Inhibition of T Cell Costimulation Abrogates Airway Hyperresponsiveness in a Murine Model

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Abstract

Activation of naive T cells requires at least two signals. In addition to the well characterized interaction of the T cell antigen receptor with the antigen/MHC expressed on an antigen-presenting cell, T cell activation also requires costimulation by a second set of signals. The best characterized costimulatory receptor is CD28, which binds to a family of B7 ligands expressed on antigen-presenting cells. In asthma, although activated T cells play a role in the initiation and maintenance of airway inflammation, the importance of T cell costimulation in bronchial hyperresponsiveness had not been characterized. Therefore, we tested the hypothesis that inhibition of the CD28:B7 costimulatory pathway would abrogate airway hyperresponsiveness. Our results show that blockade of costimulation with CTLA4-Ig, a fusion protein known to prevent costimulation by blocking CD28:B7 interactions, inhibits airway hyperresponsiveness, inflammatory infiltration, expansion of thoracic lymphocytes, and allergen-specific responsiveness of thoracic T cells in this murine model of allergic asthma. (*J. Clin. Invest.* 1996. 98:2693–2699.) Key words: T cells • asthma • costimulation • airway hyperresponsiveness • murine

Introduction

T cells play a central role in regulating both the initial and the chronic inflammatory cascades of allergic asthma (1–3). Activation of naive T cells requires at least two signals. In addition to the well characterized interaction of the T cell antigen receptor with the antigen/MHC expressed on an antigen-presenting cell, T cell activation also requires costimulation by a second set of signals. The best characterized costimulatory receptor is CD28, which binds to a family of B7 ligands expressed on antigen-presenting cells. Inhibition of CD28:B7 signaling has been shown to prevent the activation of T cells by antigen-primed antigen-presenting cells (4).

The B7 family of costimulatory ligands includes at least two members, B7-1 and B7-2, which are differentially expressed in terms of cell type and kinetics of expression. B7-2 is expressed constitutively on some professional antigen-presenting cells including dendritic cells and is rapidly induced on activated B cells. In contrast, expression of B7-1 is upregulated later on activated antigen-presenting cells. CD28 and the homologous molecule CTLA4 bind to both B7 ligands. CTLA4 binds to B7 with an $\sim$ 16-fold greater affinity than does CD28 (5). Recent work suggests that CD28 and CTLA4 have opposing functions. CD28 has been shown to promote T cell activation with costimulatory signals; in contrast, CTLA4 may function to downregulate T cells (6–9). The different functions of CD28 and CTLA4 may be mediated, at least in part, by differential expression. Whereas CD28 is expressed constitutively on most T cells, CTLA4 is expressed at low levels on resting T cells and is highly induced after T cell activation (6, 10–12).

CTLA4-Ig, a soluble fusion protein composed of the extracellular domain of CTLA4 linked to the IgG_1 Fc region, has been shown to block the CD28:B7 pathway. In vivo administration of CTLA4-Ig has been shown to inhibit primary T cell–dependent antibody responses (13) and to improve survival of both pancreatic islet cell xenografts (14) and rat cardiac allografts (15). It has also been shown to decrease autoantibody production in a murine model of systemic lupus erythematosis (16). Depending on the kinetics of administration, CTLA4-Ig can potentially block interactions between CD28 or CTLA4 and either B7-1 or B7-2.

Although T cells play a central role in initiating and maintaining pulmonary inflammation and airway obstruction in asthma, the contribution of T cell costimulation to the pathogenesis of asthma is unknown. We have used a murine model of allergic asthma (17) to demonstrate that, when administered during aerosolized antigen challenge, CTLA4-Ig attenuates pulmonary inflammation and prevents airway hyperreactivity.

Methods

Animals. Male BALB/c mice were obtained from The Jackson Laboratories (Bar Harbor, ME) and were 4–5 wk old at entry into the pro-

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tocol. Mice were allowed free access to water and commercially pelleted mouse feed.

Experimental design. Three groups were studied. Two groups, CTLA4-Ig and L6, were sensitized to ovalbumin (OVA)[1] by intraperitoneal injection and then underwent aerosolized OVA challenge as previously reported (17), and briefly described below. During the aerosol challenges, one of these groups was treated with the costimulatory antagonist CTLA4 immunoglobulin (CTLA4-Ig), while the other was treated with L6, an IgG monoclonal antitumor antibody that serves to control for the Ig portion of CTLA4-Ig. A third group, Sham, was sensitized and challenged with PBS, and treated with PBS during the aerosol challenges.

