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The LFA-1 Adhesion Molecule Is Required for Protective Immunity during Pulmonary Mycobacterium tuberculosis Infection

Shamik Ghosh,* Alissa A. Chackerian,* Christina M. Parker,* Christie M. Ballantyne, † and Samuel M. Behar2*

Host immunity to Mycobacterium tuberculosis is mediated by T cells that recognize and activate infected macrophages to control intracellular bacterial replication. The early appearance of T cells in the lungs of infected mice correlates with greater resistance to infection. However, it is unknown whether the trafficking of T cells to the lung following infection is dependent upon the expression of certain adhesion molecules. To address this question, we infected knockout (KO) mice that have defective expression of CD11a, CD11b, CD18, CD62, CD103, or β7. We found that the integrins CD11a and CD18 are absolutely required for host resistance following infection with aerosolized M. tuberculosis. Although Ag-specific T cells are generated following infection of CD11a KO mice, T cell priming is delayed, T cell trafficking to the lung is impaired, and fewer ESAT6-specific CD4+ T cells are found in the lungs of CD11a KO mice compared with control mice. Thus, LFA-1 (CD11a/CD18) plays an essential role in immunity to M. tuberculosis infection. The Journal of Immunology, 2006, 176: 4914–4922.

The localization of naïve T cells within the LN requires CD11a/18 (LFA-1), CD62L, and CCR7 expression by the T cells and ICAM-1, P-selectin glycoprotein ligand 1, and CCL19 by the LN endothelial cells (7–9). Following the activation of T cells in organized lymphoid tissues such as the LN, T cells must exit the lymphoid tissue, recirculate, and ultimately extravasate in the peripheral tissues, where they perform their effector functions. The preferential homing of activated T cells to peripheral tissues depends upon their expression of specific patterns of adhesion molecules and chemokine receptors, and upon the expression of the appropriate counterreceptors by endothelial cells at sites of tissue inflammation (10, 11). For example, T cells must express cutaneous lymphocyte Ag to home to the skin, lymphocyte trafficking into inflamed nonintestinal tissues depends upon the αβ7 integrin (VLA-4), which binds VCAM-1, and gut-homing T cells require the expression of αβ7 integrins (12–15). In contrast, it is not clear whether adhesion molecules exist that allow selective targeting of activated T cells to the lung.

The lung is an anatomically complex structure composed of mucosal and nonmucosal compartments. After infection, DC migrate to lymphoid tissue and stimulate naïve T cells through a cognate interaction (16). These newly stimulated T cells are likely induced to express adhesion molecules that can then mediate migration to pulmonary tissues. Airway inflammation induced by intratracheal instillation of sheep RBC leads to up-regulation of P-selectin, E-selectin, and VCAM-1 on lung vasculature and the recruitment of circulating T cells is dependent upon selectin ligands and α4 integrin (17). Although the accumulation of CD8+ T cells and B cells in the lungs following intratracheal challenge with sheep RBC is clearly dependent on endothelial selectins, this effect is less dramatic for CD4+ cells (18). Other studies have shown that LFA-1 and ICAM-1 are important for the retention by the lung of adoptively transferred alloreactive and Ag-specific T cells (19, 20). Finally, because pulmonary epithelial cells express E-cadherin, the ability of α4β7 to bind to E-cadherin is one mechanism that could lead to the retention of T cells in lung tissue (21–23). Animal studies have shown a strong correlation between the early appearance of activated CD4+ T cells in the lungs of...
C57BL/6 (B6) mice and resistance to tuberculosis (3, 24, 25). However, little is known about the molecular signals that are required for the recruitment of Ag-specific T cells to the lung and to other sites of infection. It is likely that multiple mechanisms facilitate the migration of effector T cells into actively infected sites, and some of these molecular pathways may be redundant or only induced following tissue inflammation. Defining specific adhesion molecules that are required for optimum host resistance against tuberculosis may provide insight into the molecular requirements for the migration of activated T cells into the lung during inflammation. By comparing integrin knockout (KO) and normal control mice following respiratory infection with Mtb, we sought to determine which adhesion molecules are critical for immunity to tuberculosis.

Materials and Methods

Mice

Six- to 8-wk-old female B6, B6.PL-Thy1.2/Cy, and BALB/c mice were obtained from The Jackson Laboratory. CD11a KO (26) (>N6 backcross to B6, from C.M.B.), CD11b KO (27) (>N6 backcross to B6, from C. M. Ballantyne), CD18 KO (28) (N8 backcross to B6, Jax 003329), CD62 KO (B6;129S6-Selectm1Hyn Selltm1Hyn Selptm1Hyn Jax 003807), CD11b KO (29) (>N14 backcross to B6, Jax 003807), CD103 KO (14) (>N10 backcross to BALB/c, from C. M. Parker), and β7 KO (>N11 backcross to B6, Jax 003807) mice have been previously described and were bred at our institution. All mice were housed under specific pathogen-free conditions and used in a protocol approved by the institution.

