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Vaccination against Lymphocytic Choriomeningitis Virus Infection in MHC Class II-Deficient Mice

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The impact of prophylactic vaccination against acute and chronic infection in a Th-deficient host has not been adequately addressed because of difficulties in generating protective immunity in the absence of CD4+ T cell help. In this study, we demonstrated that a broad CD8+ T cell immune response could be elicited in MHC class II-deficient mice by vaccination with adenovirus encoding lymphocytic choriomeningitis virus (LCMV) glycoprotein tethered to MHC class II-associated invariant chain. Moreover, the response induced conferred significant cytolytic CD8+ T cell-mediated protection against challenge with a high dose of the invasive clone 13 strain of LCMV. In contrast, vaccination with adenovirus encoding unlinked LCMV glycoprotein induced weak virus control in the absence of CD4+ T cells, and mice may die of increased immunopathology associated with incomplete protection. Acute mortality was not observed in any vaccinated mice following infection with the less-invasive Traub strain. However, LCMV Traub infection caused accelerated late mortality in unvaccinated MHC class II-deficient mice; in this case, we observed a strong trend toward delayed mortality in vaccinated mice, irrespective of the nature of the vaccine. These results indicated that optimized vaccination may lead to efficient protection against acute viral infection, even in Th-deficient individuals, but that the duration of such immunity is limited. Nevertheless, for select immunodeficiencies in which CD4+ T cell deficiency is incomplete or transient, these results are very encouraging.

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The induction and maintenance of antiviral immune responses in the absence of CD4+ T cells are still not completely understood. Thus, studies in Th-deficient mice revealed a complete dependence on CD4+ T cells for the acute and long-term control of infection with viruses normally causing chronic infections (1, 2). In contrast, transient virus control may be observed following infection of CD4+ T cell-deficient mice with viruses, which in immunocompetent mice causes only acute and self-limiting infection (3). In contrast to live infections in which an acute CD8+ T cell response is often elicited in the absence of CD4+ T cell help, CD8+ T cell responses against inert protein Ags, peptide-pulsed dendritic cells (DCs), or Ags expressed from replication-deficient viral vectors are usually absolutely dependent on CD4+ T cell help (4–8). Seemingly, however, the latter requirement may be bypassed by Ag engineering, presumably by increasing the density of MHC/Ag complexes on the individual APC (8, 9). Thus, it seems that signal strength, via the TCR and/or additional signaling pathways activated by viral replication in infected cells, modulates the CD4+ T cell dependency of acute CD8+ T cell responses.

The extent to which CD8+ T cells activated in the absence of CD4+ T cell help (often termed helpless CD8+ T cells) are able to persist, expand, and control a secondary challenge or a chronic infection is also hotly debated. Memory CD8+ T cells have been postulated not to persist in MHC class II-deficient mice and to require CD4+ T cell help during initial priming to be able to undergo secondary expansion (5, 10, 11). In contrast, we recently demonstrated significant acute virus control and secondary expansion in Th-deficient mice vaccinated 60 d previously with an adenovirus-vectored Ag engineered for enhanced presentation (8), but the question of chronic virus control was not addressed.

The extent to which virus or vaccine induced CD8+ T cells can contribute to the control of chronic virus infection in the absence or presence of CD4+ T cell help has been addressed by transfer experiments of sorted cell populations into carriers of different strains of lymphocytic choriomeningitis virus (LCMV) (12, 13). A conclusion from these studies has been that, on their own, CD8+ T cells only mediate transient virus control, whereas permanent eradication of the infection requires the help of Ag-specific CD4+ T cells in a B cell-commence mouse (14). Primate infection with SIV may represent a similar system in which an immune response primed in the presence of CD4+ T cells eventually fails as CD4+ T cell help is reduced (15). Although useful conclusions can be drawn from the LCMV-transfer system, the hitherto applied model systems failed to incorporate the impact of an intact immune system in the infected host, because the virus carrier recipients are themselves tolerized against the virus. Similarly, infection with normally resolving strains fails to address how the impact of a pre-existing immune response affects peak viral replication levels, dissemination, and, ultimately, disease progression. Accordingly, there is a need to investigate how a pre-existing immune response fares in the absence of CD4+ T cell help in the same animal.
In this study, we demonstrated that a broad CD8+ T cell immune response could be elicited in MHC class II-deficient (MHC II−/−) animals by vaccination with replication-deficient adenovirus encoding the viral glycoprotein of LCMV linked to the MHC class II-associated invariant chain (Ad-IiGP). The induced response was effective in reducing viral replication during the acute stage of infection in challenged mice, and vaccinated mice maintained substantially increased LCMV-specific responses during the subsequent chronic infection. Furthermore, LCMV infection accelerates the lethal wasting disease previously reported for old MHC class II-deficient mice (16, 17), and there is a strong trend suggesting that vaccination may delay this accelerated mortality in LCMV-infected mice.

