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Plasmid DNA Encoding CCR7 Ligands Compensate for Dysfunctional CD8+ T Cell Responses by Effects on Dendritic Cells

Seong Kug Eo, Udayasankar Kumaraguru, and Barry T. Rouse

Lymphotixin α-deficient (L.Tα−/−) mice, which lack lymph nodes and possess a disorganized spleen, develop dysfunctional CD8+ T cells upon HSV infection and readily succumb to herpes encephalitis. Such mice do develop apparently normal peptide-specific CD8+ T cell responses, as measured by MHC class I tetramer staining, but the majority of cells fail to become cytotoxic or express peptide-induced IFN-γ production. In the present study, we demonstrate that functional defects of CD8+ T cells in L.Tα−/− mice can be largely rectified by the administration of plasmid DNA encoding CCR7 ligands before HSV infection. Treated mutant mice developed increased peptide-specific cytotoxic responses, enhanced numbers of CD8+ T cells capable of producing IFN-γ, as well as improved resistance to HSV challenge. The corrective effect of chemokine treatment appeared to result from improved dendritic cell-mediated Ag presentation. Thus, a major consequence of the treatment was an increase in splenic dendritic cell number in CCR7 ligand-treated L.Tα−/− mice with such splenocyte populations showing improved APC activity in vitro. Our results document that functional defects of CD8+ T cells can be corrected, and indicate the value of plasmid vector encoding appropriate chemokines to achieve such immunotherapy. The Journal of Immunology, 2001, 167: 3592–3599.

CD8+ T cells act as important effectors in defense against several viruses and certain tumors. Recently, with the development of multiple assays to detect and quantify CD8+ T cells, it has become evident that such cells may be functionally heterogeneous. Several examples have now been described in which cells detectable by novel sensitive assays such as avidin-linked MHC tetramers show incomplete activation markers and lack one or more functions responsible for effector activity (1–3). Examples have been described for persistent virus infections, especially under circumstances in which animals have some form of immune suppression (4–6). One of the earliest reports came from the Ahmed (5) laboratory, which observed that the minor specificity of lymphocytic choriomeningitis virus-specific CD8+ T cells in persistently infected helper-cell-deficient mice failed to express cytotoxicity or to produce IFN-γ. Such cells were termed “Sisyphean” to describe their effector cell futility. Other examples of effector cell dysfunction were observed in SIV infection in macaques (6, 7) and in the brains of mice infected with coronavirus (8). In the cancer field, too, CD8+ T cells may be detectable by MHC class I tetramer-binding assays, yet such cells lack effector function (9). In all instances in which dysfunctional CD8+ T cell effectors were noted, no attempts were made to correct such defenses and to observe the outcome of such maneuvers.

Our laboratory recently reported that the CD8+ T cell response in lymphotixin α-deficient (L.Tα−/−)4 mice may also fail to functionally mature upon infection with HSV or immunization with OVA protein (10). Such mice were highly susceptible to challenge with HSV as previously observed with some other pathogens (11–13). The reasons for their increased susceptibility to infection probably relate to the fact that L.Tα−/− mice have defective lymphoid tissue (14–19). Accordingly, they lack lymph nodes and Peyer’s patches, and their splenic white pulp is disorganized in structure and contains greatly reduced numbers of mature dendritic cells (DCs) (20). L.Tα−/− spleens also lack normal expression of certain chemokines such as CCR7 ligands (21). Because these chemokines are known to mediate the interaction between mature DCs and naive T cells (22–24), it could be that their reduced expression in L.Tα−/− spleens accounts for the dysfunctional CD8+ T cell response observed in such animals. Consequently, we reasoned that if L.Tα−/− mice were given expression plasmids encoding CCR7 ligands, CD8+ T cell function could be restored. Our results support this notion, although functional restoration was not fully complete. Accordingly, systemic administration of plasmid DNA encoding either CCL21 (formerly secondary lymphoid tissue chemokine) or CCL19 (formerly EBV-induced molecules 1 ligand chemokine) to L.Tα−/− mice before infection with HSV resulted in enhanced CD8+ T cell responses. More importantly, such responses were functionally superior to those of control animals, as measured by cytotoxicity and immunodominant epitope peptide-induced intracellular IFN-γ production. In addition, treated animals were more resistant to HSV-induced encephalitis. Our novel observations document that dysfunctional CD8+ T cell responses can be corrected, and indicate the value of expression plasmids encoding appropriate chemokines to achieve this objective. The mechanisms by which chemokine immunotherapy most likely functions to compensate for the immune defects in L.Tα−/− mice were also described.