Sensitization. On day 0, each mouse in the CTLA4-Ig and L6 treatment groups was immunized via intraperitoneal injection with 20 μg chicken ovalbumin (Grade III; Sigma Chemical Co., St. Louis, MO), and 2 mg $Al(OH)_3$ (J.T. Baker, Inc., Phillipsburg, NJ) in 0.4 cc PBS (Sigma Chemical Co.), followed by a boosting injection on day 7 with 10 μg OVA and 1 mg Alum. PBS control mice were immunized and boosted with identical amounts of PBS and Alum.

Antigen challenge. CTLA4-Ig and L6 mice underwent aerosolized antigen challenge with 6% OVA, dissolved in 0.5× PBS (pH 7.35–7.45), for 25 min per d for 7 d. This series of aerosols began 16 to 17 d after the initial immunization. Mice were placed in a plastic chamber (23 × 23 × 11 cm) and the OVA solution was aerosolized via an ultrasonic nebulizer (5000; DeVilbiss Co., Somerset, PA) attached directly to the chamber. Continuous air flow (< 1.0 liter/min) was supplied to the nebulizer to drive the aerosol into the chamber, and small ventilation holes were created in the opposite side of the chamber. Control mice (PBS) were challenged with 0.5× PBS alone.

Determination of lung resistance and airway reactivity. Mice were studied 1 d after the final aerosol, as previously described (17, 18). Each mouse was anesthetized with an intraperitoneal injection of pentobarbital sodium (70–80 mg/kg), (Anthony Products Co., Arcadia, CA). After acceptable anesthesia was achieved, the metal portion of a 19-gauge tubing adapter was inserted into the trachea and secured in place with sutures. An internal jugular vein was cannulated with a saline-filled Silastic catheter (0.06 cm o.d., 6–8 cm in length, < 0.005 ml vol) attached to a 0.1-ml Hamilton microsyringe (Hamilton Co., Reno, NV) and used to administer methacholine (MCh) (Acetyl-β-methylcholine chloride; Sigma Chemical Co.). Pulmonary resistance and dynamic compliance were determined as previously described (18). Dose-response curves to methacholine were obtained by administering sequentially increasing doses of MCh (33 to 3,300 μg/kg) in a 20- to 35-μl vol, and each animal's dose-response curve was subjected to regression analysis and then log-transformed to calculate the dose required for a twofold increase in lung resistance (log $ED_{200}\,R_L$).

Collection of samples. Mice were removed from the plethysmograph chamber and killed by drawing blood by cardiac puncture. Lungs were removed from the thoracic cavity and immersed in K2 fixative for 24 h, transferred into sodium cacodylate (pH 7.3), stained with hematoxylin and eosin, and examined by light microscopy. The remaining mice in each group underwent bronchoalveolar lavage (BAL) and lymph node dissection immediately after plethysmography. BAL fluid was obtained by instilling and withdrawing lavage solution (1.0 cc PBS with 0.6 M ethylenediaminetetraacetic acid) three times via the tracheal cannula. BAL samples were centrifuged at 2,000 rpm for 5 min, the supernatant discarded, and cell pellets resuspended in Hanks' Balanced Salt Medium (JRH Biosciences, Lenexa, KS). Slides were prepared by spinning samples at 800 rpm for 10 min (Cytospin 2; Shandon Inc., Pittsburgh, PA). BAL specimens were fixed (Leukostat fixative solution; Fisher Diagnostics, Pittsburgh, PA) and stained with methylene blue and eosin Y (Leukostat solutions I and II; Fisher Diagnostics). BAL differentials were determined by counting cells using a hemocytometer, based upon two counts of 100 cells each for each sample. The investigator counting the cells was blinded to the treatment groups.

Lymphocyte isolation and quantification. Lymph nodes were obtained by dissection from the paratracheal, peri-hilar, and -bronchial regions, and placed in RPMI 1640 medium at 4°C (Whittaker M.A. Bioproducts, Inc., Walkersville, MD). Single-cell suspensions were created by gently pressing the lymph nodes through a steel mesh (stainless steel screen 60 mesh) with a rubber plunger. Cell suspensions were centrifuged at 1,800 rpm for 5 min at 4°C, the supernatant was discarded, and the cells were resuspended in PBS with 3% bovine calf serum (Irvine Scientific, Santa Anna, CA). Lymphocytes were counted with a hemocytometer.

