT-NP6
100 : 1	54·2	92	64·12	74·17	18·72	42·64
50 : 1	35·46	89·75	44·48	102·8	16·33	47·57
25 : 1	23·73	92·73	29·21	97·4	18·50	41·98
12 : 1	16·48	87·04	15·54	84·21	8·86	38·12
6 : 1	10·33	67·86	5·12	64·75	0	36·33
3 : 1	4·17	51·79	2·76	40·43	0	16·18

Adjuvant effect of CTB after intranasal vaccination

It has been suggested that the adjuvant effect of CTB might be due to the presence of trace amounts of holotoxin.[16] To test this possibility groups of mice were immunized intranasally with TT-NP6 peptide with either commercial CTB, recombinant CTB or in saline. As shown in Fig. 4, splenocytes from all three groups of mice can effectively lyse L929 target cells pulsed with NP6 peptide. However, the CTL responses observed in the group of mice in which commercial CTB was used as an adjuvant were much higher even at 3 : 1 E : T ratio than in groups of mice in which recombinant CTB was used as an adjuvant or peptide was administered in saline. In the group of mice in which recombinant CTB was used, the observed responses were almost equivalent to those of the group immunized with saline. Splenocytes from mice immunized with TT-NP6 peptide in saline could also lyse effectively NS20Y target cells pulsed with NP6 peptide or persistently infected with MV NS20Y/MS targets (Fig. 5) but not non-pulsed cells or targets pulsed with the Th epitope 288–302. No such response was detected in mice immunized with T-NP6 peptide in saline (data not shown).

Evaluation of the protective efficacy of intranasally administered TT-NP6 chimeric peptide

The protective efficacy of the immune response to the TT-NP6 peptide induced following intranasal immunization with CTB as an adjuvant, was tested in CBA mice. Peptide-immunized mice mounted a good CTL response 2 weeks after priming (data not shown). Following intracranial challenge with neuroadapted MV, the peptide-immunized group survived longer than mice in the control group (Fig. 6). By day 30, 37·5% of peptide-immunized mice had survived whereas all mice in the control group were dead. However, this difference was not statistically significant using the Fisher–Irwin exact test.

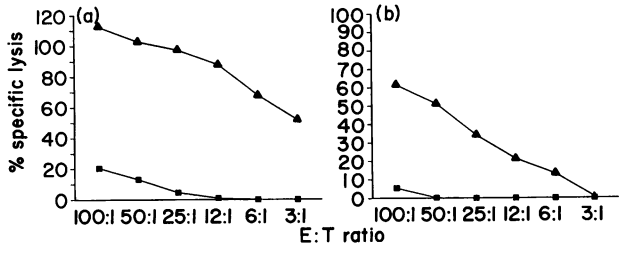

Figure 3. CTL activity after intranasally priming CBA mice with: (a) TT-NP6, or (b) NP6-TT chimeric peptides with CTB. After *in vitro* restimulation of splenocytes with NP6 peptide, effector cells were co-cultured with L929 target cells pulsed with the NP6 peptide (▲) or non-pulsed targets (■), at the indicated E : T ratios.

DISCUSSION

As the nasal mucosa is the first site of contact with inhaled antigens, the development of novel strategies to allow mucosal immunization by well-defined antigens via the nasal mucosa will be of particular importance for the development of new vaccines. Several approaches for immunization via the mucosal route have been employed. These include the use of mucosal

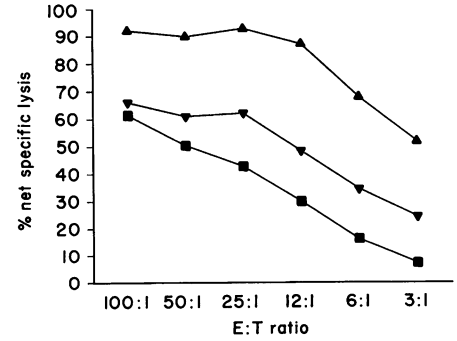

Figure 4. CTL activity induced after intranasally priming CBA mice with TT-NP6 chimeric peptide with commercial CTB (▲) (background lysis ranged from 20·47%–0·9% at 100 : 1 and 3 : 1 E : T ratio respectively), recombinant CTB (▼) (background lysis ranged from 12%–2·1% at 100 : 1 and 3 : 1 E : T ratio), or in saline (■) (background lysis ranged from 10·81%–0·15% at 100 : 1 and 3 : 1 E : T ratio respectively). After *in vitro* restimulation of splenocytes with NP6 peptide, effector cells were co-cultured with L929 target cells pulsed with the NP6 peptide at the indicated E : T ratios. Data are presented as the percentage net specific lysis by subtracting the background lysis.