Bacteria and aerosol infections

All of the experiments were performed using virulent Mtb (Erdman) grown as previously described (24). It should be noted that a total five lots of bacteria were used for the experiments reported herein (Erd 1.1, 1.2, 1.3, 1.5, and 1.6) by our nomenclature. The experiments depicted in Fig. 1 were infected with Erd 1.1, Erd 1.3, Erd 1.5, respectively. The experiments depicted in Fig. 2, A and B, were done with Erd 1.3. The vast majority of other experiments were done using either Erd 1.5 or 2.1. Just before infection, an aliquot was thawed, sonicated twice for 10 s with a cup horn sonicator, and then diluted in 0.9% NaCl-0.02% Tween 80. A 15-ml suspension of Mtb was loaded into a nebulizer (MiniHEART nebulizer; Vornado, an isotype-matched control Ab, conjugated to FITC, PE, or CyChrome (all from BD Pharmingen) for 20 min on ice. Cells were washed and fixed overnight at 4°C in 1% paraformaldehyde in PBS. After being washed twice, the cells were analyzed with a FACSort (BD Biosciences). The FlowJo software program was used to analyze the data (Tree Star).

In vitro restimulation assays

CD4+ T cells were purified from PLN or from the lungs of infected mice, by a two-step procedure. Using the pan-T cell isolation kit, total T cells were purified by negative selection, followed by positive enrichment using anti-CD4 immunomagnetic beads (Miltenyi Biotec). In all experiments, the purity of the cells was 90–95% as determined by flow cytometry. Purified CD4+ T cells (0.1 × 10^6 cells/well) were stimulated with mycobacterial sonicate in the presence of irradiated naive C57BL/6 splenocytes (0.4 × 10^6 cells/well) for 48 h in vitro. Culture supernatants were assayed for INF-γ by ELISA.

ELISPOT assay for INF-γ

An ELISPOT was used to detect INF-γ secretion by individual CD4+ T cells specific for ESAT6_1–15 as previously described (30). ELISPOT plates were coated with the capture anti-INF-γ mAb overnight at 4°C. The capture mAb was discarded, and the plates were washed and blocked with complete media for 2 h at room temperature. The number of purified CD4+ T cells was varied and cultured with 10-μm ESAT6_1–15 and irradiated naive splenocytes (0.4 × 10^6 cells/well) and cultured for 40 h at 37°C. The cells were discarded, and after washing the plates with deionized water and PBS/Tween 20, the biotinylated anti-INF-γ mAb was added for 2 h at room temperature. The plate was washed and streptavidin-alkaline phosphatase was added for 1 h followed by washing and development of the color reaction using the AEC substrate reagent kit (BD Biosciences). The reaction was stopped with water after the spots developed, and they were enumerated using an Immunospot image analyzer (Cellular Technologies).

Adaptive transfer of immune cells

CD4+ T cells were purified from PLN of five to seven infected B6 or CD11a KO mice 3 wk after infection as described. CD4+ T cells from both groups of mice were mixed at a 1:1 ratio and 10 × 10^6 cells were injected i.v. via the tail vein into three to four infected B6.PL-Thy1.2/Cy mice. Each mouse was analyzed 18 h after transfer and donor cells were identified in the lung and blood by staining the cells with CD11a-FITC, Thy1.2-PE, and CD4-CyChrome. The donor CD4+ T cells were Thy1.2 CD11a or Thy1.2-CD11a−.

Results

CD18 is required for host resistance following pulmonary infection with Mtb

To determine which adhesion molecules are required for immunity to Mtb following aerosol infection, we compared the survival of normal inbred BALB/c or B6 mice to genetically engineered mice lacking CD18 (β2), CD103 (αε), or β7. The survival curve for αε KO mice closely paralleled that of congenic BALB/c mice, with a median survival time of 252 and 246 days, respectively. These findings demonstrate that the αεβ7 integrin is not required for optimum resistance to infection (Fig. 1A). Because β7 pairs with both the αε and αα, we then tested whether mice deficient in the β7 integrin have impaired immunity to Mtb. Similar to the results with αε KO mice, the survival of β7 KO mice is nearly identical to

Preparation of cells

Leukocytes were isolated from the lung, PLN, and spleen from infected or uninfected mice. The PLN was identified by its location along the trachea near the bifurcation of the mainstem bronchi. In general, a single enlarged LN was reproducibly identified in this location. Mononuclear cells (MNC) from the PLN and spleen were obtained by mechanical disruption through a 70-μm nylon Falcon cell strainer (Fisher). Contaminating RBC were lysed in 0.15 M NaCl, 1 mM KHCO3, 0.1 mM Na EDTA (pH 7.2–7.4). Lung tissue was finely minced and digested for 2 h at 37°C in 125–150 U/ml type IV collagenase (Sigma-Aldrich). The digested lung tissue was pressed through 70-μm nylon Falcon cell strainers.

Flow cytometry

Lung MNC were washed in staining buffer (5% FBS/0.02% NaN3, in PBS). Cells were stained with Abs specific for mouse CD4, CD8, CD22, NK1.1, an isotype-matched control Ab, conjugated to FITC, PE, or CyChrome (all from BD Pharmingen) for 20 min on ice. Cells were washed and fixed overnight at 4°C in 1% paraformaldehyde in PBS. After being washed twice, the cells were analyzed with a FACSort (BD Biosciences). The FlowJo software program was used to analyze the data (Tree Star).

Histological analysis

Lung from four to six mice per group was examined at each time point. Tissue was preserved in Z-fix (Amitech) embedded in paraffin, sectioned and stained either with H&E or Mason’s trichrome. Images were obtained using a Leica DMLB microscope and a Nikon Coolpix 9500 digital camera. The images were adjusted and assembled in Adobe Photoshop 7.0.
control B6 mice (Fig. 1B). These results exclude a critical role for the αβ, and αβ integrins in survival following respiratory infection. Because the α KO and β KO mice are embryonic lethal, their importance could not be assessed using this approach (31, 32).

