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*J Immunol* 1998; 160:2425-2432; 
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Induction of CD8\(^+\) T Cell Responses to Dominant and Subdominant Epitopes and Protective Immunity to Sendai Virus Infection by DNA Vaccination\(^1\)

Yongjin Chen,\(^*\)\(^+\) Robert G. Webster,\(^*\)\(^+\) and David L. Woodland\(^2\)\(^*\)\(^+\)

While recent studies have demonstrated that DNA vaccination induces potent CD8\(^+\) T cell memory in vivo, it is unclear whether this memory is qualitatively and quantitatively comparable with that induced by natural viral infection. In the current studies, we have investigated the induction of CD8\(^+\) memory CTL responses to Sendai virus nucleoprotein (NP) in C57BL/6 mice following gene gun vaccination. The data demonstrate that this mode of vaccination induces potent long-lived memory CTL precursors (CTLp) specific for both the dominant (NP\(_{324-332}/K\(^b\)) and the subdominant (NP\(_{324-332}/D\(^b\)) epitopes of NP. The frequencies of T cells specific for each of these epitopes in the spleen is about 1:2000 CD8\(^+\) T cells, similar to those induced by intranasal infection with Sendai virus. Moreover, the induction of memory CTLp by DNA vaccination is independent of MHC class II molecules or Ab, as is the case for memory CTLp induction by live Sendai virus infection. CTLp specific for both epitopes are capable of migrating to the lung following Sendai virus infection and express potent cytotoxic activity at the site of infection. Consistent with this activity, DNA vaccination with Sendai virus NP induced a substantial degree of Ab-independent protection from a challenge with a lethal dose of Sendai virus. Taken together, these data demonstrate that for the parameters tested, DNA vaccination is indistinguishable from live virus infection in terms of priming functional memory CTLp with broad specificity for both dominant and subdominant T cell epitopes. The Journal of Immunology, 1998, 160: 2425–2432.

Viruses-specific CD8\(^+\) T cells play a central role in the immune response to acute respiratory virus infection (1–3). For example, the control of Sendai virus (a murine paramyxovirus-1 virus) infection in C57BL/6 mice is mediated principally by CD8\(^+\) CTL. Elimination of CD8\(^+\) T cells prior to or during an infection with Sendai virus results in a significant delay in viral clearance, and control of the virus in this situation is dependent on functional CD4\(^+\) T cells (4).

It has been established that CD8\(^+\) T cells recognize peptide Ags in the context of self-MHC class I glycoproteins through the TCR (5). Recognition of a relevant peptide/MHC complex triggers the specific lysis of virally infected cells and the secretion of cytokines, such as IFN-\(\gamma\) and TNF-\(\alpha\), involved in the noncytopathic inactivation of virus (6,7). The number of epitopes involved in the effector CTL response to viral infection is small relative to the total number of epitopes potentially available. This is because effector CTL responses to dominant epitopes tend to suppress responses to subdominant epitopes, a phenomenon referred to as immunodominance (8). For example, the effector CTL response to Sendai virus infection is predominantly directed against a single immunodominant NP epitope, NP\(_{324-332}/K\(^b\)) (9–11). However, CTL responses to subdominant epitopes are able to readily compensate for the loss of an effector CTL response to the dominant epitope (12–15). In addition, potent memory CTLp specific for subdominant epitopes are elicited by primary virus infection, despite the apparent absence of effector CTL responses to these epitopes (16–21).

The observation that CD8\(^+\) T cell responses tend to be focused on relatively conserved internal viral components has led to interest in vaccines designed to emphasize cell-mediated immunity (8). In this regard, both peptide- and vaccinia-based vaccines that activate CD8\(^+\) T cells have been shown to be protective against respiratory virus infections (9, 22–24). Recently, it has been established that immunization with naked DNA is an effective way of inducing both cellular and humoral immunity to viruses, parasites, bacteria, and tumor Ags (25–37). For example, it has been shown that DNA vaccination primes potent MHC class I-restricted CTL activity specific for respiratory virus Ags (38, 39). In the case of influenza nucleoprotein (NP), DNA vaccination has been shown to confer protection against infection with other strains of influenza virus (39, 40).