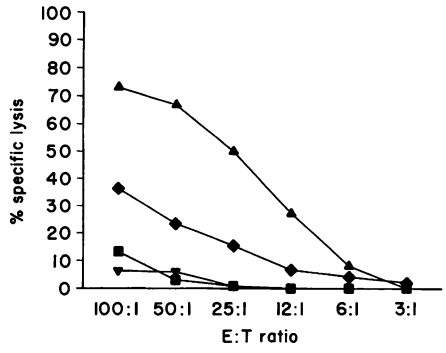

Figure 5. Priming with TT-NP6 in saline generates CTL in CBA mice. CBA mice were immunized intranasally with TT-NP6 chimeric peptide in saline. After *in vitro* restimulation of splenocytes with NP6 peptide, effector cells were co-cultured with NS20Y target cells pulsed with NP6 peptide (▲), unrelated NP9 peptide (288–302) (■), non-pulsed targets (▼) or persistently infected with MV, NS20Y/MS cells (◆) at the indicated E : T ratios.

adjuvants,[17] the linkage of antigens to mucosal immunogens,[18] the use of attenuated pathogens expressing recombinant proteins[19] and the use of different delivery systems.[20] The results presented here show that the intranasal administration of a synthetic peptide immunogen can induce systemic MV-specific CTL responses. Moreover, for the successful induction of these responses the linkage of the CTL epitope to a T-helper epitope in the appropriate orientation was required.

There is a growing body of evidence showing the successful generation of CTL responses after systemic immunization with small synthetic peptides.[21,22] However, little is known about the generation of CTL responses after intranasal administration of synthetic peptides. Although there is considerable evidence to suggest that T-helper activity is needed,[23,24] its necessity for the *in vivo* induction of CTL responses has been questioned.[25] The results presented here support the view that T-cell help is necessary. Unlike the situation of B–Th cell collaboration where extensive knowledge has been accumulated[26] there is little known about Th-CTL interaction. Based on the MHC restriction requirements for T-helper cells and CTLs, a three-cell cluster between Th, CTL and antigen-presenting cells (APCs) has been proposed in which the same APCs may present both the CTL and the T-helper epitopes through class I and class II respectively.[27,28] Activation of $CD4^+$ cells results in the secretion of cytokines such as IL-2 and interferon-γ (IFN-γ) which are essential for the generation of cell-mediated cytotoxicity[29,30] and could result in upregulation of adhesion molecules to allow direct Th : CTL contact. Such adhesion molecules could also act as modulators of T-cell receptor (TCR)-triggered signalling or they could induce additional signals.[27] However, the possibility exists that the amphipathic character of the Th epitope in the chimeric construct may facilitate cytosolic delivery and class I presentation. The essential requirement for Th and CTL epitope linkage for the *in vivo* induction of CTL responses is further highlighted by the observation that the linkage of two copies of the Th epitope to a CTL epitope can render it more immunogenic given that the orientation of epitopes is the appropriate one. This is illustrated by the strong CTL responses observed after immunization with TT-NP6 peptide as compared with the responses induced by the T-NP6 peptide (Table 2). Moreover, TT-NP6 peptide could induce peptide-specific and MV-specific CTL responses in saline. The presence of two helper epitopes likely to increase the binding affinity of the Th epitope to class II molecules[31] thus rendering the chimeric construct of the Th CTL epitopes more immunogenic. A similar effect has been demonstrated with a peptide in which two copies of a Th epitope in a Th : B-cell construct resulted in the induction of antibodies of significantly higher titre and affinity for the B-cell epitope as compared with a peptide containing one copy of the Th epitope.[32]