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2 S.K.E. and U.K. contributed equally to this work.

Abbreviations used in this paper: L.Tα−/−, lymphotixin α-deficient; DC, dendritic cell; wt, wild-type; CD62L, CD62 ligand; CD40L, CD40 ligand.
Materials and Methods

Mice and virus

Female 5- to 6-week-old C57BL/6 (H-2b) mice were purchased from Harlan Sprague-Dawley (Indianapolis, IN) and housed in the animal facilities at the University of Tennessee. LTα−/− mice were maintained on a pure C57BL/6 background, as described (15). All investigations follow guidelines of the Committee on the Care of Laboratory Animals Resources, Commission on Life Science, National Research Council. HSV-1 strain KOS was grown in Vero cells obtained from American Type Culture Collection (Manassas, VA). The viruses were concentrated, triturated, and stored in aliquots at −80°C until use. Titters were measured in Vero cells and expressed as PFU per milliliter.

Synthetic peptide

HSV gBgsos peptide (SSIEFARL) specific for MHC class I (H-2b)-restricted CD8+ T lymphocytes (25, 26) was chemically synthesized, purified, and quantitated by Genemed Synthesis (South San Francisco, CA).

Plasmid DNA preparation

Plasmid DNA encoding CCL21 or CCL19 was kindly provided by A. Zlotnik (Dana-Farber Research Institute, Palo Alto, CA) and J. G. Cyster (University of California, San Francisco, CA), respectively, and then inserted into the pCI-neo eukaryotic expression vector (Promega, Madison, WI). Plasmid DNA encoding gB (gB DNA) of HSV-1 KOS under the CMV promoter has been described in detail elsewhere (27). The 28S rRNA DNAs were purified by polyethylene glycol precipitation, as described previously (28).

Immunization

Groups of wild-type (wt) and LTα−/− mice (5- to 6-week-old female mice) previously given 200 μg of plasmid DNA encoding CCR7 ligand via the i.m. route were immunized with a sublethal dose (105 PFU) of live HSV-1 KOS in the hind footpad 5 days later. In some experiments, wt and LTα−/− mice were injected i.p. with plasmid DNA encoding CCR7 ligand to preferentially direct plasmid DNA into spleen, and then 5 days later immunized with live HSV-1 KOS in the hind footpad. The control mice were injected with 200 μg of empty pCI-neo control vector.

SSIEFARL-specific CD8+ T cell proliferation

CD8+ T cell proliferation was evaluated following in vitro restimulation of splenocytes with MHC class I (H-2b)-restricted SSIEFARL peptide. Briefly, the splenocytes collected from immunized mice were in vitro restimulated with irradiated SSIEFARL peptide (5 μg/ml)-pulsed syngeneic splenocytes for 3 days. [3H]Thymidine (1 μCi/well) was added to each well 18 h before harvest. Harvested cells were measured for radioactivity using a beta scintillation counter (Beckman, Fullerton, CA). They were examined daily starting from day 3 for signs of HSV infection that included mobility, wasting, limb paralysis, and encephalitis.

Statistics

Significant differences between groups were evaluated using Student’s t test.

Results

LTα−/− mice develop functionally inferior CD8+ T cell responses corrollable by CCR7 ligands

As shown in Table I, following footpad infection of wt and LTα−/− mice with a sublethal dose (105 PFU) of HSV-1 KOS, splenocytes collected 14 days after infection possessed similar numbers of SSIEFARL-specific CD8+ T cells following in vitro stimulation with peptide (5.2% for wt and 4.3% for LTα−/−). However, when testing tetramer-positive sorted cells for intracellular IFN-γ production after brief peptide stimulation, whereas 82.4% of tetramer-positive wt cells scored positive, only 10.6% of tetramer-positive LTα−/− cells produced IFN-γ (Table I). Furthermore, when measuring peptide-specific CTL activity, the response of LTα−/− splenocytes (4 LU) was markedly less than was evident in wt splenocyte populations (81 LU) (Table I). Similar data were reported in a previous publication (10), in which it was suggested that the functional defect was the consequence of inappropriate Ag
The table shows the average results from six expanded splenocytes.