The β integrins are another major family of leukocyte integrins. In contrast to the results obtained using α KO and β KO mice, the median survival time for β KO mice was 134 days and all of them died within 150 days after infection (Fig. 1C). In contrast, the median survival time of B6 control mice was >320 days (p < 0.0001). These results show that β integrins play a critical role in the host response following respiratory infection with Mtb.

Control of bacterial replication and host survival is dependent on LFA-1

The β2 integrin (CD18) associates with four different α-chains to form heterodimeric proteins that are expressed at the cell surface. For example, CD18 associates with CD11a (α), to form the heterodimer known as LFA-1, which is expressed by nearly all leukocytes (11). CD18 also associates with CD11b (α), and the resulting heterodimer, known as Mac-1, is expressed on myeloid cells and polymorphonuclear leukocytes (PMN) (11). Finally, CD18 also associates with CD11c and CD11d (α and α, respectively) (11). Finding that CD18 KO mice are more susceptible to tuberculosis implied one or more members of the β integrin family are critical for immunity to Mtb. To determine which α-chain is required, we compared the survival of B6, CD11a KO, and CD11b KO mice (Fig. 2A). As previously reported, the survival of CD11b KO mice is not significantly different from control mice (33). In contrast, CD11a KO mice succumb prematurely following infection with Mtb by the respiratory route (p < 0.0001) (Fig. 2A).

The ability of B6, CD11a KO, and CD11b KO mice to control Mtb replication in the lung was measured 4 and 12 wk after respiratory infection. CD11b KO mice control bacterial replication as efficiently as B6 mice (data not shown). In contrast, CD11a KO mice were unable to limit bacterial replication in the lung as efficiently as B6 mice (Fig. 2B). For example, 4 wk after infection, 2.3 ± 2.3 × 10^6 CFU/lung were found in B6 mice. In contrast, a higher average bacterial burden was found in the lungs of CD11a KO mice (8.4 ± 2.2 × 10^6 CFU; p = 0.0023). After 12 wk of infection, the CFU in the lungs of B6 mice fell slightly (0.97 ± 0.27 × 10^6 CFU). At this time point, the CFU in the lungs of CD11a KO mice was nearly 25-fold greater (24.3 ± 17.7 × 10^6 CFU; p < 0.0001), demonstrating a significant impairment in the ability of CD11a KO mice to contain the infection (Fig. 2B).

We also examined whether mice that are triply deficient in CD62L, -P, and -E (triple selectin KO (TSKO)) have impaired control of bacterial replication. Compared with control mice, TSKO mice had slightly higher CFU in the lung 28 days after aerosol infection (means of 2.6 × 10^9 vs 0.82 × 10^6; p = 0.0295) (Fig. 2C). Similar results were observed in the spleen and livers of these mice (data not shown). However, this small difference was transient. Measurement of the CFU 12 wk after infection failed to demonstrate any significant differences between TSKO and control mice in the number of CFU in the lung (Fig. 2C). Thus, among the cell adhesion molecules examined in this study, CD11a and CD18 are both required for survival and control of bacterial replication, whereas αβ, β, CD11b, and CD62 are not. These findings lead us to conclude that LFA-1 (e.g., αβ) is required for immunity following respiratory infection with Mtb.

Granuloma formation in CD11a KO mice

Although it is well accepted that LFA-1 is critical for the trafficking of naive T cells into LN (9), we wondered whether LFA-1 may also be required for the trafficking of activated T cells into the lung following infection. Therefore, we examined the development of pulmonary granulomas in mice chronically infected with Mtb. Although numerous granulomas were observed in the lungs of B6 and CD11a KO mice 9 wk postinfection, their histological features were strikingly distinct (Fig. 3). The lesions in B6 mice were typical granulomas with small focal areas of granulomatous inflammation which were characterized by a central macrophage rich region (black asterisk) surrounded by a dense lymphocytic infiltrate (white asterisk) (Fig. 3, A–C). In contrast, although discrete granulomas were identified in the lungs from susceptible CD11a KO mice (Fig. 3A, middle and right panel), their central regions were more diffuse, and while there were abundant macrophages, neutrophils were also prominent (Fig. 3C, black arrowheads), which can be associated with early central necrosis (Fig. 3A, arrows). The relative paucity of lymphocytes was striking. Although scattered lymphoid cells could be identified, some in small clusters (Fig. 3B, middle panel, white asterisk), the dense lymphocytic infiltrates characteristically observed in B6 mice were absent. The areas of pale eosinophilic staining in the granulomas of CD11a KO mice were further evaluated using a trichrome stain that colors extracellular matrix components such as collagen blue. Although minimal blue staining was observed in the pulmonary granuloma in B6 mice, the extensive blue staining observed in the
lungs of CD11a KO mice is consistent with the development of fibrosis in these lesions (Fig. 3D). This difference highlights how the evolution of granulomas in B6 and CD11a KO mice is qualitatively different.

In parallel with the histological analysis (Fig. 3), the infiltrating leukocytes in the lungs of these infected B6 and CD11a KO mice was analyzed by flow cytometry. The lungs of infected B6 mice had 8-fold more B cells and 3- to 4-fold more CD4$^+$ and CD8$^+$ T cells than CD11a$^{-/-}$ mice (Table I). Interestingly, infected C57BL/6 and CD11a$^{-/-}$ mice had similar numbers of NK cells and PMN. Together, these data demonstrate that although leukocytes infiltrate the lung and form granuloma in the absence of LFA-1, LFA-1 is required for the efficient accumulation of T and B lymphocytes into the lung and possibly their migration into the microenvironment of the granuloma following Mtb infection.

