

Leukotriene B4 Receptor-1 Is Essential for Allergen-Mediated Recruitment of CD8⁺ T Cells and Airway Hyperresponsiveness¹

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Recent studies in both human and rodents have indicated that in addition to CD4⁺ T cells, CD8⁺ T cells play an important role in allergic inflammation. We previously demonstrated that allergen-sensitized and -challenged CD8-deficient (CD8^{-/-}) mice develop significantly lower airway hyperresponsiveness (AHR), eosinophilic inflammation, and IL-13 levels in bronchoalveolar lavage fluid compared with wild-type mice, and that all these responses were restored by adoptive transfer of in vivo-primed CD8⁺ T cells or in vitro-generated effector CD8⁺ T cells (T_{EFF}). Recently, leukotriene B4 and its high affinity receptor, BLT1, have been shown to mediate in vitro-generated T_{EFF} recruitment into inflamed tissues. In this study we investigated whether BLT1 is essential for the development of CD8⁺ T cell-mediated allergic AHR and inflammation. Adoptive transfer of in vivo-primed BLT1^{+/+}, but not BLT1^{-/-}, CD8⁺ T cells into sensitized and challenged CD8^{-/-} mice restored AHR, eosinophilic inflammation, and IL-13 levels. Moreover, when adoptively transferred into sensitized CD8^{-/-} mice, in vitro-generated BLT1^{+/+}, but not BLT1^{-/-}, T_{EFF} accumulated in the lung and mediated these altered airway responses to allergen challenge. These data are the first to show both a functional and an essential role for BLT1 in allergen-mediated CD8⁺ T_{EFF} recruitment into the lung and development of AHR and airway inflammation. *The Journal of Immunology*, 2005, 174: 4979–4984.

Allergic asthma is a complex syndrome that has been characterized by airway obstruction, airway inflammation, and airway hyperresponsiveness (AHR)³ (1, 2). Ag-specific memory T cells, especially CD4⁺ T cells, have been considered pivotal in the development of AHR and eosinophilic inflammation (1–4) through the production of cytokines, especially IL-13 (5, 6). However, there is now increasing evidence for the role of CD8⁺ T cells in these responses as well. Increased numbers of CD8⁺ T cells have been shown in the lungs of asthmatic patients (7) and in animal models of allergic asthma (8). In addition, CD8⁺ T cells in the lung are a source of IL-13 and may be required for the full development of allergen-induced AHR and inflammation (9). Moreover, in vitro-generated Ag-specific effector CD8⁺ T cells (T_{EFF}) contribute to these responses through their migration into lung tissue and local production of IL-13 in sensitized and challenged mice (10).

Leukotriene B4 (LTB4) is an arachidonic acid-derived proinflammatory lipid, rapidly generated from innate immune cells (11, 12). LTB4, interacting through a specific G protein-coupled cell surface receptor, BLT1 (13, 14), leads to granulocyte and macrophage accumulation at sites of inflammation (15, 16). Because BLT1 is expressed by T_{EFF}, the LTB4-BLT1 pathway may be essential for effector CD8⁺ T cell movement to sites of acute inflammation (17, 18). However, its role in the development of allergen-induced AHR and inflammation has not been defined.

In the present study we investigated the requirement for BLT1 expression on CD8⁺ T cells and Ag-specific T_{EFF} in the development of allergen-induced AHR and airway inflammation. We show that BLT1-deficient (BLT1^{-/-}) CD8⁺ T cells or BLT1^{-/-} T_{EFF} are not capable of restoring these responses in CD8^{-/-} mice. The absence of BLT1 resulted in the reduced accumulation of CD8⁺ T cells in the lungs and decreased IL-13 production in sensitized and challenged CD8^{-/-} mice. These data demonstrate that BLT1 is essential for allergen-mediated CD8⁺ T cell recruitment to the lung and induction of eosinophilic airway inflammation and AHR.

Materials and Methods

Animals

Homozygous CD8^{-/-} mice, generated by targeting the CD8 α -chain gene in C57BL/6 mice (19), OT-1 mice (C57BL/6 strain) expressing a transgenic TCR that is specific for OVA_{257–264} (SIINFEKL) peptide (20), and wild-type (WT) C57BL/6 mice were purchased from The Jackson Laboratory. BLT1^{-/-} mice, F₁ hybrids of C57BL/6 and 129Sv/J genetic background (21), were backcrossed into the C57BL/6 genetic background for nine generations. BLT1-deficient OT-1 mice were generated by mating BLT1^{-/-} mice with OT-1 mice. These mice were housed under specific pathogen-free conditions and maintained on an OVA-free diet in the Biological Resources Center at the National Jewish Medical and Research Center. Male and female mice, 6–12 wk of age, were used in these experiments. Controls were matched with the deficient mice with regard to age and gender in each experimental group. All experimental animals used in

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³ Abbreviations used in this paper: AHR, airway hyperresponsiveness; BAL, bronchoalveolar lavage; BLT1, leukotriene B4 receptor 1; LTB4, leukotriene B4; MNC, mononuclear cell; PBLN, peribronchial lymph node; PI, phorbol myristate acetate/ionomycin; RL, lung resistance; T_{CM}, central memory CD8⁺ T cell; T_{EFF}, effector CD8⁺ T cell; WT, wild type.

this study were under a protocol approved by the institutional animal care and use committee of the National Jewish Medical and Research Center.

