Antibody-antigen interaction in the airway drives early granulocyte recruitment through BLT1

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Antibody-antigen interaction in the airway drives early granulocyte recruitment through BLT1. Am J Physiol Lung Cell Mol Physiol 290: L170–L178, 2006. First published August 26, 2005; doi:10.1152/ajplung.00212.2005.—Antibody-antigen interactions in the airway initiate inflammation in acute asthma exacerbations. This inflammatory response is characterized by the recruitment of granulocytes into the airways. In murine models of asthma, granulocyte recruitment into the lung contributes to the development of airway hyperresponsiveness (AHR), mucus production, and airway remodeling. Leukotriene B4 (LTB4) is a mediator released following antigen challenge that has chemotactic activity for granulocytes, mediated through its receptor, BLT1. We investigated the role of BLT1 in granulocyte recruitment following antigen challenge. Wild-type mice and BLT1−/− mice were sensitized and challenged with ovalbumin (OVA) to induce acute allergic airway inflammation. In addition, to explore the relevance to antibody-antigen interactions, we injected OVA bound to anti-OVA IgG1 or anti-OVA IgE intratracheally into naive wild-type and BLT1−/− mice. Cell composition of the lungs, cytokine levels, histology, and AHR were determined. After sensitization and challenge with ovalbumin, there was significantly reduced neutrophil and eosinophil recruitment into the airways of BLT1−/− mice compared with wild-type animals after one or two daily antigen challenges, but this difference was not seen after three or four daily antigen challenges. Mucus production and AHR were not affected. Intratracheal injection of OVA bound to IgG1, or IgE induced neutrophil recruitment into the airways in wild-type mice but not in the BLT1−/− mice. We conclude that BLT1 mediates early recruitment of granulocytes into the airway in response to antigen-antibody interactions in a murine model of acute asthma.

Lung inflammation; asthma; lipid mediator

ACUTE ASTHMA EXACERBATIONS are life-threatening complications of allergic asthma that result in significant morbidity and health care costs (31). Inhalation of allergen initiates airway inflammation in part by binding of allergen to antigen-specific antibodies, which then bind to antibody receptors on innate immune cells, such as macrophages and mast cells. In response, these cells release mediators, which initiate inflammation and induce the recruitment of neutrophils and eosinophils into the airways (5, 7, 13, 25). Granulocytes are important sources of lipid mediators, cytokines, and cytotoxic proteins and thus could play an important role in the propagation of airway inflammation and airway remodeling. Consistent with this, recent studies in murine models of asthma have demonstrated that eosinophils are crucial for the development of airway hyperresponsiveness (AHR), mucus production, and airway remodeling (8, 23, 26, 46). Furthermore, studies in humans have suggested that neutrophil and eosinophil recruitment into the airways may lead to airway fibrosis (9, 10, 14). Thus measures that inhibit the accumulation of granulocytes in the airway during asthma attacks could have profound effects on the development of airway inflammation and long-term complications of the disease.

A commonly used murine model of asthma utilizes mice sensitized and then challenged via the airway with ovalbumin (OVA) to recreate many of the pathological and physiological changes seen in acute allergic asthma exacerbations, including the recruitment of granulocytes into the airway (32, 47, 48). The initial recruitment of neutrophils has been shown to be induced in part through antigen-antibody complex stimulation of FcγRIII (47). Leukotriene B4 (LTB4) is a lipid mediator released following allergen challenge in humans (39, 41, 42, 49) and mice (20) that has potent chemotactic activity for granulocytes mediated through its G protein-coupled seven transmembrane-spanning receptor, BLT1 (18, 22, 44, 52). Because allergen-antibody complexes can stimulate mast cells (15, 19, 27) and macrophages to release LTB4 (11, 21, 36, 38), these data suggest that the LTB4/BLT1 pathway may be an important mechanism of granulocyte recruitment into the airways following allergen challenge and in acute asthma exacerbations.