While the efficacy and utility of DNA vaccines have been established, relatively little is known about their mechanism of action or the quality of the CD8\(^+\) T cell response elicited. For example, it is not clear whether concomitant Ab responses are necessary for the efficient priming of CTL precursors (CTLp) in vivo. In addition, it is not clear whether DNA vaccination elicits the same breadth of response as natural infection in terms of the specificity of the T cells. In the current study, we analyzed the CD8\(^+\) T cell response induced following vaccination with a plasmid construct encoding the Sendai virus NP. Various parameters of the response were investigated including the establishment of memory to both dominant and subdominant epitopes, the trafficking of primed CD8\(^+\) T cells to sites of infection, and the role of Ab.

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\(^{1}\) Abbreviations used in this paper: NP, nucleoprotein; BAL, bronchoalveolar lavage; CTLp, CTL precursors; CTM, complete tumor medium; EID\(_{50}\), 50% egg infectious dose; i.n., intranasal.
in establishing CD8+ T cell immunity. The data show that vaccination with DNA encoding Sendai NP can prime strong CTL responses specific for both dominant and subdominant epitopes, and that neither MHC class II+ cells or Ab are required for the priming of these T cells. In addition, primed CD8+ memory T cells are able to migrate to the lung following intranasal (i.n.) Sendai virus infection. Finally, DNA vaccination with Sendai NP confers Ab-independent protection against challenge with a lethal dose of Sendai virus.

**Materials and Methods**

**Mice**

Female C57BL/6 (H-2b), B6CBA/F1/J (H-2b/b), and B6.C-H-2bm1/BjJ (H-2bm1) mice were purchased from The Jackson Laboratory (Bar Harbor, ME). C2 (H-2b) mice, which lack MHC class II molecules (41), and µMT (H-2b) mice, which lack mature B cells (42), were bred under license at St. Jude Children’s Research Hospital (Memphis, TN). All mice were held under specific pathogen-free conditions until infection or immunization.

**Cell lines and culture conditions**

The L929, L929-Kb, L929-Dd, SVBM1, and MC57G cell lines have been described previously (43–46). SVBM1 cells were a kind gift of Dr. Linda Gooding (Emory University School of Medicine, Atlanta, GA), and Cos7 cells were provided by Dr. Mary Ellen Conley (St. Jude Children’s Research Hospital). All cell lines were maintained in complete tumor medium (CTM) (47) at 37°C in the presence of 10% CO2. L929-Dd and L929-Kb were cultured in CTM supplemented with 0.5 mg/ml G418 (Life Technologies, Grand Island, NY) to maintain Dd and Kb expression.

**Synthetic peptide**

Sendai virus NP peptide, NP324-332 (FAPGNYPAL), was synthesized at St. Jude Children’s Research Hospital Center for Biotechnology on an Applied Biosystems model 433A peptide synthesizer (Berkeley, CA). Peptide purity was monitored by reverse phase HPLC analysis. Peptide stock solutions were prepared in PBS and diluted in CTM to the appropriate concentration.

**Generation of polyclonal CTL lines and limiting dilution analysis**

Short-term CTL lines were generated from the spleens of immunized mice as described previously (16). Briefly, spleen cells were teased through nylon meshes to generate single-cell suspensions and then depleted of erythrocytes by suspension in Gey's solution for 5 min. Spleen cells (6 x 10^7) from immunized mice were incubated with 6 x 10^6 irradiated syngeneic splenocytes in 2 ml CTM in the presence of 0.5 µg/ml NP324-332 and 10 U human rIL-2 in 24-well plates for 5 to 6 days. It should be noted that the NP324-332 peptide binds to Kb and Dd with approximately equal affinity and stimulates both NP324-332-Kb- and NP324-332-Dd-specific T cells in this system. Limiting dilution analysis was used to determine CTLp frequencies as described previously (16, 48, 49). Briefly, titered numbers of spleen cells from vaccinated mice were cocultured with 5 x 10^7 irradiated C57BL/6 splenocytes in the presence of 0.5 µg/ml NP324-332 peptide and 10 U/ml human rIL-2 in a final volume of 200 µl in round-bottom microtiter wells. After 7 days, the contents of individual wells were split and tested in a 6-h cytotoxicity assay on 2 x 10^3 Cr-labeled target cells. Minimal estimates of CTL frequency were obtained according to the Poisson equation of the slope of the line (cell number vs log fraction of nonresponsive wells) using \( x^2 \) analysis. This yields the minimal frequency estimate and 95% confidence intervals.