Sensitization and airway challenge

CD8^{-/-} and WT mice were assigned to control (C) and treatment groups (S/C) based on the following treatments: 1) airway challenge with OVA nebulization alone (three times; C group), and 2) i.p. sensitization with OVA and OVA airway challenge (S/C). Mice were sensitized by i.p. injection of 20 μ g of OVA (grade V; Sigma-Aldrich) emulsified in 2.25 mg of alum (AlumImject; Pierce) on days 1 and 14. Mice were subsequently challenged via the airways by inhalation exposure to aerosols of OVA (1% in saline) for 20 min on days 28, 29, and 30. OVA aerosols were produced by an ultrasonic nebulizer (particle size, 1–5 μ m; DeVilbiss). On day 32, airway function was measured as described below, followed by collection of samples for further analyses.

Purification of CD8⁺ T cells

Spleens of BLT1^{-/-} mice or BLT1^{+/+} mice, which were sensitized twice (days 1 and 14) with OVA plus alum, were removed 14 days after the last sensitization (day 28), and purification of CD8⁺ T cells was performed by negative selection using MACS (Miltenyi Biotec) as previously described (9). To assess purification, cells were incubated with allophycocyanin-conjugated anti-CD3; FITC-conjugated anti-CD4, anti-CD8, or anti-B220 Abs; and PE-conjugated anti-CD11c, anti-mouse NK1.1, anti- $\gamma\delta$ TCR, or anti- $\alpha\beta$ TCR (BD Pharmingen), then analyzed by flow cytometry (FACSCalibur; BD Biosciences). The proportion of the transferred cells that stained for CD3⁺CD8⁺ from BLT1^{+/+} or BLT1^{-/-} mice exceeded 93%. They were >99% $\alpha\beta$ CD8⁺ T cells, with <0.5% of cells being $\gamma\delta$ CD8⁺ T cells in preparations from both groups. Contamination by CD4⁺ cells, $\gamma\delta$ ⁺ cells, CD11c⁺ cells, or NK1.1⁺ cells in transferred cells was, in total, <0.5%.

Effector CD8⁺ T cell generation

Differentiation of T_{EFF} cells in vitro was conducted as previously described (10). Lymph node and spleen cells collected from BLT1^{+/+} and BLT1^{-/-} OT-1 TCR transgenic mice were cultured with 1 μ M SIINFEKL peptide. After culture for 2 days, cells were washed and cultured with IL-2 (20 ng/ml). After 7 days, it was determined that the cultures contained >99% $\alpha\beta$ CD8⁺ T cells. CD4⁺ cells, CD11c⁺ cells, NK1.1⁺ cells, or $\gamma\delta$ ⁺ cells were <0.1%. The cells were >90% CD8⁺ effector memory cell phenotype, as shown by the CD122^{high}/CD44^{high}/CD62L^{low} cell surface phenotype. T_{EFF} derived through this protocol showed phenotypic and functional characteristics of effector memory CD8⁺ T cells in vivo (22, 23).

Adoptive transfer recipient mice

Recipient CD8^{-/-} mice were sensitized twice with OVA plus alum on days 1 and 14. OVA-primed CD8⁺ T cells (5×10^6) from BLT1^{+/+} or BLT1^{-/-} mice were administered i.v. via the tail vein to OVA-sensitized CD8^{-/-} mice 14 days after the last sensitization (day 28). In vitro-generated CD8⁺ T_{EFF} cells (5×10^6) from BLT1^{+/+} or BLT1^{-/-} mice were transferred in the same way. After transfer, the mice were exposed to three allergen challenges via the airways on days 28, 29, and 30. Assays were conducted on day 32.

Assessment of airway function

Airway function was assessed as previously described by measuring changes in lung resistance (RL) in response to increasing doses of inhaled methacholine (24). Data are expressed as the percentage of change from baseline RL values obtained after inhalation of saline.

Bronchoalveolar lavage (BAL)

Immediately after assessment of AHR, lungs were lavaged via the tracheal tube with HBSS. Total leukocyte numbers were counted by cell counter (Coulter). Cytospin slides were stained with Leukostat (Fisher Diagnostics) and differentiated by standard hematological procedures.

Measurement of cytokines

Cytokine levels in the BAL fluid and cell culture supernatants were measured by ELISA as previously described (25). IFN- γ , IL-4, IL-5 (BD Pharmingen), and IL-13 (R&D Systems) ELISAs were performed according to the manufacturer's directions. The limits of detection were 4 pg/ml for IL-4, IL-5, and IL-13 and 10 pg/ml for IFN- γ .

Cell isolation

Mononuclear cells (MNC) from spleen and peribronchial lymph node (PBLN) were purified by Ficoll-Hypaque gradient centrifugation (Organon Teknika) (9). Lung cells were isolated as previously described using collagenase digestion (26). Cells were resuspended in HBSS, and MNC were purified by Ficoll-Hypaque gradient centrifugation.

