Protection by CD4 or CD8 T Cells against Pulmonary Mycobacterium bovis Bacillus Calmette-Guérin Infection

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Mice deficient in CD8 T cells demonstrated levels of Th1 cytokines and granulomatous responses in the lungs very similar to those demonstrated by normal control mice and were fully capable of controlling pulmonary mycobacterial infection by Mycobacterium bovis BCG as assessed at day 37 postinfection. In comparison, mice deficient in CD4 T cells had similar levels of interleukin-12 (IL-12) and tumor necrosis factor alpha but lower levels of gamma interferon in the lungs and were still able to mount tissue granulomatous responses and control pulmonary mycobacterial infection. In contrast, IL-12−/− mice with impaired CD4 and CD8 T-cell responses had a markedly weakened control of infection, whereas SCID mice deficient in all T cells succumbed to such pulmonary mycobacterial infections.

The cell-mediated immune response is known to be critical in host defense against intracellular mycobacterial infection. T lymphocytes, particularly CD4 T cells, are believed to play an important role in the development of protective tissue immunoinflammatory responses by secreting type 1 cytokines, including gamma interferon (IFN-γ) and tumor necrosis factor alpha (TNF-α), and implementing cytotoxic activities (2, 16). The release of type 1 cytokines, particularly IFN-γ, is triggered by interleukin-12 (IL-12), a cytokine released from antigen-presenting cells upon interaction with infectious agents (19). These cytokines have a profound activating effect on macrophages, an ultimate effector cell type in host resistance to mycobacterial infection. Increasing evidence also suggests the participation of CD8 T cells in host defense against mycobacterial infections (2). These cells are capable of not only cytotoxic activities but also cytokine release, particularly the release of type 1 cytokines (13, 14). Compared to CD4 and CD8 T cells, γδ T cells represent a very small fraction of the increased total T-cell population during mycobacterial infection, and recent studies carried out with mice have provided compelling evidence that this subset of T cells is not involved in immune protection against mycobacterial infection (5). However, the relative roles of CD4 and CD8 T cells in antimycobacterial host resistance have remained incompletely understood. In models of systemic infection by Mycobacterium bovis BCG, mice genetically deficient in, or depleted by antibodies of CD4 T cells succumbed to or failed to control infection (9, 10). On the other hand, while mice genetically deficient in CD8 T cells succumbed to systemic infection by Mycobacterium tuberculosis, they were demonstrated to be either capable or incapable of controlling systemic infection by M. bovis BCG, depending on the experimental conditions (6, 10).

Overall, these findings imply a role for both CD4 and CD8 T cells in antimycobacterial host responses. However, the natural route of infection for many strains of mycobacteria is the respiratory tract, and other studies have recently found that immune responses to mycobacterial infection in the lungs may be different from those in other tissue sites, such as the liver and spleen (3, 12, 20). This may explain why mice are more susceptible to aerogenic than to systemic mycobacterial infection (15). Thus, increasing efforts have been made to establish models of pulmonary mycobacterial infection via airway inoculation and to dissect cellular and molecular mechanisms in the lungs. Recently, it has been demonstrated that IL-12 is a critical upstream type 1 cytokine required for the initiation of downstream type 1 cytokines IFN-γ and TNF-α and for protective tissue immunoinflammatory responses in a mouse model of pulmonary mycobacterial infection by M. bovis BCG (20). In this study, it was observed that increases in the number of both CD4 and CD8 T cells in the lungs were temporally associated with the degree of immune protection, demonstrated in immunocompetent C57BL/6 mice and IL-12-deficient mice (20). However, the relative roles of CD4 and CD8 T cells in host defense against pulmonary mycobacterial infection have yet to be elucidated. In this study, we investigated tissue inflammatory responses, cytokine profiles, and immune protection in the lungs of mice genetically completely devoid of either CD4 or CD8 T cells, or both, after airway inoculation with live M. bovis BCG. We have provided experimental evidence that while CD4 T cells represent the most efficient immune-cell type, CD8 T cells may carry out a certain number of functional activities overlapping those of CD4 T cells and are capable of compensating significantly, albeit not completely, for the loss of some functions of CD4 T cells during pulmonary mycobacterial infection.