### Materials and Methods

#### Mice

C57BL/6, MHC class I-deficient (MHC I−/−) and MHC II−/− mice were obtained from Taconic M&B (Ry, Denmark). Perforin-deficient (pfp) and IFN-γ-deficient (ifn) mice were originally obtained from The Jackson Laboratory (Bar Harbor, ME), and perform/IFN-γ double-deficient (pfp/ ifn) mice were generated locally through interbreeding of these strain, as previously described (18); all of the latter mice were bred locally in the animal facility of the Panum Institute. All mice used in this study were 7–10 wk old and were housed in a specific pathogen-free facility. All experimental procedures were approved by the local animal ethics council and performed according to local experimental guidelines.

#### Viral vectors

Production of Ad-IiGP and adenoviral vector expressing LCMV glycoprotein (Ad-GP) or OVA (adenoviral vector expressing OVA linked to the MHC class II-associated invariant chain [Ad-IiOVA]) and adenoviral vector expressing OVA (Ad-OVA) was described previously (19).

#### Viruses

LCMV clone 13 was originally obtained from M.B.A. Oldstone (The Scripps Research Institute, La Jolla, CA) and amplified in-house. The Traub strain of LCMV was produced and stored as previously described (3). For infections, mice were put in a restrainer and infected with 105 or 106 PFU clone 13 or 200 PFU of the Traub strain in a tail vein in 0.3 ml PBS.

#### Organ virus titers

Organs were homogenized as a 10% organ suspension, and viral titers were determined using an immune focus assay, as previously described (19).

#### Culture and staining of bone marrow-derived DCs

Single-cell suspensions of bone marrow cells were prepared from femurs, and the cells were cultured in the presence of recombinant mouse GM-CSF for 10 d, as described previously (20). Bone marrow-derived DCs (BMDCs) were transduced with Ad-OVA, Ad-IiOVA, or Ad-IiGP at a multiplicity of infection of 250; 24 h later, surfaces were stained with FITC-conjugated anti-I-Ak Ab and Alexa 647-conjugated anti-SIINFEKL/H-2Kb Ab (clone 25.D1.16) (21).

#### Spleen cell preparations and flow cytometry

Single-cell suspensions of splenocytes were obtained by pressing the organs through a fine steel mesh, followed by centrifugation and resuspension in RPMI 1640 cell culture media. Functional epitope-specific CD8+ T cell responses were enumerated by intracellular cytokine staining (ICS) after a 5-h incubation with 0.1 μg/ml relevant peptide, as described (22). Total numbers of epitope-specific CD8+ T cells were determined by staining with relevant MHC/peptide dextramers (Immundex, Copenhagen, Denmark) (22). Data analyses were performed using Cell Quest (BD Biosciences) or Flow Jo (Treestar) software.

#### Statistical analyses

Quantitative results were compared using the Mann–Whitney U test. Survival curves after Traub virus challenge were compared using a Cox proportional Hazard regression model.

### Results

#### Linkage of Ag to MHC class II-associated invariant chain improves immunogenicity to CD8+ T cells by increasing MHC class I-restricted Ag presentation

Our original strategy for the generation of memory CD8+ T cells in the absence of CD4+ T cell help was to apply an adenoviral vector encoding a fusion protein consisting of an antigenic peptide and β2-microglobulin (8). However, because this strategy only induces CD8+ T cell responses of very limited breadth, we considered it inadequate for the study of the impact of vaccine-induced CD8+ T cells during chronic viral infection. However, linkage to β2-microglobulin is assumed to bypass the requirement for CD4+ T cell help by increasing peptide/MHC class I association. Interestingly, we recently found that if the invariant chain (Ii) is used as an Ag linker of the whole LCMV glycoprotein (GP), the immunogenicity of MHC class I-restricted epitopes is also markedly enhanced and, based on in vitro proliferation of TCR-transgenic CD8+ T cells, in a seemingly MHC class II-independent manner (9). This observation could indicate that covalent linkage of Ag to Ii somehow perturbs the normal pathways of Ag presentation, thus causing increased MHC class I-associated epitope presentation. To test this possibility directly, we took advantage of the existence of an OVA-specific Ab with the same specificity as CD8+ T cells directed toward the classical OVA epitope SIINFEKL (i.e., recognizing SIINFEKL only in association with its presenting MHC class I molecule H-2Kb). BMDCs and matured DCs were infected with Ad-IiOVA, Ad-OVA, or an irrelevant adenovirus (Ad-IiGP). Twenty-four hours later, DCs were harvested and surface stained using fluorochrome-labeled OVA-specific Ab. As can be seen in Fig. 1, much more intense staining of infected DCs was observed following infection with Ad-IiOVA than with Ad-OVA, strongly supporting that tethering to Ii increases Ag presentation.