Flow cytometry

After purification, 1×10^6 cells were incubated with allophycocyanin-conjugated anti-CD3 and FITC-conjugated anti-CD8 Abs (BD Pharmingen), then analyzed by flow cytometry (FACSCalibur; BD Biosystems) as previously described (9). The number of CD8⁺ T cells per lung was derived by multiplying the percentage of stained cells by the total number of lung cells isolated. Intracytoplasmic cytokine staining for IFN- γ (BD Pharmingen) and IL-13 (R&D Systems) was performed as previously described (9). The number of cytokine-producing CD8⁺ T cells per lung was calculated from the percentage of cytokine-producing cells and the number of CD8⁺ T cells isolated from the lung.

Statistical analysis

Values for all measurements are expressed as the mean $\pm$ SEM. ANOVA was used to determine the levels of difference between all groups. Comparisons for all pairs were performed by unpaired two-tailed Student's *t* test. Significance levels were set at *p* < 0.05.

Results

In vivo-primed CD8⁺ T cells from BLT1^{+/+}, but not BLT1^{-/-}, mice fully restore AHR in CD8^{-/-} mice

Fig. 1A shows the changes in RL in response to increasing doses of inhaled methacholine for both WT and CD8^{-/-} mice. OVA sensitization and airway challenge led to the development of increased AHR in WT mice, illustrated by significant increases in RL, as previously described in this model (23). OVA-sensitized and -challenged CD8^{-/-} mice developed small increases in RL above nonsensitized and OVA-challenged control mice, but the levels were significantly lower than those in OVA-sensitized and -challenged WT mice (9). To address whether the expression of BLT1 on CD8⁺ T cells is involved in the regulation of allergen-induced AHR, inflammation and cytokine responses, CD8⁺ T cells from BLT1^{+/+} and BLT1^{-/-} mice were injected into CD8^{-/-} mice. Recipient CD8^{-/-} mice were sensitized with OVA on days 1 and 14. On day 28, 2 h before beginning the OVA challenges, 5×10^6 CD8⁺ T cells from BLT1^{+/+} or BLT1^{-/-} mice were transferred via i.v. injection into the tail vein. As shown in Fig. 1A, reconstitution of CD8^{-/-} mice with Ag-primed CD8⁺ T cells from BLT1^{+/+} mice fully restored the development of AHR to levels comparable to those seen in WT mice. In contrast, transfer of OVA-sensitized CD8⁺ T cells from BLT1^{-/-} mice failed to restore AHR.

In vivo primed CD8⁺ T cells from BLT1^{+/+} mice, but not BLT1^{-/-}, mediate allergen-induced eosinophilic airway inflammation

Previous studies indicated that CD8^{-/-} mice failed to develop OVA-induced eosinophil accumulation in BAL, a response that was restored after adoptive transfer of Ag-primed CD8⁺ T cells (9). After sensitization and allergen challenge, the numbers of eosinophils in the CD8^{-/-} mice were significantly lower than those in WT mice (Fig. 1B). Transfer of BLT1^{+/+} CD8⁺ T cells fully restored the number of eosinophils in BAL fluid, whereas BLT1^{-/-} CD8⁺ T cell transfer failed to do so.

Cytokine levels in BAL fluid

OVA sensitization and challenge did not result in significant differences between WT and CD8^{-/-} mice in the levels of IL-4, IL-5, or IFN- γ in BAL fluid (9). However, after sensitization and challenge, the levels of IL-13 were significantly lower in BAL fluids