Mice, mycobacterial infection, and sample preparation. Mice used in this study were of the following types: C57BL/6 (Harlan, Indianapolis, Ind.), IL-12 p40−/− (kindly provided by Jeanne Magram, Hoffmann-La Roche, Inc.), CD4−/−, CD8−/− (kindly provided by Tak Mak, Princess Margaret Hospital, Toronto, Canada), and SCID (severe combined immunodeficient) (8, 11, 18, 20). All of these mice were kept in our level B pathogen-free facility prior to infection. C57BL/6 mice were used as a normal immunocompetent control, whereas IL-12−/− mice were used as an immunocompromised control (20). CD4−/− mice have been shown to be deficient in CD4 T helper cells (18). CD8−/− mice were produced by targeted disruption of the Lyt-2 gene, and the resultant homozygous mice had no CD8+ CD4− or CD3+ CD4− T cells and lacked cytolytic T-cell activities (8). SCID mice are known to be devoid of all lym-
TABLE 1. Cellular profiles in BAL fluids from various strains of mice

<table>
<thead>
<tr>
<th>Mouse strain</th>
<th>AM (10^6)</th>
<th>PMN</th>
<th>LC</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4^+/-</td>
<td>46.2 ± 10.3</td>
<td>16.6 ± 1.7</td>
<td>31.9 ± 5.1</td>
</tr>
<tr>
<td>CD8^+/-</td>
<td>34.3 ± 4.1</td>
<td>1.5 ± 0.2**</td>
<td>23.0 ± 5.1</td>
</tr>
<tr>
<td>SCID</td>
<td>40.1 ± 5.3</td>
<td>24.7 ± 6.0</td>
<td>2.0 ± 0.2**</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>41.5 ± 7.6</td>
<td>11.2 ± 0.1</td>
<td>27.3 ± 1.4</td>
</tr>
<tr>
<td>IL-12^+/-</td>
<td>19.1 ± 3.5</td>
<td>1.0 ± 0.3**</td>
<td>12.1 ± 3.7*</td>
</tr>
</tbody>
</table>

*The number of alveolar macrophages (AM), neutrophils (PMN), and lymphocytes (LC) was determined on cytopsins from BAL fluids collected at day 26 after i.t. mycobacterial infection in SCID mice and at day 37 in all other strains. Results are expressed as means ± standard errors of the means for three or four mice. **P ≤ 0.01 compared to the other strains for the same cell type. *P ≤ 0.05 compared to the other strains for the same cell type.

phocytes. CD4^+/-, CD8^+/-, and IL-12^+/- mice all share the C57BL/6 genetic background, whereas SCID mice have a BALB/c background. Airway mycobacterial infection was established as previously described (20). Briefly, mice were inoculated intratracheally (i.t.) with 5 × 10^6 CFU of live M. bovis BCG (kindly provided by Robin Harkness at Pasteur Mérieux Connaught, North York, Ontario, Canada). At day 37 postinfection, bronchoalveolar lavage (BAL) was carried out, and differential cell types obtained by BAL were determined based upon morphological characteristics of cells on cytopsins (20). The level of type 1 cytokine IL-12, IFN-γ, or TNF-α protein in BAL fluids was determined by specific enzyme-linked immunosorbent assay (R & D Systems, Minneapolis, Minn.). The number of mycobacterial bacilli in the lungs and spleen of each mouse was quantitated by a colony enumeration assay (20). Pooled BAL cells from several mice were also subjected to an immunophenotyping procedure by fluorescence-activated cell sorter (FACS) analysis to quantitate the number of immune-cell types in the lungs (20). Lung tissues were fixed by perfusion with 10% formalin and processed for histopathologic examination. The time point of day 37 postinfection was chosen since it had been observed in a previous study that lung cytokine and tissue immunoinflammatory responses peaked around day 30 after infection via airways and that the difference in the numbers of mycobacterial bacilli in the lungs and other tissue sites under immunocompetent and immunodeficient conditions became the most appreciable around day 37 (3, 9, 20).

