Cutting Edge: B and T Lymphocyte Attenuator and Programmed Death Receptor-1 Inhibitory Receptors Are Required for Termination of Acute Allergic Airway Inflammation

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T cell activation is regulated by coordinate interaction of the T cell Ag receptor and costimulatory signals. Although there is considerable insight into processes that regulate the initiation of inflammation, less is known about the signals that terminate immune responses. We have examined the role of the inhibitory receptors programmed death receptor-1 and B and T lymphocyte attenuator in the regulation of allergic airway inflammation. Our results demonstrate that there is a temporally regulated expression of both the receptors and their ligands during the course of allergic airway inflammation. Following a single inhaled challenge, sensitized wild-type mice exhibit peak inflammation on day 3, which resolves by day 10. In contrast, mice deficient in the expression of programmed death receptor-1 or B and T lymphocyte attenuator have persistent inflammation out to 15 days following challenge. Thus, these receptors are critical determinants of the duration of allergic airway inflammation. The Journal of Immunology, 2006, 176: 3909–3913.

T cell activation requires the integration of signals derived from the TCR in combination with costimulatory and/or inhibitory receptors. Positive costimulatory receptors expressed by T cells include CD28 and ICOS, which augment both the priming and effector activity of T lymphocytes (1). In contrast, CD152 (CTLA-4), B and T lymphocytes (2, 3). CTLA-4 has been thought to be important in regulating the early activation of T lymphocytes. The point of regulation at which PD-1 and BTLA influence immune responses is less clear. Both are expressed primarily on activated T cells, suggesting that they may regulate effector function (3–5). Deficiency in PD-1 results in a loss of cell tolerance characterized by the gradual acquisition of autoimmune disease (6, 7). BTLA deficiency has not yet been reported to cause spontaneous autoimmunity, but did lead to an enhanced susceptibility to experimental allergic encephalomyelitis (3).

Allergic airway inflammation is a Th2-mediated immune response in the lung. Following a single allergen challenge, sensitized mice develop an eosinophilic inflammatory cell infiltrate around airways and blood vessels, which spontaneously resolves (8). Although CD28 and ICOS mediate the initiation and maintenance of airway inflammation, the basis for resolution has not been fully explored (9–12). In this study, we examined the role of BTLA and PD-1 in acute allergic airway inflammation by analysis of mice deficient in these inhibitory receptors. Although there was a minor role for these in regulating the intensity of acute airway inflammation, the receptors were crucial in limiting the duration of inflammation. This result correlated with the relatively late induction of the ligand for BTLA, herpes virus entry mediator (HVEM), and enhanced expression of the ligands for PD-1 at later times. These results suggest that the inhibitory receptors BTLA and PD-1 act as terminators of an established immune response, and may be important for limiting the extent and duration of inflammation at peripheral sites.

Materials and Methods

**Mice**

BTLA-deficient mice (strain Btlatm1kmm) in the C57BL/6 background were generated as described previously (3). PD-1-deficient mice (strain Pdcd1tm1shg) in the C57BL6/J background were obtained from Tasuka Honjo (Kyoto University, Kyoto, Japan). C57BL/6 mice were purchased from The Jackson Laboratory. All mice were housed in specific pathogen-free facilities at Washington University School of Medicine, 660 South Euclid Avenue, Box 8052, St. Louis, MO 63110. E-mail address: jgreen@im.wustl.edu

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3 Abbreviations used in this paper: BTLA, B and T lymphocyte attenuator; PD-1, programmed death receptor-1; HVEM, herpes virus entry mediator; RPA, RNase protection assay; mTEC, murine tracheal epithelial cell; BAL, bronchoalveolar lavage.
in the bronchoalveolar lavage (BAL) fluid at days 1, 3, 4, or 7 following challenge. CD4+ T cells appeared in the BAL by day 3 and peaked on day 7 (Fig. 1). Staining for PD-1 and BTLA revealed that PD-1 expression gradually increased, being detectable on day 3 and reaching its maximum on day 7 following challenge. BTLA expression exhibited a reciprocal pattern with expression being greatest on day 3 and nearly undetectable by day 7 (Fig. 1).

We next examined the allergic response of mice deficient in either BTLA or PD-1 at 3 days following allergen challenge (Fig. 2). Both BTLA-deficient and PD-1-deficient mice showed an increase in inflammatory cell recruitment compared with wild-type mice (Fig. 2, A and B). All genotypes had a mixed inflammatory cell infiltrate; however, there was an increased percentage of neutrophils and eosinophils in the BTLA-deficient mice (Fig. 2, A and B). Examination of the lung tissues revealed an increase in the intensity of inflammatory infiltrates in PD-1 and BTLA-deficient animals compared with wild-type controls (Fig. 2C). Goblet cell metaplasia was apparent in all genotypes as determined by immunostaining for Muc5AC (Fig. 2D). Thus, consistent with PD-1 and BTLA functioning as inhibitory receptors in other settings, we found that loss of their function caused a small increase in the initial inflammatory response to allergen.