Monoclonal antibodies. FITC-conjugated anti–CD4 (GK1.5) and anti–CD45 (B220), and phycoerythrin (PE)-conjugated anti–CD45 (B220) and anti–CD4 (GK1.5) mAbs were obtained commercially (PharMingen, San Diego, CA). The mAb specific for CD8 was purified from 53.6 hybridoma supernatant and conjugated to Cy5 as described (19).

Flow cytometry. Flow cytometry was performed as previously described (20) on thoracic lymphocytes after isolation as described above. Cells were washed twice in PBS with 3% BCS (wash buffer). After counting, samples were pooled as needed to obtain 5×10^5 cells per well. Each cell sample was suspended in 50-μl aliquots of wash buffer and incubated for 30 min at 4°C with saturating concentrations of fluorochrome-labeled mAb. Cell samples were washed twice with 200 μl wash buffer and resuspended in 50 μl wash buffer and fixed by resuspension in 0.5% paraformaldehyde, and stored in the dark at 4°C. Samples were analyzed on an Epics Elite fluorescence activation cell sorter (Coulter Immunology, Hialeah, FL), using 488 nm (FITC, PE, and R613) and 633 nm (Cy5) excitation wavelengths. Lymphocytes were gated according to size in a forward and side scatter plot. Fluorescence was detected at 525 (FITC), 590 (PE), 613 (R613), and 670 (Cy5) nm. Listmode data was analyzed with the Coulter Elite Software. Populations were counted based on discrete histogram populations. To determine the average number of cells per animal (see Fig. 6), the cell number in each pooled sample was divided by the number of animals in that sample, and then multiplied by the proportion of each cell type. For the purposes of statistical analysis, the estimated mean cell number thus obtained for each pool was considered a single data point, regardless of the number of animals within that pool.

Proliferation assay. Thoracic and peripheral (inguinal) lymph nodes were harvested by dissection from CTLA4-Ig– and L6-treated mice, and single cell suspensions were created as described above. Lymphocyte proliferative responses were determined as previously described (21). Briefly, the cells were plated in a half-area 96-well plate at 4×10^5 cells per well with conditioned media or serial dilutions of antigen (OVA) or irrelevant antigen cytochrome C in a final volume of 100 μl. After 48 h, 1 μCi [^{3}H]thymidine in 50 μl was added to each well. The cells were harvested 8–12 h later, and thymidine incorporation determined.

Immunohistology. Cryostat sections of lung specimens snap-frozen after inflation with OCT Compound (Miles Laboratories, Inc., Elkhart, IN) were immunostained for CD4, CD8, and CD11b antigens with rat Ig used as background control (PharMingen). After fixation with 2% paraformaldehyde and methanol, a standard immunoperoxidase protocol with diaminibenzidine as chromogen was used as previously described (22).

Serum IgE levels. Serum IgE levels were measured by an ELISA assay as previously described (23) using serum obtained from peripheral blood by cardiac puncture.

Statistical analyses. All data are reported as mean±SEM. Data were analyzed using the JMP 3.0 statistical package (SAS Institute, Cary, NC). Parametric data was analyzed by the Tukey-Kramer test, and nonparametric data was analyzed by the Wilcoxon/Kruskal-Wallis rank-sum test. $P < 0.05$ was taken as the threshold for significance.

1. *Abbreviations used in this paper:* AHR, airway hyperresponsiveness; BAL, bronchoalveolar lavage; OVA, ovalbumin.

Table I. Experimental Design

Group	Sensitization and challenge	Treatment
L6	OVA	L6
CTLA4-Ig	OVA	CTLA4-Ig
Sham	PBS	PBS

Three groups of male BALB/c mice were analyzed. L6 and CTLA4-Ig groups were sensitized to OVA with adjuvant via intraperitoneal injections, while the Sham group was sensitized with equivalent amounts of adjuvant and PBS. On days 16 and 17, L6 and CTLA4-Ig groups began seven consecutive days of OVA aerosols, and Sham mice received PBS aerosols in the same manner. Immediately before the first aerosol, mice were treated with 200 μg of CTLA4-Ig or L6, a control IgG monoclonal antitumor antibody (24), while Sham mice were treated with PBS only. Equivalent amounts of CTLA4-Ig, L6, or PBS were administered via intraperitoneal injection before the first, third, fifth, and seventh aerosols. All measurements were performed 1 d after the final aerosol treatment.