**Virus infection, virus challenge**

The Enards strain of Sendai virus was prepared and stored as described previously (4, 50). Mice were anesthetized by i.p. injection with Avertin (2,2,2-tribromoethanol) and infected i.n. with either 1 x 10^5 50% egg infectious doses (EID50) of Sendai virus for the generation of CTL responses, or lethal doses of Sendai virus for the protection studies (1.32 x 10^6 EID50 for C2 and C57BL/6 mice and 6.6 x 10^5 EID50 for µMT mice). Lymphocytes responding in the lungs of infected mice were collected 7 or 8 days after infection by bronchoalveolar lavage (BAL), as described previously (49). The cells were allowed to adhere to a tissue culture flask at 4°C for 1 hr at room temperature to remove the adherent cells. The nonadherent BAL cells were depleted of erythrocytes with Geys' solution and then used directly in the cytotoxicity assay. For the protection studies, mice were observed for 21 days following challenge with a lethal dose of Sendai virus.

**Cytotoxicity assay**

Cytotoxic activity was determined as described previously (16). Target cells (L929, L929-Kb, L929-Dd, MC57G, or SVBM1) were labeled with Na251CrO4 (Amersham Life Science, Arlington Heights, IL) and either infected with Sendai virus or pulsed with NP324-332 peptide. Various numbers of effector CTL were incubated with 2 x 10^3 target cells in 96-well plates at 37°C in the presence of 10% CO2 for 5 h. The percent of specific release was calculated by the formula: % of specific release = ([experimental release - spontaneous release]/[maximum release - spontaneous release]) x 100 cpm. Spontaneous release was typically <20% of the total release induced by 1% Triton X-100.

**Construction and purification of DNA vaccine**

The pJW4303 vector has been described previously (51). It contains a CMV immediate/early gene promoter that drives the transcription of the encoded gene. The vector was linearized with BamH1 and HindIII, and the ends were filled in with T4 DNA polymerase. An EcoRI/BamH1 fragment encoding the entire cDNA of Sendai virus NP was isolated from pTF1-SNP, kindly provided by Drs. A. Portner and T. Takimoto (St. Jude Children’s Research Hospital). The ends of the NP gene were filled in with T4 DNA polymerase and the fragment was blunt-ligated to pJW4303 with a DNA Ligation Kit (Takara Shuzo Co., Ltd., Kyoto, Japan) to generate pJW-SNP. pJW-SNP was shown to contain the full length Sendai NP gene in the correct orientation. Both pJW4303 and pJW-SNP were transformed into C600 bacteria, cultured in Luria Broth, and purified with a Qia gen Maxiprep Kit (Chatsworth, CA).

**Transfection**

Cos7 (10^5) cells were transfected with 10 µg of the pJW-SNP plasmid by electroporation with a Gene Pulser II apparatus (Bio-Rad, Hercules, CA). Briefly, cells were trypsinized, washed, and resuspended in 0.7 ml of PBS in the presence of 10 µg pJW-SNP. Cells were then transferred to a 0.4-cm Gene Pulser Cuvette (Bio-Rad) and pulsed twice using a voltage of 850 V, a capacitor setting of 25 microfarad, and a time constant after pulse of about 0.5 milliseconds. After electroporation, the cells were rested on ice for 5 min and then transferred to 12-well plates containing 4 ml CTM. The electroporated cells were cultured for 2 days and then tested for NP gene expression using an immunobrown assay. Briefly, transfected cells were permeabilized with pure methanol for 15 min prior to the addition of a mouse mAb specific for Sendai NP, A-SVN (kindly provided by Dr. A. Portner). Then biotinylated anti-mouse Ig Ab (Vector Laboratories, Burlingame, CA) was added and incubated for an additional 30 min. The cells were then washed, and streptavidin-coupled horseradish peroxidase (Vector Laboratories) was added followed by 3,3-diaminobenzidine tetrahydrochloride (Sigma Chemical Co., St. Louis, MO). Cells expressing Sendai NP turned brown and could be identified by standard light microscopy. Transfection efficiency was ~30%.