Delayed expression of ligands for BTLA and PD-1 in acute allergic airway inflammation

We next examined the kinetics of expression of the ligands for each receptor. HVE.M, the ligand for BTLA, was nearly undetectable in the first 4 days, but became detectable by day 7 and was maximal by day 10 and 15 (Fig. 3, upper panels). PDL1 expression was detectable early and increased over time, reaching a maximum between days 10 and 15 postchallenge. Expression of PD-L2, a second ligand for PD-1, was maximal at day 4 following intranasal challenge, and declined subsequently (Fig. 3). Both HVE.M and PDL1 were detectable in RNA samples obtained from cultured mTEC, suggesting that the source of ligand may be nonimmune cells of the lung (Fig. 3, lane 8).

BTLA and PD-1 limit the duration of allergic airway inflammation

Given the observed kinetics of ligand expression, we next examined the allergic response at day 10 and day 15 following intranasal challenge (Fig. 4). Wild-type mice completely resolved the inflammation by day 10, as evidenced by a low number of cells recovered in the BAL (Fig. 4A). In contrast, BTLA-deficient and PD-1-deficient mice showed an increased inflammatory cell infiltrate in the BAL, and a marked increase in the number of eosinophils (Fig. 4B). These results suggest that BTLA and PD-1 play a critical role in limiting the duration of allergic airway inflammation.

**FIGURE 1.** PD-1 and BTLA are expressed on BAL CD4 T cells. C57BL/6 mice were sensitized and challenged with OVA. On days 1, 3, 4, and 7 following challenge, groups of mice were euthanized and the cells recovered in the BAL analyzed for expression of CD4 and PD-1 or BTLA by two-color flow cytometry. The percentage of cells positive for CD4 as a fraction of the total sample or of the lymphocyte gate as well as the total number of CD4+ cells recovered is indicated in each box. Histograms of PD-1 or BTLA expression on the CD4+ cells are shown for days 3, 4, and 7. Isotype staining is shown in black, and PD-1 or BTLA is illustrated in gray. Representative data of three independent experiments are presented.

**Results and Discussion**

Regulated expression of PD-1 and BTLA during acute allergic airway inflammation

We first determined the kinetics of lymphocyte accumulation and receptor expression in vivo by examining the cells recovered in the bronchoalveolar lavage (BAL) fluid at days 1, 3, 4, or 7 following challenge. CD4+ T cells appeared in the BAL by day 3 and peaked on day 7 (Fig. 1). Staining for PD-1 and BTLA revealed that PD-1 expression gradually increased, being detectable on day 3 and reaching its maximum on day 7 following challenge. BTLA expression exhibited a reciprocal pattern with expression being greatest on day 3 and nearly undetectable by day 7 (Fig. 1).

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in the BAL fluid and histology (Fig. 4A). By contrast, mice deficient in BTLA and PD-1 showed a persistent increase in BAL cells on day 10 following intranasal challenge. Furthermore, the composition of these cells in this fluid revealed a greater proportion of lymphocytes and eosinophils in comparison to the few cells in the wild-type mice, which consisted predominantly of macrophages (Fig. 4A). Even on day 15, examination of BTLA-deficient mice revealed the continued presence of increased numbers of lymphocytes and eosinophils. Direct histological examination of H&E-stained sections also demonstrated persistent inflammation and mucus cell metaplasia in the lungs of both PD-1 and BTLA-deficient mice at days 10 and 15, whereas the wild-type mice had complete resolution in this time frame (Fig. 4B). Thus, these inhibitory receptors are critical for resolving airway inflammation.

To determine whether there were qualitative differences in the cytokine profiles, we performed a multiprobe RPA on total lung RNA isolated at day 3, 10, and 15 after challenge. Samples obtained on day 3 postchallenge revealed expression of IL-4, IL-5, IL-10, IL-13, and γ-IFN in all mice with no difference between strains (data not shown). Overall, cytokine mRNA levels were decreased on days 10 and 15; however, there was a persistence of mRNA for IL-10 and γ-IFN in the PD-1-deficient mice (Fig. 4C and data not shown). Of the four BTLA-deficient mice, one demonstrated persistence of cytokine message, whereas none of the wild-type mice had detectable message. Similar results were seen in samples collected on day 10 following challenge (data not shown). As we have previously observed, IL-15 was detected in all samples including unchallenged mice (Ref. 10 and data not shown).

FIGURE 2. PD-1 and BTLA have a minor effect on acute allergic airway inflammation. C57BL/6, PD-1−/−, and BTLA−/− mice (n = 5 per group) were sensitized and challenged with OVA. Three days following challenge, the mice were euthanized and samples collected for analysis. A, Total cell counts in the BAL fluid. B, Differential analysis of the cell types present in the BAL. C, Representative fields of H&E-stained sections or (D) stained with anti-Muc5AC Ab (magnification, ×40). *, p < 0.05; **, p < 0.005 compared with C57BL/6 by two-tailed t test. Representative data from five independent experiments are shown.
the other. Nonetheless, these data support that the regulated expression of inhibitory receptors on lymphocytes and their ligands in the lung are critical for the proper termination of the acute inflammatory response. We propose, based on these findings, that abnormalities in this immune axis could play a role in pathologic situations such as chronic persistent asthma and may represent novel targets for therapeutic intervention.

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**Disclosures**

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