In this present study we characterized the role of BLT1 in the early inflammatory response in the airway following allergen exposure. First we demonstrate that BLT1 is necessary for early eosinophil and neutrophil recruitment into the airways following antigen challenge. This recruitment defect only exists early and for the movement of the cells into the airway lumen as lung recruitment of granulocytes is preserved in the BLT1−/− mice. We then show that BLT1 is essential for granulocyte recruitment following challenge with OVA bound to IgG1 or IgE antibodies. Our findings demonstrate that the LTB4/BLT1 pathway is the major mediator of early granulocyte recruitment into the airways following exposure to allergens and is likely an important component of the early inflammatory response in acute asthma attacks. However, the early defect in granulocyte recruitment had little effect on later
inflammatory events and the development of AHR and mucus production.

MATERIALS AND METHODS

Mice. Male 129/Sv wild-type mice and BLT1−/− mice (44) (in an 129/Sv background) were used at 6–8 wk of age. All protocols were approved by the animal studies committee at Massachusetts General Hospital.

Mouse models. Allergic airway inflammation was induced in mice as previously described (32, 43). In brief, mice were injected intraperitoneally with 10 µg of OVA (Sigma-Aldrich, St. Louis, MO) and 1 mg of aluminum hydroxide (Sigma-Aldrich) suspended in 0.5 ml of PBS (Mediatech, Herndon, VA) on days 0 and 7. Mice underwent aerosol challenge with OVA (10 mg/ml in PBS) or with PBS alone on days 14, 14–15, 14–16, or 14–17. OVA challenge was performed by placing mice in a Plexiglas box (dimensions: 22 × 14 × 14 cm) and aerosolizing OVA with a nebulizer (DeVilbiss, Health Care Division, Somerset, PA), driven by compressed air for 20 min. Mice were killed 20–24 h after the last aerosol challenge.

In some mice, AHR was measured with a whole-body plethysmograph (Buxco, Sharon, CT) as described (32). AHR was expressed as the enhanced pause (Penh), a number based on inspiratory and expiratory times and pressures that is calculated by software made by the manufacturer. The average Penh over 3 min was determined after exposure for 2 min to aerosolized PBS as a baseline. We then determined average Penh over 3 min after exposing the mice for 2 min to aerosolized methacholine (Sigma-Aldrich) at increasing concentrations (3, 6.125, 12.5, and 25 mg/ml in PBS) and expressed it as the percent change from the baseline. For the intratracheal injections, mice were anesthetized with ketamine (80 mg/kg)-xylazine (12 mg/kg). The trachea was exposed by a ventral incision in the neck, and the trachea was injected with a mixture of 80 mg/kg xylazine and 12 mg/kg ketamine. The trachea was exposed and cannulated with polyethylene tubing. The lungs were lavaged with six 0.5-ml aliquots of PBS containing 0.6 mM EDTA. Lavage fluid recovered from the first 1 ml of instilled PBS/EDTA was collected separately from the rest of the BAL. Both BAL fractions were centrifuged, and the pellet cells from both fractions were pooled for analysis. The supernatant of the BAL recovered from the first 1 ml instilled was kept frozen at −80°C for subsequent analysis. The cells from both BAL fractions were exposed for 30 s to Tris (0.014 M)/NH4Cl (0.14 M) to lyse red blood cells, and the remaining live cells, as determined by trypan blue exclusion, were washed in PBS and enumerated in a hemocytometer.

Whole lung digests. The right lung was excised and minced into small pieces with a scissors. The pieces were digested for 45 min in RPMI with 0.28 Wünsch U/ml Liberase Blendzyme (Roche, Indianapolis, IN) and 30 U/ml DNase (Sigma-Aldrich, St. Louis, MO) for 45 min at 37°C. The digested lungs were then extruded through a mesh strainer, and the collected cells were washed once with PBS. Live cells were enumerated by a hemocytometer as determined by trypan blue exclusion.