**Immunization of mice**

The Accell helium-driven particle delivery device (gene gun) (Auragen Inc., Middleton, WI) was used in the experiments. 40 µg of pJW-SNP or pJW4303 was coated onto 25 mg of 2.1-diameter gold beads (Auragen Inc.) in the presence of 100 µl of 1 M CaCl2 and 100 µl of 0.05 M spermidine. Following precipitation of DNA onto the gold beads, the DNA was washed three times with ethanol and then resuspended in 3.6 ml ethanol in the presence of 0.1 mg/ml polyvinyl pyrrolidone. The DNA/gold was loaded onto the interior surface of Tefzel tubing (McMaster-Carr, Chicago, IL) using a tube loader. The tubing was cut into 0.5-inch segments, each of which contained about 0.4 µg to 0.6 µg of DNA. In general, 8- to 12-wk-old mice were vaccinated twice (at a 3-wk interval) on a shaved region of the abdomen.

**Results**

**DNA immunization elicits strong CTL response specific for both dominant and subdominant epitopes**

Previous studies have shown that infection of C57BL/6 mice with Sendai virus, a murine parainfluenza-1 virus (52), elicits a potent effecter CTL response in the lung that appears to be exclusively directed to an immunodominant NP epitope, NP324-332-Kb (9–11). However, the pool of memory CTLp that are established following viral clearance include populations specific for both the dominant
NP324–332/Kb and subdominant NP324–332/Db epitopes (16). Importantly, CTLp specific for each of these epitopes are present at similar frequencies, indicating that potent memory CTLp specific for subdominant epitopes can be induced in the absence of a detectable effector response. To investigate whether a similar spectrum of CTLp is elicited by DNA immunization, C57BL/6 mice were immunized twice (at 3-wk intervals) with a plasmid containing the Sendai virus NP gene, pJW-SNP. Control animals were immunized with the empty vector, pJW4303. Two weeks after the second immunization, splenocytes were restimulated in vitro with the NP324–332 peptide under limiting dilution conditions. As shown in Figure 2A and Table 1, the frequency of CTLp specific for the dominant NP324–332/Kb epitope was ~1:9000 spleen cells (equivalent to 1:1500 CD8+ T cells). This is similar to the frequency of memory NP324–332/Kb-specific CTLp elicited by Sendai virus infection (~1:1000 CD8+ T cells) (16). The frequency of CTLp specific for the subdominant NP324–332/Db epitope was ~1:24,000 spleen cells (equivalent to 1:3500 CD8+ T cells). Again, this is very similar to the frequency of NP324–332/Db-specific CTLp elicited by Sendai virus infection (~1:2000 CD8+ T cells) (16). Thus, these are the first data to clearly demonstrate that DNA vaccination can induce high frequency T cell memory to subdominant epitopes in the face of a competing response to a dominant epitope. Notably, memory CTLp populations specific for both dominant and subdominant epitopes were long-lived and could be readily recovered 5 mo after the initial vaccination (Table 1). In addition, similarly high frequencies of memory CTLp were also generated following only a single immunization with the appropriate DNA construct (data not shown).

**DNA-primed CTLp migrate to the lung following Sendai virus infection**

Although the data demonstrated that DNA vaccination was able to prime CTLp specific for both dominant and subdominant T cell epitopes, it was not clear that such cells had the appropriate avidity, longevity, or homing properties to effectively participate in a subsequent immune response. To investigate this, we took advantage of a system that allowed us to track primed T cells to the site of infection.
of infection. Previous studies have shown that H-2b-restricted T cells dominate the effector CD8\(^+\) T cell response to Sendai virus infection in the lungs of B6CBAF1 mice (H-2\(^{bk}\)) (16). Despite the presence of the K\(^b\) MHC molecule, there is little to no detectable effector response to the NP\(_{324-332}/K^b\) epitope, which has been rendered subdominant in this strain of mice. However, priming of NP\(_{324-332}/K^b\)-specific T cells prior to infection (for example, by infection with a recombinant vaccinia virus that expresses NP) results in a switch in the specificity of effector cells in the lung induced by subsequent Sendai virus infection (16). Thus, to demonstrate that CTLp primed by DNA vaccination could be recruited to the site of infection, B6CBAF1 mice were vaccinated with either pJW-SNP or pJW4303. Two weeks after the second vaccination, the mice were infected i.n. with Sendai virus, and the specificity of the BAL was assessed 8 days later. As shown in Figure 3A, prior vaccination with the control vector (pJW4303) resulted in the expected dominance of H-2\(^{b}\)-restricted effector CTL in the lung following Sendai virus infection. However, prior vaccination with pJW-SNP resulted in a complete switch in specificity such that the response was now dominated by NP\(_{324-332}/K^b\)-specific effector CTL (Fig. 3B). These data demonstrate that memory CTLp induced by DNA vaccination are able to traffic to and function at the site of infection.