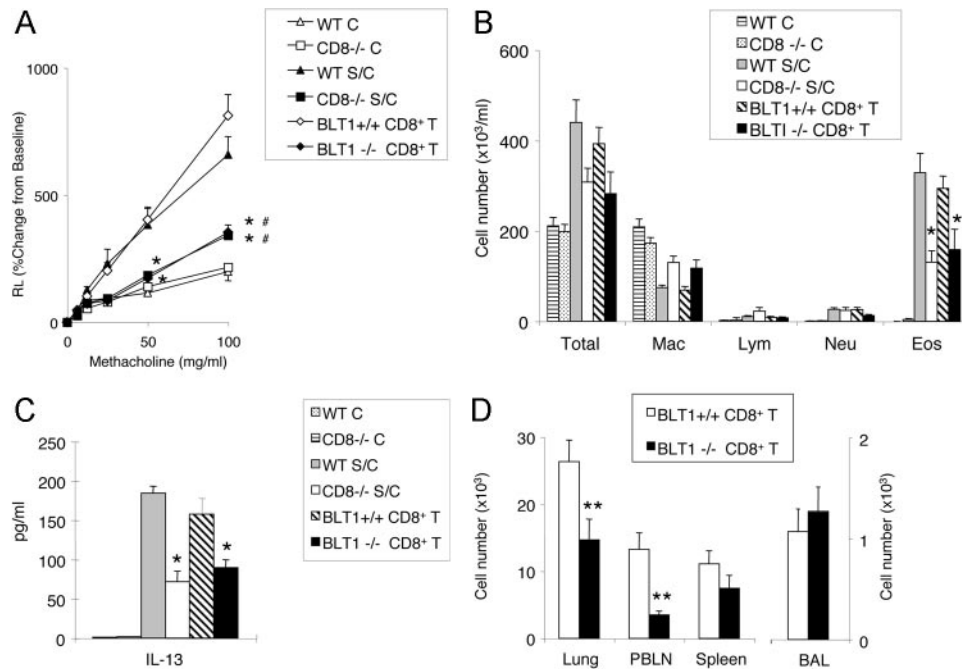


FIGURE 1. Reconstitution of $CD8^{-/-}$ mice with in vivo-primed $CD8^{+}$ T cells from BLT1-deficient mice fails to restore the development of AHR and inflammation. OVA-sensitized $CD8^{-/-}$ mice (recipient mice) received 5×10^6 $CD8^{+}$ T cells i.v. via the tail vein 2 h before the first airway challenge with aerosolized OVA. Recipient mice were comprised of two groups. In one group, mice received $CD8^{+}$ T cells from spleens of OVA-sensitized, BLT1-sufficient mice (BLT1 $^{+/+}$ $CD8^{+}$ T group; $n = 8$). In the other group, mice received $CD8^{+}$ T cells from OVA-sensitized, BLT1-deficient mice (BLT1 $^{-/-}$ $CD8^{+}$ T group; $n = 8$). WT mice and $CD8^{-/-}$ mice receiving no cells are also shown (challenged-only groups: WT C and $CD8^{-/-}$ C; sensitized and challenged groups, WT S/C and $CD8^{-/-}$ S/C; $n = 12$ in each group). **A**, AHR was monitored by measuring RL as described in *Materials and Methods*. **B**, Cell composition in BAL fluid after transfer of $CD8^{+}$ T cells. Groups are the same as in **A**. Total, total cell counts; Mac, macrophages; Lym, lymphocytes; Neu, neutrophils; Eos, eosinophils. **C**, Levels of IL-13 in BAL fluid after transfer of BLT1 $^{-/-}$ $CD8^{+}$ T cells. Groups are the same as in **A**. Cytokine levels were measured in supernatants by ELISA, as described in *Materials and Methods*. **D**, Numbers of $CD8^{+}$ T cells in the lung, BAL, PBLN, and spleen in $CD8^{-/-}$ mice after transfer of $CD8^{+}$ T cells from BLT1 $^{+/+}$ or BLT1 $^{-/-}$ mice. Lung, PBLN, and spleen MNC and BAL cells were isolated and stained with anti-CD3 and anti-CD8, and the numbers were calculated as described in *Materials and Methods* ($n = 8$ in each group). The results for each group are expressed as the mean $\pm$ SEM. *, Significant differences ($p < 0.05$) are indicated between the BLT1 $^{-/-}$ $CD8^{+}$ T group and the $CD8^{-/-}$ S/C group vs the WT S/C and BLT1 $^{+/+}$ $CD8^{+}$ T groups. #, Significant differences ($p < 0.05$) are indicated between the BLT1 $^{-/-}$ $CD8^{+}$ T group and the $CD8^{-/-}$ S/C group vs the WT C and $CD8^{-/-}$ C. **, Significant differences ($p < 0.05$) are indicated compared with BLT1 $^{+/+}$ $CD8^{+}$ T.

recovered from $CD8^{-/-}$ mice compared with WT mice (Fig. 1C). Reconstitution of $CD8^{-/-}$ mice with $CD8^{+}$ T cells from BLT1 $^{+/+}$, but not BLT1 $^{-/-}$, restored the levels of IL-13 in BAL fluid to those seen in WT mice.

Migration of BLT1 $^{+/+}$ and BLT1 $^{-/-}$ $CD8^{+}$ T cells

We next determined whether transferred, in vivo-primed $CD8^{+}$ T cells migrated to the lung 48 h after challenge of sensitized $CD8^{-/-}$ mice. Under this background, transferred cells were easily identified as $CD3^{+}CD8^{+}$ cells. Transferred BLT1 $^{+/+}$ $CD8^{+}$ T cells amounted to $1.2 \pm 0.2\%$ (mean $\pm$ SEM) of the total T cells in lungs after OVA sensitization and challenge, whereas transferred BLT1 $^{-/-}$ $CD8^{+}$ T cells were found at a lower percentage ($0.6 \pm 0.1\%$ of total lung T cells). Fig. 1D summarizes the numbers of transferred $CD8^{+}$ T cells in the lungs, BAL, PBLN, and spleens of recipient mice. Higher numbers of transferred BLT1 $^{+/+}$ $CD8^{+}$ T cells were recovered from lung and PBLN compared with BLT1 $^{-/-}$ $CD8^{+}$ T cells; the numbers in BAL and spleen were not different between the two groups. Together, these data identify a role for BLT1 in the accumulation of $CD8^{+}$ T cells in the lung after sensitization and challenge.