### Table I. The number of leukocytes in the lungs of infected B6 and CD11a$^{-/-}$ mice

<table>
<thead>
<tr>
<th>Marker</th>
<th>B6</th>
<th>CD11a$^{-/-}$</th>
<th>p$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD22</td>
<td>$1.67 \pm 1.09^{b}$</td>
<td>$0.11 \pm 0.07$</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>CD4</td>
<td>$1.15 \pm 0.67$</td>
<td>$0.37 \pm 0.13$</td>
<td>$&lt;0.05$</td>
</tr>
<tr>
<td>CD8</td>
<td>$0.77 \pm 0.51$</td>
<td>$0.29 \pm 0.09$</td>
<td>NS</td>
</tr>
<tr>
<td>NK1</td>
<td>$0.38 \pm 0.27$</td>
<td>$0.40 \pm 0.29$</td>
<td>NS</td>
</tr>
<tr>
<td>Gr-1</td>
<td>$0.26 \pm 0.14$</td>
<td>$0.19 \pm 0.04$</td>
<td>NS</td>
</tr>
</tbody>
</table>

$^a$ P Value, by two-way ANOVA  
$^b$ Mean ± s.e.; cells $\times 10^6$/lung; $n = 5$ mice/group.

The appearance of T cells in the lungs of B6 and CD11a KO mice following infection

The pathological differences between B6 and CD11a KO mice led us to more precisely define how T cell recruitment to the lung differed between the two mouse strains. Cells from the PLN and lungs of B6 and CD11a KO mice were isolated following respiratory infection and their phenotype analyzed by flow cytometry. B6 mice had earlier and more rapid appearance of CD4$^+$ and CD8$^+$ T cells in the PLN following infection compared with CD11a KO mice. Two weeks after infection, B6 mice had 4- to 5-fold more CD4$^+$ T cells and 3- to 4-fold more CD8$^+$ T cells than CD11a KO mice. For example, $134 \times 10^6$ CD4$^+$ cells and $71 \times 10^6$ CD8$^+$ cells were found in each B6 PLN on average, while CD11a KO mice had only $33 \times 10^6$ CD4$^+$ cells and $23 \times 10^6$ CD8$^+$ T cells per PLN (Fig. 4A). This early difference was transient and by 3 wk after infection, there was only a 1.5-fold difference in CD4$^+$ cells and no significant difference in the number of CD8$^+$ cells. When examined 5 and 9 wk after infection, the absolute number of CD4$^+$ and CD8$^+$ T cells in the PLN of both B6 and CD11a KO mice fell significantly, but more T cells persisted in the PLN of B6 mice at these later time points.

The kinetics with which T cells appeared in the lungs of these mice was significantly different from that observed for the PLN. When examined 2 wk after infection, the number of CD4$^+$ and CD8$^+$ T cells was very low, similar in both B6 and CD11a KO mice, and not different from uninfected mice (Fig. 4B). However,
CD4+ T cells were purified from the draining LN and lungs of infected B6 and CD11a KO mice to normalize for any differences in the number of CD4+ T cells present in these tissues. The CD4+ T cells were stimulated in vitro with mycobacterial Ag in the presence of irradiated B6 splenocytes. Under these conditions, the responses of CD4+ T cells from the draining LN to mycobacterial Ag is similar in B6 and CD11a KO mice 3 wk after infection (Fig. 5). Thus, we conclude that Ag-specific CD4+ T cells are primed following infection in CD11a KO mice. In contrast to the PLN, IFN-γ production by pulmonary CD4+ T cells from CD11a KO mice remained significantly less, particularly through week 5 after infection, despite normalizing the number of CD4+ T cells used in the in vitro assay. These data suggest that the frequency of Ag-specific CD4+ T cells in the lungs of CD11a KO mice is decreased compared with B6 control mice. Therefore, although activation of Ag-specific T cells does occur in the draining PLN, the expression of Ag-specific T cell mediated immunity in the lungs of CD11a KO mice is defective.

Primting of Ag-specific CD4+ T cells in CD11a KO mice

To quantify the Ag-specific T cell response more accurately, we determined how the frequency of ESAT61–15-specific CD4+ T cells changes in the lung and PLN of B6 and CD11a KO mice following Mtb infection. The results shown in Fig. 6 are the average of three independent experiments in which the total CD4+ T cell number, and frequency and absolute number of ESAT61–15-specific CD4+ T cells was determined in the lung and PLN of B6 and CD11a KO mice. Compared with normal mice, CD11a KO

![Image](http://www.jimmunol.org/)

**FIGURE 5.** Kinetics of the ESAT6-specific CD4+ T cell response in B6 and CD11a KO mice infected with Mtb. CD4+ T cells were purified from the PLN 3 wk, or from the lung 3, 5, and 9 wk after infection of B6 (closed circles and bars) and CD11a KO (open circles and bars) mice with aerosolized Mtb. The CD4+ T cells were stimulated with the indicated dilutions of Mtb sonicate in the presence of irradiated naive B6 splenocytes or in presence of anti-CD3 and anti-CD28 mAb. The amount of IFN-γ produced after 48 h was measured by ELISA, and the error bars represent the SE. Similar results were obtained in four independent experiments.