**Ad-IiGP vaccination induces cytotoxic CD8+ T cell-mediated protection against LCMV infection**

In a previous report, we showed that mice vaccinated with Ad-IiGP harbored more GP-specific CD8+ T cells compared with mice vaccinated with the unlinked construct and that this correlated with increased resistance to challenge with live virus. To confirm a direct role for Ad-IiGP–induced CD8+ T cells in vivo, we vaccinated pfp, ifn, and pfp/ifn mice, as well as MHC I−/− mice, and challenged them 80–90 d later with 107 PFU of LCMV clone 13. Spleens were harvested 5 d later for determination of viral titers. From the results of these experiments (Fig. 2), it was clear that protection was completely dependent on CD8+ T cells and primarily involved direct cell killing of virus-infected cells, because no protection was seen in pfp, pfp/ifn, or MHC I−/− mice.

**Ii-linked adenoviral vaccine induces strong CD8+ T cell responses in MHC class II-deficient hosts**

Having confirmed that Ad-IiGP–induced virus control directly reflects cytolytic CD8+ T cell activity, we focused on whether the Ii-linked vaccine Ad-IiGP, unlike the unlinked Ad-GP vaccine, could induce memory CD8+ T cells in MHC II−/− mice. MHC II−/− and wild-type (WT) mice were vaccinated with 2 × 107 infectious units in the right hind foot pad, and the CD8+ T cell response was analyzed 21 and 60 d later by ICS of peptide-stimulated splenocytes. As anticipated, the Ad-IiGP vaccine induced a broad CD8+ T cell response that was almost as good in MHC II−/− mice as in WT mice at the early time point (Fig. 3A). However, the number of functional (i.e., IFN-γ-producing) CD8+ T cells declined at a faster pace in the former mice, resulting in...
A reduced response at 60 d after vaccination (Fig. 3B). Nevertheless, numbers of functional virus-specific CD8+ T cells in Ad-IiGP–vaccinated MHC II−/− mice 60 d after vaccination matched those in conventionally (Ad-GP) vaccinated WT mice. In contrast, the Ad-GP vaccine, as previously reported (8), failed to induce a response in MHC II−/− mice that could be detected with certainty by ICS (Fig. 3B).

Although the primary focus of this study was clearly on CD8+ T cell function, we performed an additional analysis on day 60 after vaccination, in which we compared numbers of functional gp33-specific CD8+ T cells with numbers of H-2Db/gp33-binding CD8+ T cells. In this case (Fig. 3C), we confirmed earlier data that the ratio of functional gp33-specific CD8+ T cells to MHC/peptide-binding cells is consistently 1:1 in WT mice (22), reflecting the fact that while the peptide used for ICS in immunocompetent mice triggers two subsets of CD8+ T cells, restricted to H-2Kb and H-2Db, only the H-2Db–restricted subset is detected by our MHC/peptide staining (23). Notably, in MHC class II–deficient mice, the ratio is 1:1, indicating that functional silencing may precede a decline in numbers of helpless CD8+ T cells in Ad-IiGP–vaccinated mice.

Ad-IiGP–induced immune response in MHC class II–deficient mice protects against acute infection with LCMV clone 13

Because a functional CD8+ T cell response was readily induced in MHC II−/− mice, as well as WT mice, we tested the protective capacity of the vaccine-induced response by challenge with a high dose (106 PFU) of the invasive LCMV clone 13 strain 60 d after vaccination. LCMV clone 13 normally induces rapid CD8+ T cell exhaustion and causes a chronic infection, with virus persisting for 2–3 mo (1, 2, 24). Some of these mice were sacrificed 5 and 10 d after virus challenge, and spleen and lungs were harvested for determination of viral titers. Consistent with our previous report (9), Ad-IiGP vaccination of WT mice resulted in much reduced levels of infection in both organ sites at 5 d after challenge. In MHC class II–deficient mice, Ad-IiGP vaccination was also associated with a significant (∼2 log) reduction in viral load (Fig. 4A); the latter reduction matched that seen following conventional (Ad-GP) vaccination of WT mice. Notably, Ad-GP vaccination of MHC class II–deficient mice was associated with a small, but significant, reduction in the viral load in the spleen (Fig. 4A), suggesting that some CD8+ T cell priming had occurred in the Ad-GP–vaccinated mice that was barely detectable by ICS or did not involve IFN-γ–producing cells (Fig. 3C). An in vivo impact of such subthreshold responses was seen with DNA vaccines (25).