We also analyzed the effect of pJW-SNP vaccination in B6.C-H-2\(^{bm1}\) mice that lack the K\(^b\) molecule and normally mount a Sendai virus-specific effector CTL response that is dominated by HN/K\(^bm1\)-specific CTL (Fig. 3C). Sendai virus infection of B6.C-H-2\(^{bm1}\) mice that had been vaccinated with pJW-SNP elicited a potent effector CTL response to the subdominant NP\(_{324-332}/D^b\) epitope (Fig. 3D). It should be noted in this case that the SVBM1 target cells coexpress K\(^bm1\) and D\(^b\) so that the true level of K\(^bm1\)-restricted lysis cannot be determined in Figure 3D. Again, the data demonstrate that memory CTLp primed by DNA vaccination are fully functional in the context of a subsequent live virus infection. As a control for these experiments, we also vaccinated C57BL/6 mice with pJW4303 and pJW-SNP (Fig. 3, E and F). Vaccination with pJW-SNP in this case will prime CTLp specific for both the dominant NP\(_{324-332}/K^b\) and subdominant NP\(_{324-332}/D^b\) epitopes (Fig. 1). As expected, prior vaccination did not alter the specificity of the effector response in the lung, except to allow detection of a minor NP\(_{324-332}/D^b\)-specific effector response (Fig. 3F) that is never seen upon primary virus challenge. Thus, the normal pattern of immunodominance between NP\(_{324-332}/K^b\) and NP\(_{324-332}/D^b\) is maintained.

**Immunization with DNA encoding NP confers protection against viral challenge**

Having shown that memory CTLp primed by DNA vaccination could participate in an effector CTL response to subsequent Sendai virus infection, we next asked whether this vaccination protocol induced any degree of protection against virus challenge. Cohorts of 10 C57BL/6 mice were vaccinated twice with either pJW4303 or pJW-SNP. An additional cohort of 10 control mice was left unimmunized. Two weeks later, all of the mice were challenged i.n. with a lethal dose of live Sendai virus (1.32 × 10\(^5\) EID\(_{50}\), equivalent to 41 LD\(_{50}\)). As shown in Figure 4, all of the mice that were either left untreated or vaccinated with the blank pJW4303 vector died between days 7 and 9. In contrast, the pJW-SNP-vaccinated mice showed substantial protection, with 6 of 10 mice surviving for 21 days. This is a substantial degree of protection for a CD8\(^+\) T cell protection against respiratory virus following vaccination with either peptides or vaccinia-recombinants (9, 22–24). These data are also consistent with studies in mice demonstrating protection from a lethal dose of influenza virus following DNA vaccination with an influenza NP construct (39).

**DNA immunization confers protection against viral challenge, which is independent of Ab**

The experiments described above as well as other published reports have demonstrated that DNA immunization with genes encoding internal viral components can confer protective immunity (39, 40). While this protection appears to be mediated by CD8\(^+\) T cells, the role of humoral responses in protection has not been completely excluded. To determine whether Ab is required for this protective response, we took advantage of B cell-deficient (µMT, H-2\(^b\))(42) and MHC class II\(^-\) (C2\(^-\), H-2\(^b\)) (41) mice that are both...
profoundly deficient in generating Ab responses. In initial experiments, we assessed whether DNA vaccination was able to induce CD8+ T cell responses in these mouse strains. mMT and C22m mice were vaccinated with pJW-SNP, and splenocytes were subsequently restimulated in vitro with the NP324–332 peptide. As shown in Figure 5, CTLp were primed against both dominant and subdominant epitopes, although this priming appeared to be less efficient compared with intact C57BL/6 mice in terms of lytic activity. In the case of mMT mice, there was a specific reduction in the lytic activity of CTL specific for the subdominant NP324–332/Db epitope (Fig. 5A). Nonetheless, these data demonstrate that neither class II+ cells nor functional B cell responses are essential for the priming of CD8+ memory CTLp by DNA vaccination. This is consistent with the observation that these cells are not essential for the priming of CD8+ responses to live virus infection and further demonstrates that DNA vaccination induces responses similar to those induced by live virus (4, 53, 54).