![Image](http://www.jimmunol.org/)

**FIGURE 4.** Lymphocyte numbers in the PLN and lungs of B6 and CD11a KO mice differ following aerosol Mtb infection. The cell numbers obtained from the PLN (A) and lungs (B) of infected B6 (○) and CD11a KO (■) mice. The symbols indicate means and error bars indicate the SE determined how the frequency of ESAT61–15-specific CD4+ T cells in the PLN occurs normally by measuring IFN-γ production following in vitro stimulation of PLN CD4+ T cells with mycobacterial sonicates.

![Image](http://www.jimmunol.org/)

**Detection of Ag-specific CD4+ T cell responses in CD11a KO mice**

To further delineate whether the increased susceptibility of CD11a KO mice against Mtb arises from an inability to prime Ag-specific T cells, we investigated whether the generation of Ag-specific T cells is differs in B6 and CD11a KO mice following infection. We first determined whether the priming of Ag-specific CD4+ T cells in the PLN occurs normally by measuring IFN-γ production following in vitro stimulation of PLN CD4+ T cells with mycobacterial sonicates.

CD4+ T cells were purified from the draining LN and lungs of infected B6 and CD11a KO mice to normalize for any differences in the number of CD4+ T cells present in these tissues. The CD4+ T cells were stimulated in vitro with mycobacterial Ag in the presence of irradiated B6 splenocytes. Under these conditions, the responses of CD4+ T cells from the draining LN to mycobacterial Ag is similar in B6 and CD11a KO mice 3 wk after infection (Fig. 5). Thus, we conclude that Ag-specific CD4+ T cells are primed following infection in CD11a KO mice. In contrast to the PLN, IFN-γ production by pulmonary CD4+ T cells from CD11a KO mice remained significantly less, particularly through week 5 after infection, despite normalizing the number of CD4+ T cells used in the in vitro assay. These data suggest that the frequency of Ag-specific CD4+ T cells in the lungs of CD11a KO mice is decreased compared with B6 control mice. Therefore, although activation of Ag-specific T cells does occur in the draining PLN, the expression of Ag-specific T cell mediated immunity in the lungs of CD11a KO mice is defective.

Because T cells play an absolutely critical role in immunity to tuberculosis, we hypothesize that the inability of CD11a KO mice to recruit T cells to the lung is responsible for their increased susceptibility to Mtb infection. A priori, the failure of T cells to accumulate in the lungs of infected CD11a KO mice could reflect a problem in T cell priming and proliferation, either because naive T cells fail to be recruited to the draining LN where T cell priming occurs, or because LFA-1 is required as an accessory signal for the efficient proliferation of Ag-specific T cells. Alternately, LFA-1 could be required for the trafficking of T cells to sites of inflammation in the lung and for their proper integration into granulomas.
mice have a delayed increase in CD4+ T cells in the draining LN (Fig. 6A, left panel). By week 3 after infection, similar numbers of CD4+ T cells are found in the PLN, but the increase in CD4+ T cells observed in CD11a KO mice is transient. In contrast to the LN, B6 and CD11a KO mice have similar numbers of pulmonary CD4+ T cells during the first 2 wk of infection. Subsequently, there is a more rapid accumulation of CD4+ T cells in the lungs of B6 mice, and ultimately, a lower steady state number of total CD4+ T cells are found in the lungs of infected CD11a KO mice (Fig. 6B, left panel). Based on these results, one might conclude that CD11a KO mice have a defect in T cell recruitment to the lung.

The measurement of Ag-specific CD4+ T cells leads to a different interpretation. Although the priming of ESAT6-specific CD4+ T cells in the pulmonary LN is more efficient in B6 mice, ultimately the frequency of ESAT6-specific CD4+ T cells in CD11a KO mice approaches 0.1%, which is indistinguishable from normal mice (Fig. 6A, middle panel). Interestingly, there is already a difference in the frequency and absolute number of ESAT6-specific CD4+ T cells in the lungs of B6 and CD11a KO mice by 2 wk after infection (Fig. 6B). Then, between 2 and 5 wk after infection, a rapid increase in the number of ESAT6-specific CD4+ T cells occurs in the lungs of CD11a KO mice and control B6 mice (84-fold increase vs 66-fold increase, respectively) (Fig. 6B, middle panel). Although the absolute number of ESAT6-specific CD4+ T cells is 10-fold greater in the lungs of B6 mice compared with CD11a KO mice, these data show that Ag-specific CD4+ T cells have a similar fold increase in the lungs of both B6 and CD11a KO mice following T cell priming. We know from our previous studies that T cell priming occurs first in the draining LN on days 10–11 postinfection and subsequently Ag-specific CD4+ T cells are first detected in the lung between days 12–14. Therefore, if we assume that priming initially occurs in the draining LN, followed by T cell trafficking to the lung, then the difference observed in the lung 2 wk after infection could be a consequence of defective T cell trafficking or a difference in the kinetics of T cell priming in the draining LN.

**Bacterial dissemination from the lung occurs independently of LFA-1**

The above results indicate that although priming of Ag-specific T cells does occur in CD11a KO mice, there is a delay in the expansion of ESAT6-specific CD4+ T cells, particularly in the draining LN. We have previously demonstrated that Mtb dissemination from the lung to the PLN is linked to initiation of the T cell immune response, and may be correlated with host resistance to tuberculosis (3). As CD18 is required for the dissemination of other intracellular bacteria (34), we considered whether the delay in initiation of the immune response and susceptibility of CD11a KO mice might be a consequence of delayed dissemination following respiratory infection. Therefore, we determined whether bacterial dissemination from the lung to the PLN requires β2 integrins.