Cell differential counts. The differential cell count on cells isolated from the BAL and lung was determined by enumerating macrophages, neutrophils, eosinophils, and lymphocytes on cytospin slides from BAL, lung, and ear swelling. BAL granulocyte recruitment is reduced early after antigen challenge. We determined the number of eosinophils and neutrophils in the BAL taken from OVA-immunized wild-type or BLT1−/− mice after one, 2, 3, or 4 daily OVA challenges. There was a statistically significant reduction in the numbers of eosinophils (18-fold) and neutrophils (32-fold) in the BAL of BLT1−/− mice compared with wild-type mice after one OVA challenge (Fig. 1, A and B). After two daily OVA challenges there continued to be a significant reduction in both eosinophil and neutrophil recruitment into the airways (three- and fivefold respectively). However, this difference was not seen with repetitive challenges on days 3 and 4 (Fig. 1, A and B) or days 3 after a single OVA challenge (data not shown). These data suggest that BLT1 mediates early granulocyte recruitment into the airways, whereas alternative mediators are induced later in response to antigen challenge.
Lung granulocyte recruitment is not different in BLT1<sup>−/−</sup> mice following antigen challenge. We also determined the number of eosinophils and neutrophils recruited into the lung tissue of immunized wild-type or BLT1<sup>−/−</sup> mice following one, two, three, or four daily OVA challenges. As opposed to the BAL data, we did not see any difference in the number of lung eosinophils or neutrophils between the two groups (Fig. 2, A and B). Lung histology confirmed the presence of peribronchial eosinophils and neutrophils in both wild-type and BLT1<sup>−/−</sup> mice after one OVA challenge (Fig. 3, A and B). Evaluation of the level of inflammation around the airways based on blinded scoring of histological sections as outlined in MATERIALS AND METHODS was also not significantly different between the two mouse groups (data not shown). These data suggest a selective role for BLT1 in the recruitment of granulocytes into the airway and alternative mechanisms for lung recruitment of these cells.

AHR and mucus production were not different between wild-type and BLT1<sup>−/−</sup> mice. To determine the effect of an airway granulocyte recruitment defect on the phenotypic changes seen in asthma we evaluated the production of mucus and the development of AHR. Mucus production as assessed by blinded scoring of PAS staining of lung sections as outlined in MATERIALS AND METHODS was not different between the two mouse groups (data not shown). AHR was measured in these mice using methacholine-induced changes in the Penh, a non-invasive surrogate for airway resistance (16). After one, two, three, or four daily OVA challenges, immunized wild-type and BLT1<sup>−/−</sup> mice had similar elevations in their Penh following methacholine challenge (Fig. 4, A and B, and data not shown).

BAL fluid from OVA-immunized and -challenged animals induced more chemotaxis for granulocytes from wild-type mice compared with those from BLT1<sup>−/−</sup> mice. We wanted to better define the mechanism of the granulocyte recruitment defect seen in these experiments. First we attempted to measure LTB<sub>4</sub> levels in the lavage fluid from wild-type and BLT1<sup>−/−</sup> mice after one or two OVA challenges. The levels were at or below the limit of detection of the assay as reported by others (37) (data not shown). This did not rule out a role for LTB<sub>4</sub> as it has potent activity at low concentrations, so we used an in vitro chemotaxis assay as a functional test of LTB<sub>4</sub> activity in the BAL. Granulocytes were isolated from the bone marrow of wild-type and BLT1<sup>−/−</sup> mice by gradient separation. This preparation yielded ~80% early granulocyte precursors as assessed by cytopsins (data not shown). BAL fluid from wild-type and BLT1<sup>−/−</sup> mice that had been sensitized and challenged with OVA for 2 days was used to induce chemotaxis of the isolated cells. There was a significant decrease in chemotaxis of the BLT1<sup>−/−</sup> granulocytes compared with wild-type granulocytes from BAL from both wild-type and BLT1<sup>−/−</sup> mice (Fig. 5A). Interestingly, BLT1<sup>−/−</sup> BAL fluid had less chemotactic activity for wild-type granulocytes than BAL from wild-type mice. Because granulocytes are a major source of LTB<sub>4</sub> (53), the defect in granulocyte recruitment into the airways seen in the BLT1<sup>−/−</sup> mice could lead to reduced LTB<sub>4</sub> levels in the BAL compared with BAL from wild-type mice.