We next investigated whether DNA vaccination with the pJW-SNP construct was protective in MHC class II-deficient or B cell-deficient mice. Cohorts of 10 mMT and C22m mice were vaccinated with pJW4303, pJW-SNP, or left unimmunized. Mice were subsequently challenged i.n. with a lethal dose (1.32 ± 10^5 EID50 for C22m mice and 6.6 ± 10^4 EID50 for mMT mice) of live Sendai virus. As shown in Figure 6, vaccination with pJW-SNP induced significant protection in both MHC class II-deficient and B cell-deficient mice. This level of protection was similar to that observed following pJW-SNP immunization of intact mice. Thus, these data strongly suggest that humoral responses are not required for the induction of protective responses by DNA vaccination with pJW-SNP.

**Discussion**

While several studies have shown that DNA vaccines are capable of inducing potent effector and memory CTL responses, the underlying mechanisms has not been established. It is believed that DNA elicits local inflammatory responses by virtue of immunostimulatory sequences encoded within the vectors and that local APCs take up and express the encoded genes and present Ag to T cells in the lymph nodes and spleen (55). Consistent with this, several studies have shown that gene gun immunization results in the in vivo transfection of skin-derived dendritic cells that migrate to local lymph nodes (56), and that bone marrow-derived cells play an important role in inducing a CTL response (57, 58). Whatever the mechanism, it is unclear whether CD8+ T cell memory induced by DNA vaccination is qualitatively and quantitatively comparable with that induced by natural viral infection. In the current
studies, we investigated the induction of CD8\(^+\) memory CTL responses to Sendai virus NP following gene gun vaccination. The data indicate that this mode of vaccination induces long-lived memory CTLp specific for both dominant and subdominant epitopes at frequencies similar to those observed following i.n. infection with Sendai virus. These CTLp are fully functional and are able to participate in effector functions at the site of a subsequent Sendai virus infection. In addition, this type of vaccination provides a substantial degree of protection from a challenge with a lethal dose of Sendai virus. The induction of memory CTLp by DNA vaccination is independent of MHC class II molecules or Ab, as is the case for memory CTLp induction by live Sendai virus infection (4, 54). Thus, these data demonstrate that for the parameters tested, DNA vaccination is indistinguishable from live virus infection in terms of the establishment of functional memory CTLp.

It has been known for some time that effector CD8\(^+\) T cell responses to infection are restricted to a relatively small number of epitopes compared with the total number of epitopes available (8). Importantly, the response to the dominant epitopes suppresses, or masks, the response to subdominant epitopes (12–15, 59). Despite the immunodominance of the effector CTL response, several recent studies have shown that the specificity of the memory CTLp induced by infection is substantially broader (17–21). For example, in the case of Sendai virus infection, similar frequencies of memory CTLp are induced to both dominant (NP\(_{324–332}\)\(\text{K}^b\)) and subdominant (NP\(_{324–332}\)\(\text{D}^b\)) epitopes, despite the fact that the effector response is almost exclusively directed at NP\(_{324–332}\)\(\text{K}^b\) (16). The observation that DNA vaccination induces memory CTLp specific for subdominant epitopes at a high frequency indicates that this mode of vaccination induces a similar quality of memory to that which is normally induced by viral infection. Another study has also shown that DNA vaccination can be used to elicit CD8\(^+\) T cells specific for a subdominant T cell epitopes (40). However, in this case the subdominant response was studied using a DNA construct in which the competitive dominant epitope had been specifically eliminated. This effectively rendered the remaining epitope immunodominant in the context of this vaccine. The current studies demonstrate that memory CTLp populations specific for subdominant epitopes can be efficiently primed despite the presence of a strongly immunodominant epitope. Moreover, the frequency of memory CTLp specific for the subdominant epitope approaches that of the dominant epitope (Table 1). The capacity to induce relevant memory to subdominant epitopes may be very important in the context of viruses that induce persistent or latent infection in the host, such as HIV or EBV. In these situations, the dominant effector CTL response may be rapidly exhausted, or the virus may be able to eliminate the dominant epitope, forcing the response to the subdominant epitope. A DNA vaccine that induces broad memory to multiple epitopes is likely to be more effective against persistent infection.