Cellular profiles in BAL fluids and histopathology of the lungs. We first examined cellular profiles in BAL fluids. As shown in Table 1, the numbers of macrophages in the lungs of CD4^+/-, CD8^+/-, SCID, and C57BL/6 control mice were similarly increased, in contrast to that in the lungs of IL-12^+/- mice. Total lymphocytes markedly increased in the lungs of CD4^+/-, CD8^+/-, and C57BL/6 mice and to a much lesser degree in IL-12^+/- mice. In comparison, few cells in the lungs of SCID mice were morphologically judged as lymphocytes, in keeping with the nature of this strain of mouse. The numbers of neutrophils increased significantly to similar degrees in the lungs of CD4^+/- and C57BL/6 mice. Although the difference was not statistically significant, there was an even higher number of neutrophils in the lungs of SCID mice, which may reflect a compensatory mechanism for the lack of lymphocytes. Interestingly, there was a lack of neutrophilic response in the lungs of CD8^+/- mice which was similar to that in the lungs of IL-12^+/- mice. To validate the data from BAL analysis, we analyzed histopathologic alterations in the lungs of these mice. In keeping with cellular profiles in BAL fluids, CD4^+/- and CD8^+/- mice were fully able to mount a granulomatous response composed of typical epithelioid cells, macrophages, and lymphocytes which was morphologically almost indistinguishable from that in the lungs of immunocompetent C57BL/6 mice (Fig. 1a through c). Lymphocytes were more frequently found in clusters within granulomas in the lungs of CD4^+/- or CD8^+/- mice than those in the lungs of C57BL/6 mice. In contrast, there was only a minimum of granuloma formation in the lungs of IL-12^+/- mice (Fig. 1d), in keeping with a previous report (20). In the lungs of SCID mice, granuloma formation was further diminished (Fig. 1e). Instead, there was a striking neutrophilic accumulation together with a diffused accumulation of macrophages (Fig. 1e). Of prominence were loads of mycobacterial bacilli within alveolar macrophages (Fig. 1f).

Quantitation of mycobacterial bacilli in the lungs and spleen. To further investigate the role of CD4 and CD8 T cells in host defense against pulmonary mycobacterial infection, a colony enumeration assay with homogenized total lung tissues was performed. The colonies were enumerated at days 11 to 13 postincubation at 37°C on Middlebrook 7H10 agar (20). In agreement with previous observations, the number of bacilli in the lungs of immunocompetent C57BL/6 mice was small, in sharp contrast to that in immunocompromised IL-12^+/- mice (Fig. 3a). The number of bacilli detected in the lungs of CD8^+/- mice was similar to that in C57BL/6 mice, underscoring an important role of CD4 T cells in protective immune responses. Interestingly, the number of bacilli in the lungs of CD4^+/- mice, although it was somewhat higher, was comparable to that in the lungs of C57BL/6 mice or mice deficient only in CD8 T cells. In contrast, SCID mice deficient in both CD4 and CD8 T cells succumbed to pulmonary mycobacterial infection. Some infected SCID mice died within 3 to 4 weeks after i.t. infection and as a result, surviving mice had to be sacrificed at day 26 instead of day 37. The lungs of these mice harbored abundant bacilli, the number of which was much higher even than that in the lungs of IL-12^+/- mice at day 37. Of note, in our study, SCID mice succumbed to pulmonary
FIG. 1. Histopathology of lungs from CD4<sup>−/−</sup> (a), CD8<sup>−/−</sup> (b), C57BL/6 (c), IL-12 p40<sup>−/−</sup> (d), and SCID (e and f) mice infected i.t. with <i>M. bovis</i> BCG after 26 days (SCID) or 37 days (other strains). Lungs were fixed by perfusion in 10% formalin, sectioned, and stained with hematoxylin and eosin (a to e) or Ziehl-Neelsen staining for identification of mycobacteria (f). L, lymphocytic clusters; E, epithelioid macrophages. Arrowheads, alveolar macrophages loaded with mycobacteria. Magnifications, ×440 (a to e) and ×604 (f).
BCG infection much earlier than mice infected with a larger dose of BCG via the intravenous route (9, 20). It was also noted that although there was a high mycobacterial load in their lungs, IL-12−/− mice did not succumb to pulmonary mycobacterial infection even at a much later time in this model (20), suggesting that the immune system, although markedly compromised due to the lack of IL-12 and IFN-γ and severely impaired CD4 and CD8 T-cell responses, could still keep infection checked to a certain degree. This may be partially due also to the low virulence of M. bovis BCG, since IL-12−/− mice have been shown to succumb to systemic infection by virulent Mycobacterium tuberculosis (3).