Neutrophil chemokine expression in the lung. Other neutrophil-specific chemokines are expressed in the murine model of asthma such as CXCL1/KC and CXCL2/MIP-2 (47). To see if the expression of these chemokines was affected by disruption in BLT1 signaling we measured the RNA expression of these...
chemokines in lungs harvested from OVA-sensitized and -challenged BLT1−/− and wild-type mice. After one or two daily OVA challenges, analysis of the RNA expression of CXCL1 and CXCL2 in the lungs showed no statistically significant differences in both groups of mice (Fig. 5B). We also measured the protein levels of these chemokines in the BAL from these same mice and found no differences in the protein levels of CXCL1 and CXCL2 between BLT1−/− and wild-type mice (Fig. 5C). Protein levels of these chemokines were both upregulated in the BAL compared with unchallenged mice (undetectable levels, data not shown). We did find a higher concentration of CXCL1 than CXCL2 in the BAL, which differed from the RNA expression data. This difference may be a reflection of the different compartments of the lung in that the BAL samples only from the airways, whereas the RNA data represented lung parenchyma expression as well as the airway. Despite the differences between the RNA and protein levels both assays demonstrated that BLT1−/− mice make similar amounts of CXCL1 and CXCL2 compared with wild-type animals.

BAL levels of OVA-specific IgG1 and IgE antibodies are increased in immunized wild-type and BLT1−/− animals. Our hypothesis is that antigen-antibody interactions in the airway lead to BLT1-mediated granulocyte recruitment. Thus we wanted to confirm that OVA-specific antibodies were present in both wild-type and BLT1−/− mice at equal levels. We measured the levels of OVA-specific IgG1 and IgE in BAL fluid isolated from unimmunized mice and OVA immunized wild-type and BLT1−/− mice with an ELISA. There was a significant increase in the amount of OVA-specific IgG1 and IgE in the BAL of immunized wild-type and BLT1−/− mice compared with naive mice, in which the levels of OVA-specific antibodies were undetectable (Fig. 6, A and B). There was no significant difference in antibody levels, however, between the wild-type and BLT1−/− animals.
OVA-IgG1 and OVA-IgE antigen-antibody complexes induce granulocyte recruitment in wild-type mice but not in BLT1<sup>−/−</sup> mice. Finally, to focus the stimulus for granulocyte recruitment into the airway to antibody-antigen induced interactions, we combined OVA with OVA-specific IgG1 and IgE antibodies and then injected the mixture into the tracheae of naïve wild-type and BLT1<sup>−/−</sup> mice. These complexes induced prominent neutrophil recruitment into the airways of the wild-type animals 1 day after injection (Fig. 7). A previous study utilizing the same model had shown that granulocyte recruitment peaked at ~10–20 h and then disappeared at 48 h, so later time points were not checked (47). Injection of OVA alone, IgG1 alone, or IgE alone did not induce significant neutrophil recruitment into the BAL. The OVA-IgG1 mixture stimulated more granulocyte recruitment than the OVA-IgE mixture in wild-type mice. When the antibody-antigen mixtures were injected into BLT1<sup>−/−</sup> mice, however, there was dramatic and significant reduction in neutrophil recruitment into the airways (Fig. 7). Granulocyte recruitment after injection of OVA-IgG1 was reduced by eightfold, and after injection of OVA-IgE was reduced by over 70-fold in BLT1<sup>−/−</sup> mice compared with the wild-type mice. In fact, the number of neutrophils in the airways of BLT1<sup>−/−</sup> mice after injection of OVA-IgE was similar to the number after injection of OVA or IgE alone. These data provide strong evidence supporting a role for BLT1 in granulocyte recruitment following antigen-antibody interactions in the airway. The LTB4/BLT1 pathway seems to stimulate the majority of the granulocyte recruitment induced by IgG1-mediated stimulation and nearly all of the recruitment induced by IgE.