The targets included SSIEFARL-pulsed MHC-matched EL4 (H-2 b ), mismatched EMT6 (H-2 k), and unpulsed EL4. The table shows only the data for peptide-pulsed EL4 targets in lytic units (LU). One LU is the number of lymphocytes required to give positive cells producing IFN- γ in vitro. The values in parenthesis represent mean percentages of the indicated cells in total splenocytes.

Consequence of CCR7 ligand DNA administration on splenic DCs

The splenic architecture in LTα −/− mice is disorganized with the content of APC, particularly DCs, less than in normal mice (14–20). Because receptors for the CCR7 ligand are present on mature DCs and naïve T cells (22–24), the immunostimulatory effects of the chemokine pretreatment were anticipated to be explained by increases in cell numbers and interaction between such cells in lymphoid tissue, especially the spleen. Seven days after administration of either CCL21 or CCL19, the spleens of treated mice had given CCR7 ligand DNA (5- to 6-fold). The effect of the CCR7 ligand pretreatment had no effect on the ratio of CD4 + to CD8 + T cells (Table II). The activation status of V β 10 + CD8 + T cells for CD62L, CD44, CD25, CD40L, and CD69 was also measured in untreated and treated LTα −/− mice. As is evident in Fig. 1, whereas the majority of LTα −/−/V β 10 + CD8 + T cells in control vector-treated animals retained the nonactivation phenotype, following CCL21 or CCL19 pretreatment the percentage of such cells expressing activation markers increased, and appeared similar in number to those of wt animals.

Table II. Summary of numbers of lymphocyte subtypes in splenocytes collected from wt and LTα −/− mice infected with HSV

<table>
<thead>
<tr>
<th>Strain</th>
<th>Treatmenta</th>
<th>Viable cell</th>
<th>CD4 +</th>
<th>CD8 +</th>
<th>V β 10 +CD8 +</th>
</tr>
</thead>
<tbody>
<tr>
<td>wt</td>
<td>pCI-neo</td>
<td>59 ± 8</td>
<td>12 ± 3 (20.3)b</td>
<td>7 ± 2 (11.9)</td>
<td>0.7 ± 0.3 (1.2)</td>
</tr>
<tr>
<td></td>
<td>CCL21</td>
<td>133 ± 26</td>
<td>29 ± 8 (21.8)</td>
<td>16 ± 6 (12.0)</td>
<td>4.3 ± 2.1 (3.2)c</td>
</tr>
<tr>
<td></td>
<td>CCL19</td>
<td>115 ± 17</td>
<td>25 ± 10 (12.7)</td>
<td>15 ± 5 (13.0)</td>
<td>4.1 ± 1.7 (3.6)d</td>
</tr>
<tr>
<td>LTα −/−</td>
<td>pCI-neo</td>
<td>87 ± 14</td>
<td>16 ± 5 (18.4)</td>
<td>9 ± 3 (10.3)</td>
<td>0.7 ± 0.4 (0.8)</td>
</tr>
<tr>
<td></td>
<td>CCL21</td>
<td>165 ± 29</td>
<td>32 ± 6 (19.4)</td>
<td>18 ± 5 (10.9)</td>
<td>5.9 ± 2.6 (3.6)d</td>
</tr>
<tr>
<td></td>
<td>CCL19</td>
<td>180 ± 29</td>
<td>35 ± 10 (19.4)</td>
<td>22 ± 4 (12.2)</td>
<td>5.6 ± 2.2 (3.1)d</td>
</tr>
<tr>
<td></td>
<td>CCL3</td>
<td>113 ± 8</td>
<td>21 ± 2 (17.9)</td>
<td>14 ± 2 (12.0)</td>
<td>NDa</td>
</tr>
</tbody>
</table>

a A single dose (200 μg) of plasmid DNA encoding CCR7 ligand was administered i.m., and injected mice were then infected with HSV-1 KOS strain in the hind footpad 5 days later. The spleens from four to five mice were collected 14 days later. The total numbers of lymphocyte subtypes in splenocytes were computed following FACS analysis.

b The values in parenthesis represent mean percentages of the indicated cells in total splenocytes.

c Statistically significant differences (p < 0.01) between pCI-neo vector-treated and CCR7 ligand-treated wt mice.

d Statistically significant differences (p < 0.01) between pCI-neo vector-treated and CCR7 ligand-treated LTα −/− mice.