BLT1 $^{+/+}$, but not BLT1 $^{-/-}$, T_{EFF} fully restores AHR in $CD8^{-/-}$ mice

We previously showed that transfer of $CD8^{+}$ T_{EFF} , but not central memory $CD8^{+}$ T cells (T_{CM}), fully restored AHR and cytokine

responses in $CD8^{-/-}$ recipients (9). To address whether the expression of BLT1 on $CD8^{+}$ T_{EFF} is required for these airway and cytokine responses, $CD8^{-/-}$ mice received in vitro-generated BLT1-deficient or BLT1-sufficient $CD8^{+}$ T_{EFF} cells. Fig. 2A shows that reconstitution of the $CD8^{-/-}$ mice with T_{EFF} from BLT1 $^{+/+}$ mice fully restored AHR to levels comparable to those seen in WT mice. In contrast, transfer of BLT1 $^{-/-}$ T_{EFF} failed to restore AHR. Transfer of BLT1 $^{-/-}$ T_{EFF} also failed to restore the number of eosinophils and IL-13 levels in the BAL; transfer of BLT1 $^{+/+}$ T_{EFF} fully restored these responses (Fig. 2, B and C).

Migration of transferred T_{EFF} cells into lung

The accumulation of adoptively transferred T_{EFF} in the lungs of $CD8^{-/-}$ mice was determined 48 h after the last challenge. Transferred BLT1 $^{+/+}$ T_{EFF} cells (identified as $CD3^{+}CD8^{+}$) amounted to $11.0 \pm 2.5\%$ of the total lung MNC after OVA sensitization and challenge (Fig. 3A), whereas transferred BLT1 $^{-/-}$ T_{EFF} cells were found at a much lower percentage ($2.0 \pm 0.3\%$ of total lung MNC). A similar pattern was observed in BAL fluid (Fig. 3A). More than 95% of the BLT1 $^{+/+}$ and BLT1 $^{-/-}$ T_{EFF} in lung and BAL displayed an effector memory phenotype: $CD122^{high}/CD44^{high}/CD62^{low}$ (22). Fig. 3B summarizes the numbers of transferred T_{EFF} detected in the lungs, PBLN, BAL, and spleen of recipient $CD8^{-/-}$ mice. Lower numbers of BLT1 $^{-/-}$ T_{EFF} were detected in BAL and lung, whereas higher numbers of transferred BLT1 $^{-/-}$ T_{EFF} cells were recovered from PBLN.

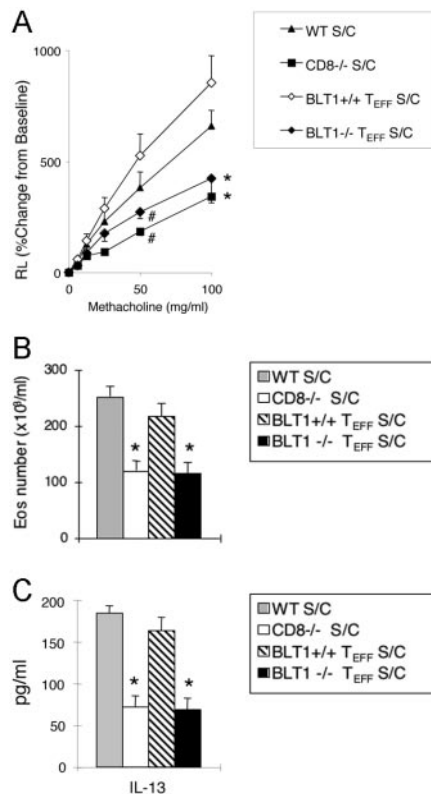


FIGURE 2. Reconstitution of CD8^{-/-} mice with T_{EFF} from BLT1^{+/+}, but not BLT1^{-/-}, mice fully restores the development of AHR and inflammation. CD8^{-/-} recipient mice received 5×10^6 T_{EFF} from BLT1^{+/+} or BLT1^{-/-} mice i.v. via the tail vein 2 h before the first airway challenge with aerosolized OVA. CD8^{-/-} recipient mice received either BLT1^{+/+} T_{EFF} (BLT1^{+/+} T_{EFF} S/C group; $n = 8$) or BLT1^{-/-} T_{EFF} (BLT1^{-/-} T_{EFF} S/C group; $n = 8$). Sensitized and challenged WT mice and CD8^{-/-} mice receiving no cells are also shown (WT S/C and CD8^{-/-} S/C; $n = 12$ in each group). **A**, AHR was monitored by measuring RL as described in *Materials and Methods*. **B**, The numbers of eosinophils in BAL fluid after transfer of BLT1^{+/+} or BLT1^{-/-} T_{EFF}. Groups are the same as in **A**. **C**, IL-13 levels in BAL fluid after transfer of BLT1^{+/+} or BLT1^{-/-} T_{EFF}. Groups are the same as in **A**. Cytokine levels were measured in supernatants by ELISA, as described in *Materials and Methods*. The results for each group are expressed as the mean $\pm$ SEM. *, Significant differences ($p < 0.05$) between BLT1^{+/+} T_{EFF} recipients after sensitization and challenge (BLT1^{+/+} T_{EFF} S/C) and WT mice after sensitization and challenge (WT S/C) vs BLT1^{-/-} T_{EFF} recipients after sensitization and challenge (BLT1^{-/-} T_{EFF} S/C) and CD8^{-/-} mice with sensitization and challenge (CD8^{-/-} S/C) group. #, Significant differences ($p < 0.05$) compared with BLT1^{+/+} T_{EFF} S/C.