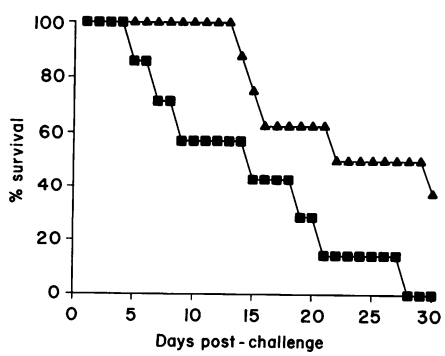

Figure 6. Survival of CBA mice immunized intranasally with PBS (■) or with TT-NP6 chimeric peptide with CTB (▲) and challenged 2 weeks later with a neuroadapted strain of MV.

In many situations it may be necessary to increase the immunogenicity of vaccine antigens by the use of an appropriate adjuvant. One such adjuvant is CTB which, when co-administered intranasally with a vaccine antigen, enhanced antibody responses.[17] In the present study, CTB was found to increase the ability of chimeric peptide TT-NP6 to induce CTL responses as compared with the responses observed when the peptide was given in saline. It has been suggested that the adjuvant effect of CTB might be due to the presence of contaminating holotoxin[16] and the data presented here are consistent with this view in that CTL activity following the use of commercial CTB was significantly higher than that observed with recombinant CTB. In these experiments administration of the TT-NP6 peptide intranasally in saline also stimulated good CTL activity, indicating the immunogenicity of this chimera in the absence of a mucosal adjuvant.

The results presented in this report also extend the observations that CTB can potentiate presentation of soluble antigens to class I-processing pathway after oral or intravenous priming[33] to small molecules such as synthetic peptides administered intranasally. The exact mechanism for the adjuvant effects of CTB are unknown but it may enhance the trasepithelial influx of the vaccine into the nasal mucosa where the immunocompetent cells are located.[34] Furthermore, CT-containing CTB can enhance the antigen presentation function of mucosal APCs.[35] Following intranasal administration, some peptide accesses the spleen where it is taken up by dendritic cells which appear to be the most important APCs for the induction of CTL responses.[36] Previous studies on the role of immune responses to MV NP in protection from MV-induced encephalitis have suggested that *in vivo* activated NP protein-specific T lymphocytes can effectively travel to MV-infected brain tissue and eliminate virus-infected cells.[37] The role of NP-specific CTLs in protection against MV-induced encephalitis in a susceptible strain of mice was

investigated by intranasally immunizing CBA mice with the immunodominant NP CTL epitope NP6 linked at the carboxyl end of two copies of a T-helper epitope with CTB as an adjuvant. Although a decrease in mortality in the peptide-immunized mice was observed, the protection achieved was not significant. This finding is in agreement with observations by Niewiesk *et al.*[38] who have suggested that strains of mice susceptible to MV encephalitis generate a poor CTL response after MV infection and the CTL induced do not recognize target cells infected with MV, despite the good lysis observed of persistently infected with MV NS20Y/MS target cells. These differences might result from variations in antigen presentation by the different APCs used or be due to low expression of MV proteins on infected cells,[38] thus resulting in a number of class I peptide complexes not sufficient enough for lysis, depending on the cell type.

The results presented in this study highlight the importance of the rational design of synthetic immunogens for the induction of CTL responses and the potential of the intranasal route for vaccination.