Fig. 5. BAL fluid granulocyte chemotactic potential and chemokine levels. A: average number of granulocytes from wild-type or BLT1<sup>−/−</sup> mice that migrated into the wells of a chemotaxis plate in response to BAL fluid isolated from wild-type or BLT1<sup>−/−</sup> mice after 2 daily OVA challenges (average of 6 BAL samples, each performed in triplicate). B: RNA expression levels of CXCL1 and CXCL2 in the lungs of wild-type and BLT1<sup>−/−</sup> mice after OVA immunization and challenge with 1 or 2 daily OVA nebulizations (n = 7 mice per group). The differences were not significant. C: protein levels of CXCL1 and CXCL2 in the BAL fluid taken from wild-type and BLT1<sup>−/−</sup> mice after OVA immunization and challenge with 1 or 2 daily OVA nebulizations (n = 7 mice per group). The differences were not significant.
Acute asthma exacerbations remain the major cause of morbidity and mortality in patients with asthma. A large number of exacerbations are caused by exposure to allergens in sensitized individuals. In the minutes following allergen inhalation, mast cells and alveolar macrophages are stimulated to degranulate via binding to IgE and IgG antibodies complexed to antigen. The released mediators induce bronchoconstriction, edema, and the recruitment of granulocytes into the airways (4, 17). Although mechanistic studies have identified an essential role for lymphocyte recruitment in the establishment of asthma (28–30, 33), recently there has been increasing realization of the importance of granulocytes in the pathogenesis of this disorder (8, 23, 26, 46, 50). Eosinophil recruitment into the lung in particular has been shown to be crucial for the development of AHR and mucus production in murine models of asthma, whereas neutrophil recruitment into the lung has been linked to severe asthma and fatal asthma attacks (1, 50). In addition to mediating acute changes in the airway, granulocytes may help establish a chronic inflammatory state and mediate some of the late complications of disease such as airway remodeling (10, 14, 23).

In the present study we use a model of acute asthma to demonstrate that early airway granulocyte recruitment was dependent on BLT1 expression. This recruitment was specific to the airway as lung recruitment of granulocytes was preserved with disruption of BLT1 expression. Furthermore, the effect was limited to the early inflammatory response and did not influence late inflammatory events, suggesting that additional mechanisms are activated later after antigen challenge. Mucus production and AHR were not diminished in BLT1−/− mice. We suspect that the preserved recruitment of granulocytes and lymphocytes into the lung was sufficient to induce AHR and mucus production. The observed differences were solely due to disruption of BLT1 signaling and not due to disruption of the low-affinity LTB4 receptor (BLT2) as BLT2 is not expressed in mouse leukocytes or in the lung (24).

We then performed a series of experiments to explore the mechanism of this recruitment defect. In an in vitro chemotaxis assay, BAL fluid isolated from sensitized and challenged animals was chemotactic for isolated murine granulocytes from wild-type animals but less so for granulocytes from BLT1−/− mice, suggesting that the chemotactic activity in the BAL was mediated in part by LTB4. Interestingly BAL fluid isolated from sensitized and challenged BLT1−/− mice induced less granulocyte chemotaxis than BAL fluid from wild-type mice. As mentioned in RESULTS, granulocytes are a major source of LTB4 (53), and LTB4 may stimulate more 5-lipoxygenase activity in neutrophils (40). Thus increased neutrophil recruitment into the airway would be expected to amplify LTB4 production and increase the chemotactic potency of the BAL. We next demonstrated that the recruitment defect was not due to differences in the expression of two other neutrophil-specific chemoattractants, CXCL1 and CXCL2. To confirm that the recruitment defect was from the absence of BLT1 on granulocytes and not due to differences in antibody production in the...
BLT1−/− mice we demonstrated equal levels of OVA-specific IgG and IgE in the airways of immunized and sensitized wild-type and BLT1−/− mice. Finally, when OVA bound to IgG1 or IgE was injected into the airways of nonsensitized mice there was BLT1-dependent recruitment of neutrophils into the airway. This did not occur if the antibodies alone or OVA alone were injected. Together, these data demonstrate that early granulocyte recruitment after antigen challenge results from antigen-antibody-induced release of LTβ into the airways and that BLT1 is an important mediator of granulocyte recruitment in asthma after allergen inhalation.