Ten days postinfection (p.i.) (Fig. 4B), very low levels of virus were also found in Ad-IiGP–vaccinated MHC class II–deficient mice. In contrast, consistent virus control in Ad-GP–vaccinated mice was only observed in WT mice. As expected, little or no virus control was observed in unvaccinated mice, irrespective of genotype.

To correlate these findings to the magnitude of the virus-induced CD8+ T cell response in challenged mice, splenocytes of vaccinated mice were analyzed for the presence of functional virus-specific CD8+ T cells on days 10 and 17 p.i. On both of these days, substantial CD8+ T cell responses were noted in all vaccinated groups, whereas unvaccinated mice presented with low responses, as expected for high-dose clone 13 infection (Fig. 4C, 4D). Mice vaccinated with the Ad-IiGP vector presented with a broader CD8+ T cell response than did Ad-GP–vaccinated mice.
vaccinated mice, consistent with earlier observations (9). Also, Ad-GP–vaccinated MHC class II-deficient mice, unlike their unvaccinated littermates, were able to raise a significant CD8+ T cell response, although this did not completely match that in similar vaccinated WT mice. Thus, vaccinated MHC class II-deficient mice were the only group of mice to present with impaired virus control and the generation of a significant CD8+ T cell response. In contrast, the infection was consistently controlled in all other vaccinated mice, irrespective of genotype, and unvaccinated mice had high viral loads but few CD8+ T cells.

**Ad-IiGP–induced immune response in MHC class II-deficient mice does not prevent chronic infection with LCMV clone 13**

Because Ad-IiGP vaccination caused robust early virus control, whereas Ad-GP vaccination was associated with impaired, albeit initially significant, virus control in vaccinated MHC class II-deficient mice following high-dose clone 13 challenge, similarly vaccinated and infected mice were used to assess the clinical course of infection in vaccinated mice, as well as the ability of these mice to control viral replication in the long term (Fig. 5). We did not observe severe clinical disease in either group of vaccinated WT mice (Fig. 5A), and no virus could be recovered from the lungs by the end of the observation period. In contrast, infectious virus was recovered from all vaccinated MHC class II-deficient mice (Fig. 5C). However, only a single Ad5-IiGP–vaccinated mouse died during the observation period. This is strikingly different from the situation in Ad-GP–vaccinated MHC class II-deficient mice, of which up to 70% died 2–4 wk after challenge (Fig. 5A). The reason for this high mortality is likely to be found in vaccine-induced immunopathology, resulting from the coexistence of moderate to high viral loads and substantial CD8+ T cell numbers. Thus, clone 13-infected Th-deficient mice are not reported to suffer any severe disease (1, 2); in contrast, there is ample evidence that preimmunization may augment LCMV-induced immunopathology in immunodeficient mice (26). The remaining 30% appeared healthy for many weeks before developing a lethal wasting syndrome. The last two moribund animals were sacrificed for determination of viral loads and FACS analysis, together with Ad-IiGP–vaccinated MHC II−/− mice and WT animals (Fig. 5B, 5C). The FACS analysis revealed that Ad-IiGP–vaccinated MHC class II-deficient mice maintained high numbers of functional LCMV-specific CD8+ T cells in their spleen, surpassing those seen in WT animals. In contrast, very few cells could be recovered from the small and fibrotic spleens of the Ad-GP–vaccinated MHC class II-deficient mice (Fig. 5B). Also, analysis of viral titers in the lungs of these mice (too little material was available from the spleens to attempt both FACS and virus titration) supported the assumption that these mice had only been able to survive for so many weeks as a result of the complete breakdown of their immune system. Thus, although virus reemergence was noted in both groups of vaccinated MHC class II-deficient mice (Fig. 5C), the viral load was kept at a moderate level in Ad-IiGP mice, whereas the two Ad-GP–vaccinated mice harbored virus at a level reminiscent of that in acutely infected, unvaccinated mice.

**Evaluation of adenovirus-induced protection against infection of MHC class II-deficient mice with LCMV Traub**

All of the above experiments were performed using high-dose infection with rapidly invasive LCMV clone 13 virus, which may represent an extreme case. Therefore, to study the control of chronic infection in a more physiological setting, we set up cohorts of WT and MHC class II-deficient mice that were left unvaccinated and infected, were vaccinated and infected, or were vaccinated with Ad-GP and infected; we used a low dose (200 PFU) of the LCMV Traub strain for these experiments. In unvaccinated MHC class II-deficient mice, this regimen resulted in low-grade chronic infection associated with partial CD8+ T cell dysfunction, whereas WT mice controlled this infection to a very low level within weeks to months p.i. (3).