Intracellular cytokine detection in transferred T_{EFF}

In addition to or as a consequence of the marked reduction in recruitment of BLT1^{-/-} T_{EFF}, we determined whether the failure to restore AHR and cytokine response by transferred BLT1^{-/-} T_{EFF} could also be due to decreased IL-13 production by these cells. MNCs were obtained from lung tissue of recipient CD8^{-/-} mice after transfer of either BLT1^{+/+} T_{EFF} or BLT1^{-/-} T_{EFF} 48 h after the last challenge. The cells were stimulated with PMA/ionomycin and incubated with Abs for intracellular staining of IL-13 as described in *Materials and Methods*. Fig. 4A illustrates the percentage of cytokine-producing T_{EFF} in recipient lungs. IL-13 was detected in $33.3 \pm 2.8\%$ of transferred BLT1^{+/+} T_{EFF} cells, whereas among the BLT1^{-/-} T_{EFF} cells, only $13.8 \pm 1.5\%$ expressed IL-13. After in vitro culture and just before transfer, there were no significant differences in IL-13 production between BLT1^{+/+} and BLT1^{-/-} T_{EFF} (5.2 ± 1 vs $4.7 \pm 0.9\%$).

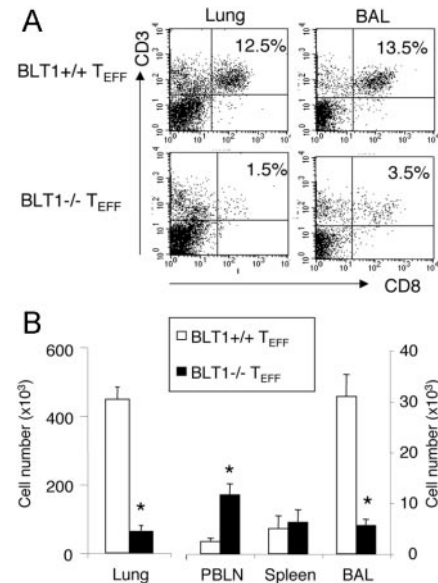


FIGURE 3. Accumulation of transferred BLT1^{+/+} and BLT1^{-/-} T_{EFF} in lung and BAL fluid. **A**, Lung MNC were isolated and stained with anti-CD3 and anti-CD8 Ab as described in *Materials and Methods*. Quadrants were based on isotype control staining. The data shown are representative of two similar experiments ($n = 8$ in each group). **B**, Numbers of CD8⁺ T cells in lung, BAL, PBLN, and spleen in CD8^{-/-} mice after transfer of BLT1^{+/+} and BLT1^{-/-} T_{EFF}. Lung, PBLN, and spleen MNC and BAL cells were isolated and stained with anti-CD3 and anti-CD8, and the numbers were calculated as described in *Materials and Methods*. Groups are the same as in **A**. *, Significant differences ($p < 0.05$) compared with BLT1^{+/+} T_{EFF} group.

In parallel, we monitored CD4⁺ T cell numbers and cytokine production. Numbers of CD4⁺ T cells in the lungs of BLT1^{-/-} T_{EFF} recipient mice were not different compared with those in BLT1^{+/+} T_{EFF} recipient mice. Similarly, the numbers of IL-4- and IL-5-producing CD4 T cells in the lungs of BLT1^{-/-} T_{EFF} recipient mice were not different from those in BLT1^{+/+} T_{EFF} recipient mice. These data suggest that CD4 recruitment or activation in the lung is not affected by the expression of BLT1.

These results demonstrate that in addition to the reduced recruitment, a smaller percentage of the transferred BLT1^{-/-} T_{EFF} cells was capable of IL-13 production after airway allergen challenge of sensitized recipients. Overall, the numbers of IL-13-producing BLT1^{-/-} T_{EFF} cells were significantly lower than the numbers of IL-13-producing BLT1^{+/+} T_{EFF} cells (Fig. 4, **B** and **C**). By contrast, the percentage of IFN- γ -producing cells did not change. These data illustrate that in addition to its role in cell trafficking to the lung, the functional activation of T_{EFF} and IL-13 production may be dependent in part on the expression of BLT1.