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REFERENCES

1. Obeid E.O., Partidos C.D., Howard C.R. & Steward M.W. (1995) Protection against Morbillivirus induced encephalitis by immunization with rationally designed synthetic peptide vaccine containing B and T cell epitopes from the fusion protein of measles virus. *J Virol* **69,** 1420.
2. Valero M.V., Amador L.R., Galindo C. *et al.* (1993) Vaccination with SPF66 a chemically synthesized vaccine against Plasmodium falciparum malaria in Colombia. *Lancet* **341,** 705.
3. Good R.A. & Zak S.J. (1956) Disturbances in gamma globulin synthesis as 'experiments of nature'. *Pediatrics* **18,** 109.
4. Kreth H.W., ter Meulen V. & Eckert G. (1979) Demonstration of HLA restricted killer cells in patients with acute measles. *Med Microbiol Immunol* **165,** 203.
5. Lucas C.J., Biddison W.E., Nelson D.L. & Shaw S. (1982) Killing of measles virus-infected cells by human cytotoxic T cells. *Infect Immunol* **38,** 226.
6. Uytdehaag F.G.C.M., van Binnendijk R.S., Kenter M.J.H. & Osterhaus A.D.M.E. (1994) Cytotoxic T lymphocyte responses against measles virus. *Curr Top Microbiol Immunol* **189,** 151.
7. Townsend A. & Bodmer H. (1989) Antigen recognition of class I restricted T lymphocytes. *Annu Rev Immunol* **7,** 601.
8. Treanor J.J., Mattison H.R., Dumyati G. *et al.* (1992) Protective efficacy of combined live intranasal and inactivated influenza A virus vaccines in the elderly. *Ann Intern Med* **117,** 625.
9. Anderson E.L., Newman F.K., Maassab H.F. & Belshe R.B. (1992) Evaluation of a cold adapted influenza B/TEXAS/84 reassortant virus (CRB-87) vaccine in young children. *J Clin Microbiol* **30,** 2230.
10. Sabin A.B., Arechiga A.F., de Castro J.F., Albrecht P., Sever J.L. & Shekarchi I. (1984) Successful immunization of infants with and without maternal antibody by aerosolized measles vaccine; ii Vaccine comparisons and evidence for multiple antibody response. *J Am Med Assoc* **251,** 2363.
11. Partidos C.D. & Steward M.W. (1990) Prediction and identification of a T cell epitope in the fusion protein of measles virus immunodominant in mice and humans. *J Gen Virol* **71,** 2099.
12. Partidos C.D., Stanley C.M. & Steward M.W. (1991) Immune responses in mice following immunization with chimeric synthetic peptides representing B and T cell epitopes of measles virus. *J Gen Virol* **72,** 1293.
13. Beauverger P., Chadwick J., Buckland R. & Wild T.F. (1994) Serotype specific and canine distemper virus cross reactive H-2^k restricted cytotoxic T lymphocyte epitopes in the measles virus nucleoprotein. *Virology* **203,** 172.
14. Rager Zisman B., Egan J.E., Kress Y. & Bloom B.R. (1984) Isolation of cold-sensitive mutants of measles virus from persistently infected murine neuroblastoma cells. *J Virol* **51,** 845.
15. Gopas J., Itzhaky D., Segev Y. *et al.* (1992) Persistent measles virus infection enhances major histocompatibility complex class I expression and immunogenicity of murine neuroblastoma cells. *Cancer Immunol Immunother* **34,** 313.
16. Tamura S., Yamanaka A., Shimohara M. *et al.* (1994) Synergistic action of cholera toxin B subunit (and *Escherichia Coli* heat-labile toxin B subunit) and a trace amount of cholera whole toxin as an adjuvant for nasal influenza vaccine. *Vaccine* **12,** 419.
17. Elson C.O. & Dertzbaugh M.T. (1994) Mucosal Adjuvants. In: *Handbook of Mucosal Immunology* (eds P.L. Ogra, M.E. Lamm, J.R. McGhee, J. Mestecky, W. Strober & J. Bienenstock), p. 391. Academic Press, San Diego, CA.
18. Hajishengallis G., Hollingshead S.K., Toshihiko K. & Russell M.W. (1995) Mucosol immunization with a bacterial protein antigen genetically coupled to cholera toxin A2/B subunits. *J Immunol* **154,** 4324.
19. Langermann S., Palaszynski S., Sadziene A., Stover K.C. & Koenig S. (1994) Systemic and mucosal immunity induced by BCG vector expressing outer surface protein A of Borrelia Burgdorferi. *Nature* **372,** 552.
20. Mowat A. & Donachi A.M. (1991) ISCOMs a novel strategy for mucosal immunization. *Immunol Today* **12,** 383.
21. Ishioka G.Y., Colon S., Miles C., Grey H.M. & Chesnut R.W. (1989) Induction of class I MHC restricted, peptide specific cytolytic T lymphocytes by peptide priming *in vitro*. *J Immunol* **143,** 1094.
22. Deres K., Schild H., Wiesmuller K.H., Jung G. & Rammensee H.G. (1989) *In vivo* priming of virus-specific cytotoxic T lymphocytes with synthetic lipopeptide vaccine. *Nature* **342,** 561.
23. Widmann C., Romero P., Maryanski J.L., Corradin G. & Valmori D. (1992) T helper epitopes enhance the cytotoxic response of mice immunized with MHC class I restricted malaria peptides. *J Immunol Methods* **155,** 95.
24. Fayolle C., Deriaud E. & Leclerc C. (1991) *In vivo* induction of cytotoxic T cell response by a free synthetic peptide requires $CD4^+$ T cell help. *J Immunol* **147,** 4069.
25. Buller R.M., Holmes K.L., Hugin A., Frederickson T.N. & Morse H.C. (1987) Induction of cytotoxic T cell responses *in vivo* in the absence of $CD4^+$ helper cells. *Nature* **328,** 77.
26. Singer A. & Hodes R.J. (1983) Mechanisms of T cell B cell interaction. *Annu Rev Immunol* **1,** 211.
27. Stuhler G. & Walden P. (1993) Collaboration of helper and cytotoxic T lymphocytes. *Eur J Immunol* **23,** 2279.
28. Rock K.L., Gamble S. & Rothstein L. (1990) Presentation of exogenous antigen with class I major histocompatibility complex molecules. *Science* **249,** 918.
29. Erard F., Corthesy P., Nabholz M. *et al.* (1985) Interleukin 2 is both necessary and sufficient for the growth and differentiation of lectin stimulated cytolytic T lymphocyte precursors. *J Immunol* **134,** 1644.
30. Simon M.M., Hochgeschwender U., Brugger U. & Landolfo S. (1986) Monoclonal antibodies to interferon inhibit interleukin 2 dependent induction of growth and maturation in lectin antigen reactive cytolytic T lymphocyte precursors. *J Immunol* **136,** 2755.
31. Sette A., Sidney J., Albertson M. *et al.* (1990) A novel approach