For part of the LCMV Traub-challenged mice, spleen and lungs were harvested for determination of viral titers 6 d p.i. (Fig. 6A). As expected, both groups of vaccinated WT mice were clearly
protected from infection of lungs and spleens, whereas unvaccinated mice had high levels of virus in both organs. In the MHC class II-deficient mice, robust protection was again observed in Ad-IiGP–vaccinated mice. Interestingly, despite the lower challenge dose used with the LCMV Traub virus, only a small, albeit significant, level of protection was observed in the spleens of Ad-GP–vaccinated MHC class II-deficient mice, underscoring the unique ability of the Ii-linked Ag to induce substantial protection in Th-deficient mice.

We followed the weight of the infected mice as a sign of acute virus-induced disease (Fig. 6B). Both vaccines prevented or reduced the transient weight loss normally associated with immune-mediated virus control in LCMV Traub virus-infected WT animals (18). Vaccination with Ad-IiGP also prevented an early weight loss in MHC class II-deficient mice, whereas Ad-GP priming did not prevent acute wasting or actually increased the weight loss compared with unvaccinated MHC class II-deficient mice. The body weights of mice in all groups were normalized after ∼2 wk.

All of the mice appeared healthy for several weeks following recovery from any acute symptoms of infection. However, starting ∼10 wk p.i., some of the unvaccinated LCMV-infected MHC class II-deficient mice started to lose weight again; with time, more and

![Diagram](image_url)
more moribund mice had to be put down (Fig. 7A). Delayed by several weeks, vaccinated MHC class II-deficient mice also started to show signs of wasting. Notably, no difference between Ad-IiGP– and Ad-GP–vaccinated mice was observed with regard to the kinetics of disease induction. Finally, ~40 wk into the experiment, age-matched, uninfected MHC class II-deficient mice developed wasting and had to be put down. No disease was observed at this time in any of the groups of WT mice included in the study. When the long-term survival of MHC class-deficient mice was fitted to a Cox proportional hazard regression model, LCMV infection was associated with a significantly reduced survival ($p = 0.015$; hazard ratio of 2.8588, with a 95% confidence interval of 1.226–6.562). This reduced survival was not significantly improved by prior vaccination (because the two vaccination groups could not be distinguished from each other, they were pooled to increase the statistical power) (hazard ratio of 0.611, with a 95% confidence interval of 0.363–1.027), although the $p$ value bordered on the level of statistical significance ($p = 0.0632$) (Fig. 7A).

To better understand the mechanisms underlying the above-described mortality patterns, we set up new cohorts of MHC class II-deficient and WT mice in which the animals were sacrificed for FACS analysis 12 and 24 wk into the experiment and evaluation of organ virus titers at 24 wk p.i. (Figs. 7B, 8). Because the control of a chronic infection is often correlated to the breadth of the antiviral CD8$^+$ T cell response, we quantified the number of CD8$^+$ T cells in infected MHC class II-deficient and WT mice responding by cytokine production to two (12 wk; Fig. 8A) or four (24 wk; Fig. 8C) different H-2$^b$-restricted LCMV GP epitopes. In WT animals, the GP-directed epitope hierarchies were similar in all groups, and the induced CD8$^+$ T cell response tended to focus primarily on gp33 and, to a lesser extent, on gp276.

With regard to MHC class II-deficient mice, FACS analysis as late as 24 wk p.i. demonstrated preservation of splenic cellularity, as well as B cell (CD19$^+$) and CD8$^+$ T cell compartments, in all three groups of LCMV-infected mice (Supplemental Fig. 1A). This was particularly apparent for Ad-IiGP–vaccinated mice, which had cell numbers clearly exceeding WT levels. Consistent with the observation in chronic clone 13-infected and vaccinated mice, FACS analysis of splenocytes from MHC class II-deficient mice at 12 and 24 wk p.i. revealed quantitatively robust and broad virus-specific CD8$^+$ T cell responses in Ad-IiGP–vaccinated mice; however, following LCMV Traub infection, the Ad-GP–vaccinated mice also presented with some improvement of the induced CD8$^+$ T cell responses. In addition to the described differences in the numbers of epitope-specific CD8$^+$ T cells, we noted differences in their functionality (Fig. 8B, 8D). Moreover, the frequency of virus-specific CD8$^+$ T cells co-producing IFN-γ and IL-2 (only analyzed at 12 wk p.i.) was reduced in MHC class II-deficient mice, particularly in the unvaccinated animals (Fig. 8E, 8F).