Discussion

There is now increasing evidence that CD8⁺ T cells contribute to the development of allergic disease (7–10), but their function in allergic airway disease, especially how they are recruited and activated in the lung, is not well defined. LTB₄ has been shown to be a potent chemoattractant for effector CD8⁺ T cells, and LTB₄ production by activated leukocytes, such as neutrophils, mast cells, and macrophages, may be important in the recruitment of T_{EFF} cells to sites of inflammation (17, 18, 27, 28). BLT1, the high affinity receptor for LTB₄, has been induced substantially on CD8⁺ T_{EFF}, with less expression on CD8⁺ T_{CM} (17). LTB₄ triggers BLT1-dependent chemotaxis of T_{EFF}, but not naive T cells or T_{CM} (17, 27),

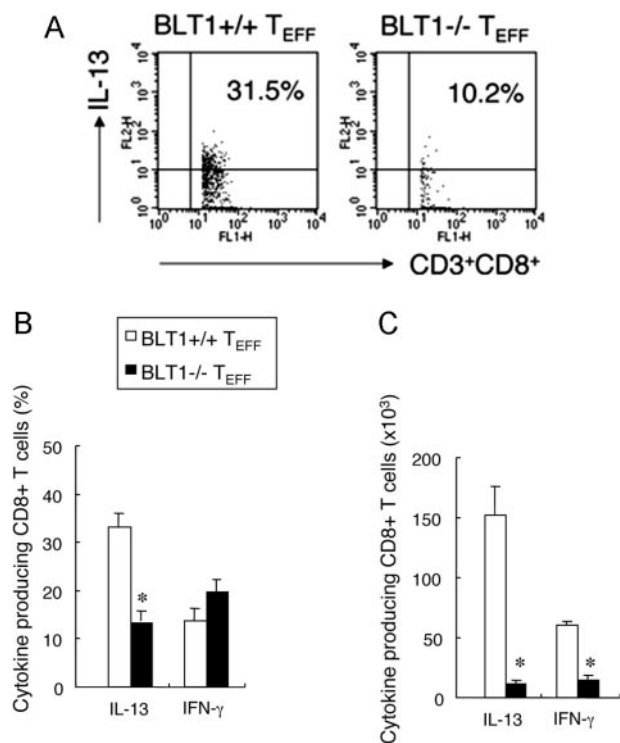


FIGURE 4. Intracellular staining of IL-13 in lung CD8⁺ T cells from recipient CD8^{-/-} mice after transfer of BLT1^{+/+} and BLT1^{-/-} T_{EFF}. *A*, Lung MNC were isolated and stimulated with PMA and ionomycin, then cells were fixed, permeabilized, and stained with anti-mouse IL-13 Ab as described in *Materials and Methods*. CD3⁺CD8⁺ cells were gated and analyzed for intracellular detection of IL-13 protein. Quadrants were set based on isotype control staining. The data shown are representative of two similar experiments ($n = 8$ in each group). *B*, Percentages of cytokine-producing CD8⁺ T cells in the lungs of CD8^{-/-} mice after transfer of BLT1^{+/+} and BLT1^{-/-} T_{EFF}. Groups are the same as in *A*. *C*, Absolute numbers of cytokine-producing CD8⁺ T cells in the lungs of CD8^{-/-} mice after transfer of BLT1^{+/+} and BLT1^{-/-} T_{EFF}. The numbers were calculated as described in *Materials and Methods*. Groups are the same as in *A*. *, Significant differences ($p < 0.05$) compared with BLT1^{+/+} T_{EFF} group.

suggesting that the LTB₄-BLT1 interaction is a potent nonchemokine pathway for effector CD8⁺ T cell accumulation.

In the present study we investigated the role of this LTB₄-BLT1 pathway in effector CD8⁺ T cell function in a mouse model of allergen-induced AHR and airway inflammation. We have reported that CD8^{-/-} mice develop significantly lower AHR and eosinophilic inflammation compared with WT mice, and that these deficient responses were restored by transfer of in vivo-activated CD8⁺ T cells from Ag-primed donors or by transfer of in vitro-generated CD8⁺ T_{EFF}, identifying a critical role for CD8⁺ T cells (9, 10). In the present study we prepared in vivo-primed CD8⁺ T cells and in vitro-generated, Ag-specific CD8⁺ T_{EFF} from BLT1^{-/-} mice to address the question of whether BLT1 expression on CD8⁺ T cells affects their migration and effector function and, consequently, the development of AHR and airway inflammation. After transfer of these cells into CD8^{-/-} mice, a significant number of the transferred BLT1^{+/+} CD8⁺ T cells and BLT1^{+/+} T_{EFF} cells were detected in the lung, and this was associated with the development of AHR, eosinophilia, and IL-13 production. Transfer of BLT1^{-/-} CD8⁺ T cells or BLT1^{-/-} T_{EFF} cells was not effective in mediating any of these responses. This appeared to be due to the absence of two components of the response: 1) failure to accumulate in the lung, and 2) decreased functional activation in the lung, at least for IL-13 production.