Viable Mtb is detected in the PLN of both B6 and CD18 KO mice beginning 8 days after aerosol infection with Mtb (Fig. 7). By day 11, nearly all the mice in both groups had disseminated to the PLN, and the viable bacterial count in the PLN had increased nearly 100-fold compared with day 8. Similarly, no delay in dissemination was apparent in CD11a KO and CD11b KO mice (data not shown). These results indicate that bacterial dissemination in the absence of β2 integrins is unaltered, and thus, cannot explain the delay in T cell priming observed in CD11a KO mice.

**Homing of immune CD11a KO CD4+ T cells to the lung is impaired in infected mice**

To address whether CD11a is required for trafficking of primed CD4+ T cells to the lungs of Mtb-infected mice, we determined how efficiently adoptively transferred CD4+ T cells obtained from the PLN of infected B6 and CD11a KO mice traffic to the lungs of infected B6 mice. The choice of B6.PL-Thy1a/Cy congenic (Thy1.1) mice as recipients allowed us to use the Thy1.2 Ag expressed by both B6 and CD11a KO mice to track the donor CD4+ T cells. Furthermore, because CD11a is uniformly expressed by T cells, we distinguished CD4+ T cells from B6 and CD11a KO mice based on their expression of CD11a. Thy1.2 CD4+ CD11a+ T cells and Thy1.2 CD4+ CD11a− T cells purified from the PLN of mice 3 wk after infection were mixed at a 1:1 ratio and transferred into infected recipient Thy1.1 mice. We chose this time point because a similar frequency and number of ESAT6-specific CD4+ T cells are found in the LN of B6 and CD11a−/− mice 3 wk after infection (see Fig. 6). Purified CD4+ T cells were transferred into congenic Thy1.1 B6 mice that were infected for at least 3 wk so that sufficient inflammation and mycobacterial Ag would be present in recipient mice to induce T cell homing to the lung. We analyzed the blood and the lungs of infected recipient mice by flow cytometry 18 h later. There was a skewing of the ratio of Thy1.2 CD4+ CD11a+/−+ to Thy1.2 CD4+ CD11a−/− cells recovered from the lungs compared with the cells found in the blood of recipient Thy1.1 B6 mice (Fig. 8). This data indicate that the migration of activated CD4+ T cells to the lung following infection is partly dependent on CD11a and supports the hypothesis that...
CD11a expression by CD4+ T cells is important for the trafficking of Ag-experienced cells to infected nonlymphoid tissue.

Discussion

Following priming of Ag-specific T cells in the draining LN, activated T cells preferentially migrate to the sites of inflammation from which the Ag-laden DC emigrated. The recruitment and retention of T cells by inflamed tissue beds is mediated in large part by chemokines and cell adhesion molecules (10, 35). Although many adhesion molecules required for lymphocyte trafficking are well characterized, the candidate molecules that mediate specific homing to the lung have not been clearly delineated. Our interest in defining the adhesion molecules required for T cell migration to the lung following Mtb infection stemmed from our previous observation correlating T cell migration to the lung with resistance to tuberculosis in inbred mouse strains (3, 24).

Our strategy was to determine whether KO mice that lack certain adhesion molecules were more susceptible to Mtb. This strategy was based on the hypothesis that the absence of a critical adhesion molecule would impair T cell trafficking to the lung following infection, and consequently would impair host resistance to tuberculosis. Control of bacterial replication is impaired in mice deficient in CD18. B6 (closed symbols) and CD18 KO (open symbols) mice were sacrificed at the indicated times after aerosol infection with 169 CFU of Mtb, and the number of bacteria in the lung and PLN were determined. Each symbol represents an individual mouse. All of the time points have six mice per group (except for day 8 CD11a KO mice; n = 5), but some of the symbols are superimposed. None of the differences between the two groups of mice were statistically significant, as determined using a two-way ANOVA and a Bonferroni posttest.

FIGURE 7. Dissemination of Mtb following aerosol infection is independent of CD18. B6 (closed symbols) and CD18 KO (open symbols) mice were sacrificed at the indicated times after aerosol infection with 169 CFU of Mtb, and the number of bacteria in the lung and PLN were determined. Each symbol represents an individual mouse. All of the time points have six mice per group (except for day 8 CD11a KO mice; n = 5), but some of the symbols are superimposed. None of the differences between the two groups of mice were statistically significant, as determined using a two-way ANOVA and a Bonferroni posttest.

CD11a expression by CD4+ T cells is important for the trafficking of Ag-experienced cells to infected nonlymphoid tissue.

Discussion

Following priming of Ag-specific T cells in the draining LN, activated T cells preferentially migrate to the sites of inflammation from which the Ag-laden DC emigrated. The recruitment and retention of T cells by inflamed tissue beds is mediated in large part by chemokines and cell adhesion molecules (10, 35). Although many adhesion molecules required for lymphocyte trafficking are well characterized, the candidate molecules that mediate specific homing to the lung have not been clearly delineated. Our interest in defining the adhesion molecules required for T cell migration to the lung following Mtb infection stemmed from our previous observation correlating T cell migration to the lung with resistance to tuberculosis in inbred mouse strains (3, 24).