The breadth of CD8$^+$ T cell responses, as well as the expression of certain surface markers (e.g., CD27, CD127, and KLRG1), has been associated with efficient control of chronic virus infections (27–32). For this reason, we performed multicolor flow cytometry analyzing the expression of these markers on gp33-responding (Fig. 8E, 8G) and gp276-responding (Fig. 8F, 8H) IFN-γ–producing CD8$^+$ cells. This analysis revealed that although the majority of LCMV-specific cells in WT mice expressed markers associated with high-quality memory cells (CD27$^{high}$, CD127$^{high}$, KLRG1$^{low}$), the vast majority of gp33- or gp276-specific cells in MHC class II-deficient mice, regardless of vaccination, showed a more terminally differentiated effector memory phenotype (CD27$^{low}$, CD127$^{low}$, KLRG1$^{high}$). Interestingly, not only the epitope-specific CD8$^+$ T cells, but also the entire CD8$^+$ T cell repertoire, in MHC class II-deficient mice were dominated by these phenotypic traits (Supplemental Fig. 1B).

When assaying organ virus titers in WT and MHC class II-deficient mice at 24 wk p.i., no WT animal harbored detectable levels of virus (data not shown). In contrast, virtually all MHC class II-deficient mice harbored detectable levels of virus in their organs, and a trend was found with titers in Ad-IiGP < Ad-GP < unvaccinated mice (Fig. 7B). However, these differences were not significant. Thus, in contrast to the situation in clone 13-infected...
mice, it seems that all LCMV Traub-infected, MHC class II-deficient mice maintained a substantial measure of virus control (compare Fig. 7B with Fig. 5C). Moreover, the trend toward prolonged survival in vaccinated mice may be correlated to an increased breadth and magnitude of the LCMV-specific CD8+ T cell response.

**Discussion**

Although several studies have addressed whether a viral infection can be contained and controlled in various CD4+ T cell-deficient models or which cell types can impact an established chronic infection in WT animals, to our knowledge, this is the first study to address whether it is possible to vaccinate and control a subsequent chronic viral infection in a CD4+ T cell-deficient host. It is important to make this distinction when considering vaccination against opportunistic infections in the immunodeficient host, because the immunodeficiency may impact the response against both vaccine and challenge. The absence of potent vaccine regimens inducing substantial protection in MHC class II-deficient hosts is a major reason why it has not been possible to perform this kind of study previously. In the absence of MHC class II, Ab responses are dramatically reduced, and it is obvious that a potent CD8+ T cell immunogen would be required in this setting. We previously reported that covalent tethering of short peptides to b2-microglobulin, and hence, the MHC class I complex, enabled the induction of a CD8+ T cell response in MHC class II-deficient mice (8). Although a virus-specific response generated using this strategy could protect against acute infection, the breadth of the response was very limited; only a single epitope specificity could be targeted per vaccine, and we needed a broader response to be able to realistically combat variable and/or mutating pathogens typically causing chronic infection. Therefore, it is of interest that we also recently published that adenoviral vaccination with Ags linked to the li induced broad and potent CD8+ T cell responses, apparently by increasing MHC class I-restricted Ag presentation independently of MHC class II (9). Because it is generally assumed that T cell activation occurs when the integrated value of signals delivered through the TCR (signal 1) and various costimulatory molecules (signal 2) exceeds a certain threshold, we considered it very likely that increased Ag presentation on APCs would bypass the normal requirement for CD4+ T cell help. As a first step to test this, we confirmed that tethering to li chain significantly improved MHC class I-restricted Ag presentation, as expected. With this information available, it was logical to go on and test whether li linkage might also induce protective CD8+ T cell immune responses in MHC class II-deficient hosts, although a previous study on the anticancer effect of an li-linked DNA vaccine pointed to an MHC class II dependence of protection (33). Our finding in this study, that only the adenoviral li-linked vaccine induced a potent CD8+ T cell response and robust short-term protection in MHC class II-deficient hosts, could support the assumption that a high level of Ag presentation on the vaccine vector-transduced cells, as is achieved with li linkage, in combination with innate signals induced through recognition of molecular patterns of the adenoviral vector, might act in concert to activate CD8+ T cells in the absence of CD4+ T cell help. Until now, this has only been possible with live vectors (see Ref. 34 for our recent review of the principal differences between DNA vaccines and viral-vector vaccines).