ND, Not done.
increased in total cell numbers (~2- to 3-fold), as well as increases in both total T cells and especially DCs. Thus, as shown in Fig. 3A, the content of CD11c^{high} class II^{bright} cells per spleen in control vector-treated LTα^{−/−} mice was 0.7%. This contrasts with values of 2.8% and 3.1% in CCL21- and CCL19-treated LTα^{−/−} mice, respectively (Fig. 3A). CCR7 ligand treatment also significantly increased the content of mature DCs in the spleen of wt mice (Fig. 3A).

To study whether pretreatment of CCR7 ligand preferentially regulates the migration of one or the other of DC subset, FACS analysis was used to compare the content of both myeloid (CD8α^{+}CD11b{^+}) and lymphoid (CD8α^{−}CD11b{^−}) DC subsets in untreated and treated LTα^{−/−} mice. The ratio of myeloid to lymphoid DCs in LTα^{−/−} spleen was unchanged by treatment, and in fact was approximately the same as observed in wt mice (Fig. 3B). Hence, the consequence of CCR7 ligand administration appeared to increase the quantity of DCs in spleen, but not to change the subset ratio.

In Fig. 4, the data demonstrate that the splenic cells from CCR7 ligand-treated mice had superior APC function to that of control vector-treated mice. To demonstrate this, spleens from both wt and LTα^{−/−} mice were subjected to collagenase digestion to completely release DCs from the architecture of spleen. The collagenase-treated peptide-pulsed splenocytes from untreated or treated wt and LTα^{−/−} mice were then used as stimulators for enriched gB DNA-primed T cells. In such experiments, the population of CCR7 ligand-treated splenocytes induced higher peptide-specific CD8^{+} T cell proliferation and CTL responses than that of control vector-treated LTα^{−/−} splenocytes (Fig. 4, A and B). Interestingly, the increased APC activity of CCR7 ligand-treated LTα^{−/−} splenocytes was ~3- to 4-fold, correlating closely with the content of extra-DCs in the treated mutant population (Fig. 4, A and B). These results indicate that CCR7 ligand administration increases APC activity, and that this effect may be dependent on the increased number of DCs induced by CCR7 ligand treatment.

Histological consequence of CCR7 ligand administration on splenic architecture

With the observation that CCR7 ligand administration restored immunocompetence and in vitro APC activity, ectopic expression of CCR7 ligand on the surface of splenic tissue resulted in the restoration of normal lymphoid neogenesis (33). To assess the influence of CCR7 ligand administration on the disorganized lymphoid tissue, the architecture of the spleen was

### Table III.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Treatment</th>
<th>No. of Tet^{+} CD8^{+} T Cells (×10^{3})</th>
<th>Sorted Tet^{+} Cell Producing IFN-γ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>wt</td>
<td>pCl-neo</td>
<td>45</td>
<td>82</td>
</tr>
<tr>
<td>LTα^{−/−}</td>
<td>pCl-neo</td>
<td>43</td>
<td>10</td>
</tr>
<tr>
<td>CCL21</td>
<td>pCl-neo</td>
<td>119</td>
<td>28</td>
</tr>
<tr>
<td>CCL19</td>
<td>147</td>
<td></td>
<td>32</td>
</tr>
<tr>
<td>CCL3</td>
<td>42</td>
<td></td>
<td>13</td>
</tr>
</tbody>
</table>

*Equal number of splenocytes (2.5 × 10^{7} cells) collected from treated mice were used in vitro with SSIEFARL-pulsed syngeneic splenocytes for 5 days and stained to determine the number of Tet^{+} CD8^{+} T cells by FACS analysis. Numbers represent the average indicated cells from four to five mice.

* Tetramer-positive cells were sorted with FACS sorter following staining in vitro expanded splenocytes with MHC class I tetramer. To analyze the ability of each tetramer-positive cell to produce IFN-γ, sorted tetramer cells were restimulated briefly with SSIEFARL-pulsed syngeneic APC for 6 h. The number of tetramer-positive cells producing IFN-γ were determined with an intracellular cytokine staining assay. The numbers represent average percentages of IFN-γ-producing cells in tetramer-positive cells sorted from four to five expanded splenocytes.
analyzed histologically. As is evident in Fig. 5, CCR7 ligand treatment of LTα−/− mice appeared to have no discernable effect on the disorganized splenic structure. However, when the distribution and number of DCs were visualized immunohistologically, the density of DCs distributed in spleens of CCR7 ligand-treated LTα−/− mice was clearly increased (Fig. 5). This result correlated with the FACS analysis indicating increased percentages of DCs in the spleen of CCR7 ligand-treated LTα−/− mice.