Our strategy was to determine whether KO mice that lack certain adhesion molecules were more susceptible to Mtb. This strategy was based on the hypothesis that the absence of a critical adhesion molecule would impair T cell trafficking to the lung following infection, and consequently would impair host resistance to tuberculosis. Control of bacterial replication is impaired in mice deficient in αv, β2, CD11b, or CD62, and thus we conclude that these molecules are not required for optimum host resistance to Mtb. Although this approach rules out a significant contribution of αvβ2 and α6β2, we cannot address the potential role of αβ2, by this approach. Other evidence suggests that αvβ2 may be important for the trafficking of T cells to the lungs of Mtb-infected mice (36).

In contrast, mice lacking the β2 integrin (CD18) are significantly more susceptible to tuberculosis than control mice. CD18 is known to associate with four different α-chains (CD11a-d), and the cell surface expression of all four heterodimers is abrogated in the absence of CD18. CD11a/CD18 and CD11b/CD18 are the only members of this family that are significantly expressed by T cells. Therefore, CD11a KO and CD11b KO mice were infected with Mtb by the aerosol route to identify which heterodimer is required for immunity against tuberculosis.

Our data confirm a previous report that mice lacking CD11b control bacterial replication and have normal survival following infection with Mtb (33). In contrast to CD11b, absence of CD11a clearly impairs control of bacterial growth in the lung and diminishes long-term survival. Although pulmonary granulomas can be identified in CD11a KO mice, their histological structure is abnormal. Our analysis reveals a striking paucity of lymphocytes in the granulomas and surrounding lung tissue. The diminished numbers of B and T cells may explain why a higher bacterial burden is observed in the lungs of infected CD11a KO mice. These results are complementary to the findings reported by Orme and colleagues (37) that ICAM-1 KO mice are more susceptible to tuberculosis. ICAM-1 is a ligand of LFA-1 and ICAM-1 KO mice also succumb prematurely to aerosol infection. Interestingly, the pulmonary lesions in ICAM-1 KO mice contain lymphocytes but lack epithelioid macrophages (38). Thus, we speculate that β2 integrins other than LFA-1 may facilitate the trafficking of macrophages and neutrophils into the inflamed lung, and furthermore, that ICAM-1 plays a nonredundant role in the trafficking of myeloid cells into granuloma. Although multiple ligands exist for LFA-1, and some of these may be redundant in vitro, their interactions in vivo may be complex and remain to be clearly understood. What is certain is that LFA-1 is essential for host resistance to tuberculosis.

LFA-1 is known to be critical for the migration of naive T cells into LN and the mass of LN tissue is reduced in CD11a KO mice (9). Therefore, it may not be too surprising that we find fewer T cells, particularly Ag-specific T cells, in the PLN of CD11a KO mice 2 wk after infection, indicating a problem with the initiation of adaptive immune response. As we previously found that mycobacterial dissemination to the PLN precedes initiation of T cell immunity, and because dissemination of Salmonella is CD18 dependent, we considered whether dissemination of Mtb from the lung is similarly dependent upon CD18 (3, 34). We found that dissemination is unaltered in the absence of CD18, making it unlikely that the kinetics of the T cell response are affected by a change in Ag delivery to the LN. Thus, we favor the interpretation that the impairment in T cell activation in the PLN of CD11a KO mice arises from a delay in the delivery of naive T cells to the LN, or alternately from inefficient costimulation of naive T cells during the T cell-DC interaction. Although no intrinsic defect in proliferation of LFA-1-deficient T cells after TCR stimulation has been identified in CD11a KO mice, their histological structure is abnormal. Our analysis reveals a striking paucity of lymphocytes in the granulomas and surrounding lung tissue. The diminished numbers of B and T cells may explain why a higher bacterial burden is observed in the lungs of infected CD11a KO mice. These results are complementary to the findings reported by Orme and colleagues (37) that ICAM-1 KO mice are more susceptible to tuberculosis. ICAM-1 is a ligand of LFA-1 and ICAM-1 KO mice also succumb prematurely to aerosol infection. Interestingly, the pulmonary lesions in ICAM-1 KO mice contain lymphocytes but lack epithelioid macrophages (38). Thus, we speculate that β2 integrins other than LFA-1 may facilitate the trafficking of macrophages and neutrophils into the inflamed lung, and furthermore, that ICAM-1 plays a nonredundant role in the trafficking of myeloid cells into granuloma. Although multiple ligands exist for LFA-1, and some of these may be redundant in vitro, their interactions in vivo may be complex and remain to be clearly understood. What is certain is that LFA-1 is essential for host resistance to tuberculosis.

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observed (28, 39, 40), it is possible that the lack of an LFA-1-dependent T cell costimulatory signal limits the degree of clonal expansion of Ag-specific T cells in CD11a KO mice (41). Although LN are smaller in naive CD11a KO mice, they enlarge and become indistinguishable in size from B6 LN following Mtb infection. Thus, during inflammatory states such as infection, there exist CD11a-independent pathways that facilitate the migration of naive T cells into lymphoid tissue. Not only does priming of Ag-specific CD4+ T cells occur in the draining LN of infected CD11a KO mice, but by 3 wk after infection, as the immune response begins to peak, similar numbers of ESAT6-specific CD4+ T cells and total numbers of T cells are found in the PLN of B6 and CD11a KO mice.