Although Ag-specific CD8+ T cells were readily induced in Ad-liGP-vaccinated MHC class II-deficient mice, helpless CD8+ T cells were less stable over time compared with CD8+ T cell memory cells generated in the presence of CD4+ T cell help. This impaired memory is unfortunate, but it probably is inherent to the CD4 T cell-deficient environment, because even CD8+ T cells
which the majority of CD8+ T cells became exhausted following infection with LCMV Traub, which is not associated with acute exhaustion of the CD8+ T cells in MHC class II-deficient mice (41). Large groups of these mice were followed, and in agreement with earlier observations, we found that MHC class II-deficient mice have a restricted life span, even under specific pathogen-free conditions (16, 17). However, LCMV infection could further shorten the life span of these mice, and a strong trend was observed, suggesting that vaccination could delay the infection-associated mortality. In separate experiments, this trend was supported by results that pointed to broader CD8+ T cell epitope coverage, more stable CD8+ T cell memory, and lower viral loads, particularly in Ad-IiGP–vaccinated mice, but also to a lesser degree in Ad-GP–primed mice. However, despite marked differences in immunogenicity and early virus control, no clear difference in long-term virus control and survival was apparent between Ad-IiGP– and Ad-GP–vaccinated MHC class II-deficient mice. Although the power of these experiments may be too low to detect such differences, it can be suggested that differences in the early course of the infection may become less relevant with time in the MHC class II-deficient mice. At the molecular level, a reason for this may be our inability to rescue the normal phenotypic appearance of virus-specific memory CD8+ T cells in vaccinated MHC class-deficient mice, which, irrespective of vaccination, present with a more terminally differentiated phenotype. Interestingly, Ad-GP vaccination in MHC class II-deficient mice could have disastrous consequences, with high numbers of the vaccinees dying within 4 wk of challenge with a high dose of clone 13. Considering that unvaccinated clone 13-infected mice probably avoid acute lethal tissue damage through rapid inactivation of the majority of their virus-specific CD8+ T cells (37, 38), the most likely explanation for the severe disease in Ad-GP–vaccinated MHC class II-deficient mice seems to be the combination of a somewhat strengthened immune response (compared with unvaccinated mice) and still relatively inefficient virus control. A similar phenomenon was observed in vaccinated pfp mice challenged with a high dose of LCMV clone 13 (26). This interpretation was supported by the results presented in Fig. 4, as well as our analysis of the immune status in the few long-term surviving, Ad-GP–vaccinated mice (Fig. 5B, 5C), which yielded results consistent with the notion that survival of Ad-GP–vaccinated MHC class II mice though the early phase of clone 13 infection was associated with a complete breakdown of the immune system and a rebound of uncontrolled viral replication (38). In contrast, Ad-IiGP–vaccinated mice probably survived through the early phase by rapidly curtailing the infection and reducing viral replication in critical organ sites. The eventual cause of death in the Ad-GP–vaccinated MHC class II-deficient mice that survived >4 wk could be intrinsic to clone 13 infection or precipitated by the Ad-GP vaccine; the destruction of splenic architecture and cellularity clearly suggested that at least the lymphoid organs are afflicted by Ad-GP and clone 13-precipitated immunopathology. CD8+ T cell-mediated destruction of splenic architecture was previously observed during the acute phase of LCMV infection in WT mice and, during chronic infection, CD4+ T cell depletion was found to exacerbate immunopathology through loss of virus control (38–40).

Nevertheless, if an attempt should be made to resolve the long-term outcome of vaccination and LCMV infection in MHC class II-deficient mice, and in particular the importance of the strength of the CD8+ T cell vaccine, the selection bias introduced by the high mortality following clone 13 infection in Ad-GP–vaccinated MHC class II-deficient mice is confounding. Therefore, we decided to change our experimental approach and analyze the outcome of infection with LCMV Traub, which is not associated with acute exhaustion of the CD8+ T cells in MHC class II-deficient mice (41). Large groups of these mice were followed, and in agreement with earlier observations, we found that MHC class II-deficient mice have a restricted life span, even under specific pathogen-free conditions (16, 17). However, LCMV infection could further shorten the life span of these mice, and a strong trend was observed, suggesting that vaccination could delay the infection-associated mortality. In separate experiments, this trend was supported by results that pointed to broader CD8+ T cell epitope coverage, more stable CD8+ T cell memory, and lower viral loads, particularly in Ad-IiGP–vaccinated mice, but also to a lesser degree in Ad-GP–primed mice. However, despite marked differences in immunogenicity and early virus control, no clear difference in long-term virus control and survival was apparent between Ad-IiGP– and Ad-GP–vaccinated MHC class II-deficient mice. Although the power of these experiments may be too low to detect such differences, it can be suggested that differences in the early course of the infection may become less relevant with time in the MHC class II-deficient mice. At the molecular level, a reason for this may be our inability to rescue the normal phenotypic appearance of virus-specific memory CD8+ T cells in vaccinated MHC class-deficient mice, which, irrespective of vaccination, present with a more terminally differentiated phenotype. Interestingly, the fact that improved early virus control does not correlate with an improved long-term outcome is in contrast to experiences with HIV or hepatitis C virus in humans, SIV in primates, or even different strains of LCMV infection in WT mice; however, it is noteworthy that in all of these systems, a covariant in protracted or more aggressive infection is the depletion of cognate Ag-specific CD4+ T cells (42–45). Furthermore, in the HIV and SIV system, the persistence of cognate Ag-specific CD4+ T cell co-variation with the CD8+ T cell phenotype, and we do