It has recently been demonstrated that the number of mycobacterial bacilli in the spleen correlates with the extent of systemic dissemination of mycobacteria from the lungs and that the extent of such dissemination correlates inversely with the level of protective immunoinflammatory responses in the lungs (20). Therefore, we also quantitated bacilli in the spleen in the present study. As shown in Fig. 4b, only a small number of bacilli was recovered from the spleens of C57BL/6 and CD8−/− mice. The number of bacilli was also small, although somewhat higher than in C57BL/6 and CD8−/− mice, in the spleens of CD4−/− mice. These numbers were in stark contrast to a very high number of mycobacterial bacilli present in the spleens of SCID and IL-12−/− mice. These findings suggest that although lacking CD4 T cells, CD4−/− mice could significantly control local replication and systemic dissemination of mycobacteria. On the other hand, although lacking CD8 T cells, CD8−/− mice were fully capable of controlling pulmonary mycobacterial infection and systemic spread via the functional activities of CD4 T cells, and the level of such control was indistinguishable from that in C57BL/6 immunocompetent mice. Comparing CD4−/− with CD8−/− mice, however, revealed that the extent of such control of infection was clearly greater in mice with the CD4 T-cell repertoire intact. These findings indicate that CD4 T cells play a critical role in controlling pulmonary mycobacterial infection, whereas CD8 T cells have the ability to carry out some, if not all, functional activities overlapping with those of CD4 T cells and to compensate for the loss of functional activities of CD4 T cells.

Type 1 cytokine contents in the lungs. It has previously been demonstrated that the type 1 cytokines, including IL-12, IFN-γ, and TNF-α, are critical for the development of type 1 protective tissue immunoinflammatory responses and for subsequent control of pulmonary mycobacterial infection (20). Therefore, in the present study, we examined the levels of these type 1 cytokines in the lungs. As shown in Fig. 4a, the increased levels of IL-12 in the lungs of C57BL/6 and CD8−/− mice were very similar. The level of IL-12 was higher in the lungs of CD4−/− mice, which was likely involved in enhancing CD8 T-cell responses. Likewise, the levels of IFN-γ were similar in C57BL/6 and CD8−/− mice (Fig. 4b), and it was lower in the lungs of mice deficient in CD4 T cells. These results suggest that CD4 T cells were a major source of IFN-γ in this model. Like IL-12 levels, the levels of TNF-α in the lungs were similar in CD4−/−, CD8−/−, and C57BL/6 mice, suggesting a contribution from CD8 T cells and/or macrophages to TNF-α release. The severely impaired IFN-γ or TNF-α response in
the lungs of IL-12^-/- mice was in agreement with a previous report (20).

Thus, we have demonstrated in this study an important role of T cells in the development of protective immune responses against pulmonary mycobacterial infection by M. bovis BCG. Normally, as in C57BL/6 mice, it is likely that increased CD4 T cells and, to a lesser degree, CD8 T cells contribute to such protection by the release of type 1 cytokines and by cytotoxic activities (2, 4, 14, 16, 17, 21). However, in the absence of CD8 T cells, CD4 T cells themselves are functionally sufficient in mounting appropriate immune responses in the lungs which were both qualitatively and quantitatively almost indistinguishable from those in immunocompetent C57BL/6 mice. These findings indicate a functionally critical role for CD4 T cells in host resistance against pulmonary mycobacterial infection. On the other hand, in the absence of CD4 T cells, the host managed to mount a more rigorous CD8 T-cell response, perhaps partially through an increased release of immunostimulatory IL-12, which could apparently compensate to a substantive extent for the loss of CD4 T-cell functions. However, due to a restricted capacity to release IFN-γ, compared to that of CD4 T cells, upon stimulation by IL-12 (19), the level of IFN-γ response in CD4^-/- mice was not completely compensated for, which likely accounted for a lower-than-normal level of protection. IFN-γ is a potent macrophage activator and has been demonstrated to be a critical type 1 cytokine involved in the development of protective responses during mycobacterial infections (1, 7, 20). In contrast to IFN-γ, the level of TNF-α response was not weakened at all in the lungs of CD4^-/- mice, suggesting cellular sources other than CD4 T cells. It is likely that both macrophages and CD8 T cells release this cytokine.

Thus, high levels of IL-12 and TNF-α, together with a moderate level of IFN-γ, in the lungs of CD4^-/- mice may have contributed to an unexpected degree of protection against pulmonary mycobacterial infection. We have further demonstrated that either CD4 or CD8 T cells are required for protection since, when lacking all of these important T-cell subsets, the host cannot mount appropriate granulomatous responses and fails to control (as in IL-12^-/- mice) or succumbs to (as in SCID mice) pulmonary mycobacterial infection.

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REFERENCES