The change of resistance to HSV systemic challenge in CCR7 ligand-treated LTα−/− mice

Immunity to HSV involves aspects of both CD4+ and CD8+ T cell immunity (34–38). However, protection of the peripheral and CNS appears to be mainly a property of CD8+ T cells (37, 38). Because gB498-505 peptide-specific CD8+ T cell number and function were elevated following CCR7 ligand pretreatment of LTα−/−...
mice, such animals were expected to be more resistant to encephalitis following HSV systemic challenge. To evaluate the resistance of CCR7 ligand-pretreated LTα−/− mice against HSV systemic infection, LTα−/− mice previously given control vector or CCR7 ligand DNA were infected i.m. with lethal dose (5 LD50) of HSV-1 KOS. As shown in Table IV, whereas control vector-pretreated LTα−/− mice showed no protection, CCR7 ligand pretreatment of LTα−/− mice elicited resistance up to 67% for CCL21 and 83% for CCL19 at day 10 postchallenge. However, the resistance level of CCR7 ligand-pretreated LTα−/− mice was still less than that of control vector-pretreated wt mice (Table IV). These results indicate that CCR7 ligand pretreatment results in heightened resistance to HSV-induced encephalitis.

Discussion

Functionally intact CD8+ effector T cells may mediate immunity in several ways. These include target cell killing following direct contact and immunity mediated by secreted cytokines such as IFN-γ (39, 40). Under certain circumstances, such as during some persistent virus infections or limited T cell help, CD8+ T cells may be present that possess languid effector function (4–8). As noted previously, LTα−/− mice, which lack lymph nodes and possess a disorganized spleen, develop dysfunctional CD8+ T cells upon HSV infection and readily succumb to herpes encephalitis (10). Such mice do develop apparently normal peptide-specific CD8+ T cell responses, as measured by MHC class I tetramer staining, but such mice do develop apparently normal peptide-specific CD8+ T cell responses, as measured by MHC class I tetramer staining, but the majority of cells fail to become cytotoxic or express peptide-induced IFN-γ production. In the present study, we demonstrate that functional defects of CD8+ T cells, in LTα−/− mice can be largely rectified by the administration of plasmid DNA encoding CCR7 ligands before HSV infection. Treated mutant mice developed increased peptide-specific cytotoxic responses, enhanced numbers of CD8+ T cells capable of producing IFN-γ, as well as improved resistance to HSV challenge. The corrective effect of chemokine treatment appeared to result from improved DC-mediated Ag presentation. Thus, a major consequence of the treatment was an increase in splenic DC number in CCR7 ligand-treated LTα−/− mice with such splenocyte populations showing improved APC activity in vitro. Our results document that functional defects of CD8+ T cells can be corrected, and indicate the value of plasmid vector encoding appropriate chemokines to achieve such immunotherapy.

The development of multiple assays to identify specific T cell reactivity has revealed that such cells may be functionally heterogeneous. Several examples have now been described in which cells detectable by sensitive assays, such as avidin-linked MHC tetramers, may lack one or more functions, as measurable by cytotoxicity, cytokine production, or proliferation (1–3). An early example was described by the Ahmed group (5), which coined the term “Sisyphean” to describe their effector cell futility. Such cells were present in helper cell-deficient mice persistently infected with lymphocytic choriomeningitis virus. A similar circumstance was observed in macaques persistently infected with SIV and that had suppressed CD4+ Th cell function (7). In this instance, the functional defect was correctable in vitro by prolonged in vitro culture in IL-2 (7). These and other examples of functionally defective CD8+ T cells occur in conditions of extended Ag exposure and may represent examples in which cells differentiate normally, but are subsequently anergized (41). Such a situation may not be the case in LTα−/− mice used in the present study. These animals generate CD8+ T cell responses to HSV infection or to OVA protein that numerically, as detected by MHC class I tetramer staining, were similar to the responses of normal mice (10). However, most of the cells lack functions such as cytotoxicity and peptide-induced IFN-γ expression. In this instance, the dysfunctional effector phenotype most likely indicates a failure of maturation rather than the induction of anergy in previously normal cells (42).