The induction of memory CTLp by natural virus infection is poorly understood. It is not clear whether memory cells represent a subset of activated effector cells, or whether there is a separate pathway of differentiation. It is known that the induction of CD8\(^+\) T cell responses and memory CTLp does not require help from CD4\(^+\) T cells. For example, potent CTL responses are generated in MHC class II– mice that lack CD4\(^+\) T cells and helper pathways following virus infection (4, 53, 54). However, it should be noted that some studies have suggested that the absence of CD4\(^+\) T cells results in a reduced formation of memory CTLp (60, 61). Vaccination of CD4-deficient mice with pJW-SNP resulted in potent priming of CTLp specific for the dominant NP\(_{324–332}\)\(\text{K}^b\) epitope, although the priming of CTLp to the subdominant NP\(_{324–332}\)\(\text{D}^b\) epitope was less efficient. The current studies also suggest that Ab responses are not absolutely required for the induction of CTLp by DNA vaccines. This indicates that the priming of CTLp by DNA vaccination does not require Ab-mediated uptake of free Ag by FeR\(^+\) APCs using alternative processing pathways (62–67). Thus, it seems likely that direct transfection of the APCs with the DNA vaccine is the primary mechanism for CTLp induction.

Several studies have shown that primed CD8\(^+\) T cells are able to provide a moderate degree of protection from challenge with a lethal dose of virus. For example, immunization with the Sendai NP\(_{324–332}\)\(\text{K}^b\) peptide provides protection from Sendai virus infection (9). In general, this protection is substantially weaker than the protection mediated by Ab, presumably because CD8\(^+\)-mediated responses are only activated following the establishment of an infection. Nonetheless, the CTL response plays an important role in control of the degree of infection by reducing the viral load to be cleared by other mechanisms. Importantly, the CTL response has the advantage in that it tends to be directed against relatively conserved viral gene products allowing heterologous protection. Established studies by other laboratories using the influenza system have shown that DNA vaccination with genes for internal viral proteins can induce protective memory responses (39). However, while a role for NP-specific Ab was ruled out, it was not clear from these studies that the response was actually mediated by CD8\(^+\) T cells. This issue was addressed in part by demonstrating that CTL cultures generated from DNA-vaccinated mice could protect animals from subsequent viral infection. But it is possible that the extensive in vitro amplification of Ag-specific CTL was crucial for this effect. The current studies demonstrate that DNA vaccine-primed CTLp are able to traffic to the site of inflammation, in this case the lung, following Sendai virus infection. Moreover, CTLp primed against subdominant epitopes effectively out-compete unprimed T cells specific for the dominant epitope. The degree of protection induced by this type of vaccination is equivalent to that seen by other vaccination strategies, such as vaccination with vaccinia-NP (22). In addition, the data show that neither conventional CD4\(^+\) T cells nor Ab is required for this type of protection.

Collectively, the current data demonstrate that the memory CTLp pool induced by DNA vaccination is very similar to that induced by natural viral infection. Thus, the breadth of specificity, the longevity, the ability to participate in a subsequent effector response, and the requirements for T cell help are all indistinguishable from responses induced by normal viral infection. These data suggest that the underlying mechanisms of priming of memory CTLp by viral infection and DNA vaccination are likely to be the same. Importantly, DNA vaccines are likely to induce CD8\(^+\) T cell memory, which produces appropriate responses to subsequent viral infection.

Acknowledgments

The authors thank Drs. Geff Cole, Michael Coppola, and Chiu Chen Huang for much help and advice in all aspects of the work, Dr. Shantha Kodihalli for help with the gene gun, and Drs. Dana Broussard, Edward Usherwood, Charles Hardy, and Marcia Blackman for critically reviewing the manuscript.

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