to the generation of high affinity class II binding peptides. *J Immunol* **145,** 1809.

32. PARTIDOS C.D., STANLEY C.M. & STEWARD M.W. (1992) The influence of orientation and number of copies of T and B cell epitopes on the specificity and affinity of antibodies induced by chimeric peptides. *Eur J Immunol* **22,** 2675.
33. BOWEN J.C., NAIR S.K., REDDY R. & ROUSE B.T. (1994) Cholera toxin acts as a potent adjuvant for the induction of cytotoxic T lymphocyte responses with non-replicating antigens. *Immunology* **81,** 338.
34. GIZURARSON S., TAMURA S.I., AIZAWA C. & KURATA T. (1992) Stimulation of the transepithelial flux of influenza HA vaccine by cholera toxin B subunit. *Vaccine* **10,** 101.
35. TAMURA S.I., ISHIHARA K., MIYATA K., AIZAWA C. & KURATA T. (1995) Mechanism of enhancement of the immune responses to influenza vaccine with cholera toxin B subunit and a trace amount of holotoxin. *Vaccine* **13,** 339.
36. NAIR S., BUITING A.M.J., ROUSE R.J.D., VAN ROOIJEN N., HUANG L. & ROUSE B.T. (1995) Role of macrophages and dendritic cells in primary cytotoxic T lymphocyte responses. *Intern Immunol* **7,** 679.
37. BANKAMP B., BRINCKMANN U.G., REICH A., NIEWIESK S., TER MEULEN V. & LIEBERT U.G. (1991) Measles virus nucleocapsid protein protects rats from encephalitis. *J Virol* **65,** 1695.
38. NIEWIESK S., BRINCKMANN U., BANKAMP B., SIRAK S., LIEBERT U.G. & TER MEULEN V. (1993) Susceptibility to measles virus induced encephalitis in mice correlates with impaired antigen presentation to cytotoxic T lymphocytes. *J Virol* **67,** 75.