These two deficiencies probably explain why transfer of CD8⁺ T cells and T_{EFF} cells from BLT1^{+/+} mice enhanced IL-13 levels in BAL fluid and restored AHR, whereas BLT1^{-/-} cells did not. Furthermore, we demonstrated that although numbers of IL-13-producing BLT1^{+/+} T_{EFF} in the lung after transfer were significantly higher than those of BLT1^{-/-} T_{EFF}, the numbers of IL-13-producing BLT1^{+/+} T_{EFF} in vitro before transfer were not different from those of BLT1^{-/-} T_{EFF}. These data suggest that BLT1^{+/+} T_{EFF} responded in the lung after sensitization and challenge and acquired effector function (IL-13 production), whereas BLT1^{-/-} T_{EFF} failed to do so. These data demonstrate for the first time, in an in vivo model, that migration/accumulation as well as the functional activation of T_{EFF} in vivo are at least in part dependent on the LTB₄-BLT1 pathway. Together, the data indicate a critical role for BLT1-effector CD8⁺ T cells in the full development of AHR and airway inflammation. In contrast, CD4⁺ T cell numbers and cytokine production were not affected by the absence of BLT1.

T_{EFF} have been reported to produce IFN- γ in vitro (22, 29). However, after adoptive transfer, T_{EFF} in the lung displayed a Tc2 phenotype (10). After in vitro stimulation with IL-4, cytotoxic CD8⁺ T cells can be polarized to Th2-type cytokine-producing cells (30–32). Similarly, virus-specific CD8⁺ T cells may convert into IL-5-producing cells in mice sensitized to OVA plus alum, followed by challenge with virus peptide (33). Thus, the phenotype of even predominant Th1-type cytokine-producing CD8⁺ T cells may be redirected toward Th2-type cytokine production, a plasticity previously emphasized in CD4⁺ T cells (34).

IL-13 plays a central role in the development of AHR (6, 7). We showed that lung CD8⁺ T cells are a source of IL-13, and reconstitution of CD8^{-/-} mice with IL-13^{-/-} CD8⁺ T cells failed to restore AHR, confirming that CD8⁺ T cells can contribute to the development of AHR and airway inflammation through IL-13 production (9). LTB₄ is known to augment T cell cytokine secretion in vitro (35–39). In the present study we showed that IL-13 production from CD8⁺ T cells may require BLT1 expression on effector CD8⁺ T cells. Interestingly, airway responses induced by rIL-13 may also require an intact LTB₄ pathway in vivo (40). Because CD8⁺ T_{EFF} are a source of IL-13 after Ag challenge, this production of IL-13 may further activate LTB₄ production in the lung and serve to amplify or enhance the accumulation and activation of Tc2-type effector CD8⁺ T cells.

Tager et al. (18) reported that BLT1 participates in T cell trafficking into the BAL, but not lung tissue, at an early phase after challenge of sensitized mice. In contrast, T_{EFF} did not require this receptor for trafficking into the airway after adoptive transfer and airway challenge of naive (nonsensitized) recipients (18). In contrast, using sensitized, as opposed to naive, recipient mice, we show that migration of transferred BLT1^{-/-} T_{EFF} into lung as well as BAL was significantly impaired compared with that of BLT1^{+/+} T_{EFF}. In this adoptive transfer model, the recipient mice were sensitized to OVA (plus alum) before OVA challenge. LTB₄ production in the lungs of sensitized and challenged recipients should be significantly higher than that of challenged-only recipients (28), and these increased levels of LTB₄ may play a pivotal role in enhancing the recruitment of transferred BLT1^{+/+} T_{EFF} into the lung.

Different members of the chemokine family are known to be subset-selective chemoattractants for T cells (41). For example, it has been shown that CCL2 and CCL5 may be important in the recruitment of CD8⁺ T cells (42). T_{EFF} were reported to migrate in response to CCL5 as well as LTB₄ in vitro (27). In our transfer experiments we demonstrated that the migration of both BLT1^{-/-} CD8⁺ T cells and BLT1^{-/-} CD8⁺ T_{EFF} into the lung was significantly impaired compared with that of BLT1^{+/+} cells; this impairment in the T_{EFF} was more obvious than that in CD8⁺ T cells.

In the latter case, only small numbers of Ag-specific CD8⁺ T cells are probably generated compared with T_{EFF}. These results indicate that although the LTB4-BLT1 pathway regulates CD8⁺ T cell recruitment, it is essential for trafficking of T_{EFF} to sites of airway inflammation in vivo. LTB4-dependent signals contribute at least one essential link to a chain of molecular events that may be required for efficient recruitment of T_{EFF}.

Several cellular constituents of the innate immune system are capable of generating LTB4 (12, 13). Mast cells are a major source of lipid-derived mediators, and activation of mast cells through FcεRI stimulates rapid degranulation and release of LTB4 (43). LTB4 produced by mast cells induces chemotaxis of T_{EFF} in vitro (27); therefore, after sensitization and challenge with Ag, production of LTB4 from mast cells is probably a major contributor to T_{EFF} recruitment to inflamed tissues. LTB4 production from cells other than mast cells, such as neutrophils and macrophages (12, 13), may similarly be capable of recruiting T_{EFF} to the lung in this model.

In summary, we have identified a critical role for BLT1 expression on effector CD8⁺ T cells in the development of allergen-induced AHR and airway inflammation. In vivo, BLT1 expression on effector CD8⁺ T cells may be required for their migration and effector function and the full development of airway eosinophilia and AHR. The T_{EFF}-BLT1 pathway may constitute a novel therapeutic target in bronchial asthma.

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Disclosures

The authors have no financial conflict of interest.

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