B6 mice exemplify the resistant mouse phenotype and following infection with Mtb they mount a robust immune response and form granulomas in their lungs that contain dense lymphocytic infiltrates. In contrast, the lungs of infected CD11a KO mice are remarkable for the lack of lymphocytes in their granulomas. Our impression that T cells appear to be disproportionately excluded from granulomas compared with the lung as a whole, suggests the possibility that CD11a has a role in the retention of lymphocytes within the microenvironment of the granuloma. Our data shows that there are fewer total CD4+ and CD8+ T cells and ESAT6-specific CD4+ T cells in the lungs of CD11a KO mice compared with control mice; however, we cannot be certain whether this difference is due to an alteration in recruitment of T cells into the lungs of infected mice. Although CD11a KO mice had a lower frequency and absolute number of ESAT6-specific CD4+ T cells in the lung, the kinetics of their response was similar, albeit of a lower magnitude, to infected B6 mice. Because we observed a decrease in the frequency of ESAT6-specific CD4+ T cells in the PLN and lungs of CD11a KO mice compared with B6 as early as 2 wk after infection, we cannot rule out that the difference in T cell number arises from a delay in T cell priming and/or decreased proliferation of Ag-specific T cells. For example, normal trafficking but impaired T cell proliferation in the lung may give rise to a similar phenotype. To identify a specific role for LFA-1 in the trafficking of immune T cells to the lung, we compared the ability of adoptively transferred B6 and CD11a KO CD4+ T cells to traffic to the lungs of infected recipient mice. This approach showed that immune CD4+ T cells expressing CD1a trafficked to the lung with greater efficiency than CD4+ T cells from CD11a KO mice. Thus, following infection with Mtb, LFA-1 is required at multiple stages in the development of the pulmonary T cell response.

LFA-1 is an important leukocyte adhesion molecule that serves multiple functions during the development of immunity in vivo, both in model Ag systems and following infection. Consequently, the varied phenotype of mice lacking LFA-1 should not be surprising. For example, although we report that mice deficient in LFA-1 are more susceptible to Mtb, both CD1a KO and CD18 KO mice are more resistant to Listeria monocytogenes, another intracellular bacterial pathogen that infects macrophages. It appears that the increased bacterial resistance of CD11a KO mice is due to increased granulocytosis and emergence of an LFA-1-independent pathway for neutrophil migration into the liver (42). CD18 KO mice also have peripheral blood neutrophilia and enhanced IL-6 and IL-1β production, which may promote innate immunity to L. monocytogenes (43). In the absence of LFA-1, granulocyte production of IL-12 appears to stimulate a type I immune response and induce IFN-γ-producing NK cells (44). The increased resistance of CD11a KO mice is not limited to this acute infection model. CD18 KO mice are also more resistant to Leishmania major as manifested by a reduced parasite burden locally and systemically (45). It appears that while Th2 cell homing is dependent on CD18, Th1 cell homing and effector function is preserved in CD18 KO mice. Whether this is critical for long-term survival of infected mice is not clear since another study using a different strain of L. major found CD18 KO mice to be more susceptible (46). Increased expression of Th1 immunity might be expected to make CD18 KO mice more resistant to Mtb. However, there is little evidence in mice of a significant Th2 response to Mtb, or that the Th1-Th2 balance affects host resistance following Mtb infection (47). In contrast to Listeria and Leishmania, the mortality of CD18 KO mice is significantly increased following i.p. infection with Streptococcus pneumoniae (28). Although our results indicate that resistance to Mtb is entirely dependent on CD11a, both CD1a and CD11b play distinct functions in immunity to S. pneumoniae (48). Why CD1a and CD11b are required for immunity to S. pneumoniae has not been delineated, but given that it is an acute infection, it is reasonable to hypothesize that CD1a and CD11b are required for the function of cells participating in the innate immune response.

The balance of Th1-Th2 immunity does not seem to be a major determinant of resistance and susceptibility in the mouse model of tuberculosis. Consequently, the Mtb model reveals a requirement for LFA-1 in other aspects of T cell immunity against microbial pathogens other than Th1-Th2 regulation. In the absence of LFA-1, mice form granulomas that are largely devoid of lymphocytes which undergo necrosis and fibrosis, and the mice are unable to effectively control bacterial replication and succumb prematurely from infection. Our immunological analysis shows that while T cell priming does occur in CD11a KO mice, expansion of Ag-specific T cells, particularly in the lung is delayed and never reaches the magnitude observed in B6 mice. Of note, the mortality of CD1a and CD18 KO mice is delayed compared with T cell and cytokine KO mice, which is consistent with our finding that these mice generate mycobacteria-specific T cell immunity, although their response is not well expressed in the lung. Thus, we observe that LFA-1 is required for optimum expression of T cell mediated immunity in the lung following aerosol infection with Mtb, and these observations support an important role for pulmonary T cells in containing Mtb infection. In addition to providing insight into the immunological requirements for host resistance to tuberculosis, these studies are important for clinicians using anti-LFA-1 Abs for the treatment of autoimmune disease and transplant rejection in people (49, 50). Just as treatment of rheumatoid arthritis and Crohn’s disease with anti-TNF blockade led to reactivation tuberculosis in patients with latent Mtb infection, our study suggests that heightened surveillance of patients treated with anti-LFA-1 Abs for the development of reactivation tuberculosis may be appropriate (51).

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Disclosures
The authors have no financial conflict of interest.

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