This failure of CD8+ T cells to functionally mature may reflect the fact that LTα−/− mice have abnormal secondary lymphoid tissue, lacking lymph nodes and possessing architecturally disorganized spleens (14–19). In such tissue, immune induction appears inadequate, perhaps the consequence of LTα−/− spleens lacking normal numbers of DCs, the major cell type involved in Ag presentation in primary responses (20). Thus, as reported by others and confirmed in this study, LTα−/− spleens have markedly reduced number of DCs, although the DCs present appeared to represent a normal ratio of myeloid to lymphoid subsets. Others have suggested that DC recruitment to the spleen is driven by membrane-expressed LTα, this in turn perhaps driving chemokine expression by as yet unidentified producer cells (20). Furthermore, certain chemokines appear to be minimally expressed by LTα−/− spleens (21). These include the B cell-attracting chemokine CXCL13 (formerly called BCA-1), as well as the two CCR7 ligands, CCL21 and CCL19 (21). These latter chemokines may function to attract naive T cells, as well as mature DCs (22–24). Accordingly, any deficiency in CCR7 ligand production could explain the reduced accumulation of mature Ag-presenting DCs in LTα−/− spleen.

Our observations support such ideas and extend them by showing that injection of plasmid DNA encoding CCR7 ligand into LTα−/− mice results in a major increase (4- to 5-fold) in the numbers of mature DCs in LTα−/− spleens. The reconstituted DCs included both myeloid and lymphoid subtypes, with ratio between them approximately the same as observed in wt spleen. Thus, the corrective effect of CCR7 ligand administration on DC activity could be mainly quantitative, rather than qualitative. In support of this idea, a comparison of in vitro APC activity of splenocytes from LTα−/− mice treated with control vector or CCR7 ligand revealed that the greater efficacy of the latter population roughly correlated with the increased percentages of DCs.

Most importantly, CCR7 ligand-pretreated mice developed numerically and functionally improved CD8+ T cell responses against the HSV immunodominant epitope peptide SSIEFARL compared with control vector-treated mice. In CCR7 ligand-treated animals, the Vβ10+ subset of CD8+ T cells, which contains the majority of SSIEFARL-specific CD8+ T cells (26, 32),
RESTORATION OF DYSFUNCTIONAL CD8+ T CELL RESPONSES BY CCR7 LIGANDS

was increased in number, and more cells showed activation pheno-
types than was evident in control vector-treated mice. Upon ex-
pansion in vitro, a necessary procedure to demonstrate tetramer-
positive cells as well as CTL specific for epitope peptide SSIEFARL (30), LTαβ- CD8+ T cells from treated mice were
clearly more cytotical, and significantly more cells produced
eIFN-γ following brief peptide stimulation. Such responses were of
practical consequence because the outcome of immune correction
was that treated animals were significantly more resistant to viral
challenge than were control vector-treated animals.

Accumulating numbers of observations document that T cell responses may be induced that lack one or more effector functions,
explaining in some circumstances susceptibility to infection or
neoplasia (4–9). Few of any previous reports used measures to
correct such functional defects. Our studies do achieve this objective
and demonstrate significant immune reconstitution. Our study also
emphasizes the value of systemic administration of expression
plasmids encoding appropriate chemokines, as a valuable means of
achieving immunotherapy. In our investigation, although signif-
icant immune correction was accomplished, perhaps of no surprise,
the effect was incomplete and in need of improvement. It appears
likely that improvement could come as a result of more appropriate
chemokine expression in relevant tissues, or could be achieved by
using additional means of corrective immunotherapy that optimize
T cell maturation in LTαβ mice. Regarding the former, it is not
clear whether the plasmid DNA used in our study was optimally
expressed in appropriate cells in the spleen. As shown in other
studies using plasmids encoding marker proteins, i.e. adminis-
tered plasmid result in significant and quite prolonged expression
in the spleen (43). This includes expression in DCs themselves,
perhaps an unnecessary event to achieve the function required in
our investigations. Experiments with plasmids targeted to known
cells in the spleen are required to resolve the issue of appropriate
protein expression.

Preliminary experiments have also been done to address the is-
sue of the need for additional approach to cause LTαβ- CD8+ T
cell maturation. These experiments have included the use of mix-
ture of expression plasmids such as the coadministration of both
expression plasmids such as the coadministration of both

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