Our findings are consistent with prior studies that demonstrated early antigen-specific granulocyte recruitment into the airways following allergen challenge. In these experiments utilizing murine models of allergic airway disease, IgG-allergen and IgE-allergen immune complexes were able to induce granulocyte recruitment into the airways presumably through the release of various chemoattractants (47, 54). In one of the studies, upregulation of the neutrophil-specific chemokines CXCL1 and CXCL2 was found following challenge with allergen-antibody complexes (47). However, LTβ4 liberation was not examined, and it was not definitively demonstrated that these chemokines were the crucial mediators of neutrophil recruitment into the airway. Because allergen-antibody complexes can stimulate mast cells (15, 19, 27) and macrophages to release LTβ (11, 21, 36, 38), our data clearly link the early recruitment of granulocytes following allergen challenge to the LTβ/BLT1 pathway. Although our study suggests that CXCL1 and CXCL2 do not mediate early neutrophil recruitment into the airway following challenge with antigen-antibody complexes, it does not rule out a possible role for CXCL1 and CXCL2 in the early recruitment of neutrophils into the lung parenchyma. In addition, the granulocyte recruitment that occurred at later time points may have been due to the liberation of other chemoattractants, such as CXCL1, CXCL2, and the eotaxins (CCL11 and CCL24). Thus our data provide a mechanism for the granulocyte recruitment seen in the early inflammatory response following antigen exposure and during acute asthma exacerbations.

There are three prior papers reporting on the role of BLT1 in asthma. In our previous study we demonstrated similar findings for effector T cell recruitment into the airway following allergen challenge of sensitized mice (43). In these experiments, early T cell recruitment into the airway was dependent on BLT1 expression on T cells. Lung recruitment of lymphocytes and late events were not influenced by disruption of BLT1. These data are consistent with a two-compartment model of lung recruitment where different factors control recruitment of cells into the lung parenchyma vs. the airways. The findings of our studies, along with those of Taube et al. (47), suggest a coordinated process following antigen challenge whereby CXCL1 and CXCL2 recruit neutrophils into the lung and BLT1 mediates their subsequent movement into the air spaces early in the inflammatory process.

More recently it has been demonstrated that BLT1 expression on CD8+ effector T cells helps establish allergic inflammation and AHR in an adoptive transfer model of asthma (35). In these experiments, airway inflammation and AHR were reduced in immunized and challenged CD8− mice compared with wild-type animals. Transfer of in vivo primed wild-type CD8+ T cells or in vitro polarized transgenic OVA-specific CD8+ T cells into sensitized and challenged CD8− mice was able to reestablish airway inflammation and AHR, but this did not occur if the transferred CD8+ T cells were deficient in BLT1. In a subsequent study, BLT1−/− mice in a BALB/c background were used, in a similar model to the one used in the studies presented here, to demonstrate that BLT1 was important for the establishment of AHR and mucus production after three OVA aerosol challenges (34). It was shown that the reduced AHR and mucus production in BLT1−/− mice were the result of a defect in the recruitment of IL-13-producing T cells into the airways. Similar to our findings they did not see a defect in granulocyte recruitment after three OVA challenges. Importantly, the authors did not look at earlier time points or specifically at the mechanisms of early granulocyte recruitment. Unlike these last two studies, we were not able to demonstrate a decrease in AHR or mucus production in OVA-sensitized and -challenged BLT1−/− mice. However, in the two prior studies the BLT1−/− mice used were in different genetic backgrounds, and in the first study the model used was different, all of which can influence the development of AHR and allergic airway inflammation (6, 45, 51). Finally, the other studies directly measured airway resistance, whereas in our study AHR was measured noninvasively by whole body plethysmography and the Penh. The ability of this technique to assess changes in airway resistance is controversial (3), and it is possible that direct measures of airway resistance would disclose more subtle differences.

Although the disruption of BLT1 signaling had profound effects on early airway inflammation in this murine model of asthma, these effects were not sustained, and AHR and mucus production were apparently not modulated. Consistent with this, in a study of allergic asthmatics, administration of a BLT1 antagonist reduced neutrophil recruitment into the airway following allergen challenge but did not affect AHR (12). This suggests that although inhibition of BLT1 may reduce the amount of granulocytes recruited into the airway, this may not be of great benefit in the treatment of people with asthma. Our data clearly demonstrate an important inflammatory mechanism in the development of airway inflammation. The fact that there is no effect on the overall asthma phenotype suggests that the mechanisms that lead to airway inflammation in asthma are complex and redundant and that therapeutic strategies that target the LTβ/BLT1 pathway in asthma may need to be combined with other therapies. However, these studies do not address the potential benefits of long-term inhibition of BLT1. In addition, there are some subtypes of asthma that have more neutrophilic predominant inflammation (50), and it is possible that the LTβ/BLT1 pathway plays a more important role in these forms of airway inflammation.

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REFERENCES