![Graph](image-url)
indeed find a predominance of terminally differentiated T cells in infected MHC class II-deficient mice. Thus, the removal of the association between initial viral control and long-term outcome or CD8+ T cell phenotype in MHC class II-deficient mice could be taken to suggest that the CD4+ T cell associations with long-term viral control and CD8+ T cell phenotype are causal. This is promising for the HIV vaccine field because newer vaccine regimens, unlike the regimens hitherto tested in humans, have achieved efficient preservation of central and mucosal antiviral CD4+ T cell responses (46). Exactly how LCMV infection is able to precipitate the severe wasting in MHC class II-deficient mice is unknown, but it is possible that the chronic T cell activation associated with persistent viral infection may play a role (47). Interestingly, a similar association between nonspecific inflam-

FIGURE 8. Immune profiling of Traub-infected MHC class II-deficient and WT mice with or without prior vaccination. MHC class II-deficient mice and WT controls were left untreated or were vaccinated with Ad-GP or Ad-IiGP and challenged with 200 PFU of LCMV Traub 60 d later. A, Enumeration of CD8+CD44high T cells responding to the indicated peptides with IFN-γ production as determined by ICS 12 wk after challenge. Background signal of nonstimulated cells was subtracted from all samples. B, Mean fluorescence intensity of staining for IFN-γ in those CD8+ T cells described in A that are responding to gp33 and gp276. C and D, As in A and B, except that mice were challenged 24 wk previously. Fraction of gp33-specific (E) and gp276-specific (F) T cells (as determined by ICS for IFN-γ production) that coexpress TNF-α, IL-2, CD27, CD127, or KLRG1 in mice challenged 12 wk previously. Fraction of gp33-specific (G) and gp276-specific (H) T cells (as determined by ICS for IFN-γ production) that coexpress TNF-α, CD27, CD127, or KLRG1 in mice challenged 24 wk previously. All data are presented as mean ± SEM of four or five animals per group.
and pro tease has been observed in HIV-infected humans (48). That an initially well-controlled LCMV infection rebounds over time has been observed before using the Traub strain of LCMV in MHC class II-deficient mice (3), and this outcome was correlated to the partial exhaustion of the CD8+ T cell response (49). What most likely happens in this case is that the virus, unopposed by any Ab response, rapidly reaches immunoprivileged niches, which serve as reservoirs for virus persistence and rebound (3, 50). In vaccinated mice, the augmented T cell surveillance may delay virus dissemination and viral rebound (compared with unvaccinated mice), but it cannot prevent it; consequently, the final outcome is the same.

The ability of our vaccine to induce an efficient acute and prolonged CD8+ T cell response in the absence of CD4+ T cells may have implications for clinical immunodeficiencies. Most obvious is the HIV infection, which, since the introduction of highly active antiretroviral therapy, includes a continuous spectrum of more-or-less-severe CD4+ T cell deficiency. Although it is possible that a vaccine regime, such as the present one, could induce quantitatively robust CD8+ T cell responses in HIV-infected mice, it remains speculative which benefits this would raise, because the repertoire of naive CD8+ T cell precursors with high affinity for HIV or established infections may be limited, for which reason the long-term outcome might still be dependent on the preservation of antiviral CD4+ T cell response with antiviral therapy (12, 51).

Other indications are more promising. Following bone marrow transplantation, herpesviruses derived from host or donor can roam freely in the lymphopenic host and cause significant morbidity and mortality. Because the CD8+ T cell population in such patients recovers faster than does the CD4 population (52), the ability of our vaccine to induce acute protective could be exploited to curtail virus replication until the CD4+ T cell repertoire recovers.

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Disclosures

The University of Copenhagen holds a patent regarding the invariant chain vaccine strategy. The authors are entitled to a fraction of any net income that may derive from the commercialization of this patent.

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