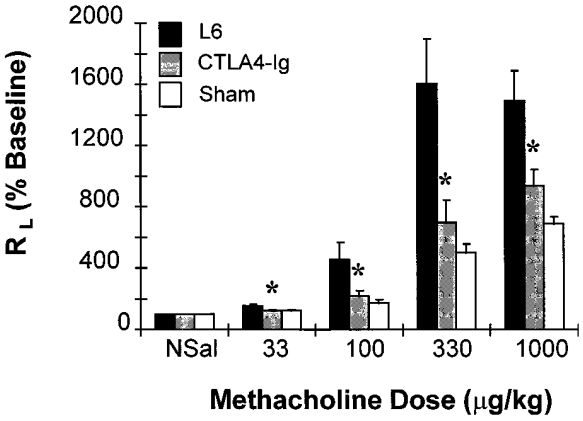

Figure 1. Effect of CTLA4-Ig on airway resistance in response to methacholine. Pulmonary resistance was measured in living, mechanically ventilated CTLA4-Ig– (n = 13), L6- (n = 11), and Sham- (n = 7) treated mice (described in Table I) in response to sequential methacholine doses, expressed as a percentage of baseline resistance±SEM. *$P < 0.05$ for CTLA4-Ig vs. L6 groups (17, 18). There were no significant differences between CTLA4-Ig and Sham groups at any methacholine dose.

Results

Effect of CTLA4-Ig treatment on allergic airway reactivity. Characteristic features consistent with allergic asthma are produced when BALB/c mice are sensitized with the antigen OVA, and subsequently challenged by inhalation of OVA aerosols (17). To examine the effects of blocking costimulation on allergic airway reactivity, we administered CTLA4-Ig to OVA-sensitized and -challenged mice (Table I). Given that allergic asthma represents a secondary immune response in a previously sensitized individual, we analyzed the effect of CTLA4-Ig during the aerosol challenge phase of the protocol, after mice had been sensitized to OVA. Control mice were treated with L6, an IgG monoclonal antibody specific for a human tumor antigen (24). Comparisons were also made with Sham mice sensitized, challenged, and treated with PBS to control for fluid effects in the aerosols and to provide baseline data in non–antigen-challenged mice.

Increased airway reactivity to methacholine is a defining feature of asthma (25), and reflects a combination of increased smooth muscle sensitivity due to inflammatory mediators such as histamine and leukotrienes and airway narrowing due to inflammation (26–28). To determine whether the development of airway hyperreactivity requires lymphocyte costimulation, pulmonary resistance and airway reactivity to intravenous methacholine were determined using plethysmography (Fig. 1). The results show that CTLA4-Ig treatment markedly decreased airway hyperresponsiveness (AHR) compared with the L6-treated group. Furthermore, CTLA4-Ig blockade of costimulation effectively inhibited AHR in OVA-sensitized and -challenged mice to levels comparable to the Sham (PBS). Of interest, CTLA4-Ig administered during sensitization alone also significantly decreased AHR and pulmonary inflammation (P. Finn, unpublished observations). Thus, both the early sensitization phase and later challenge phase are sensitive to costimulatory blockade by CTLA4-Ig.

Pulmonary pathology, bronchoalveolar lavage fluid, and IgE levels. Examination of lung tissue specimens from L6-treated mice demonstrated cellular peribronchial and perivascular infiltrates consisting of neutrophils, lymphocytes, and eosinophils, (Fig. 2, *A* and *B*). In addition, there was hyperplasia of the mucus-secreting goblet cells lining the bronchial epithelium (Fig. 2 *C*). All of these findings are consistent with the pathological features of human asthma (29) and are comparable to our observations in OVA-sensitized and -challenged mice (17). In contrast, CTLA4-Ig treatment produced a marked decrease in both cellular infiltrates and inflammatory changes within the bronchial mucosa (Fig. 2, *D* and *E*). Examination of the cells of the perivascular infiltrate by immunohistochemistry revealed CD4+ and CD8+ T lymphocytes and CD11b+ monocyte/macrophages (Fig. 3, *A–D*). Examinations of cells obtained by bronchoalveolar lavage revealed that CTLA4-Ig treatment induced a significant reduction in the proportions of neutrophils, lymphocytes, and eosinophils, with a concomitant increase in the proportion of alveolar macrophages (Fig. 4). In human asthmatics, antigen challenge has been shown to produce similar increases in neutrophils and eosinophils in BAL fluid (30) when measured, as in our model, 24 h after the most recent antigen exposure. As shown in earlier studies, the number of eosinophils in BAL fluid from asthmatics correlates with severity of disease (31). Measurements of serum IgE revealed that antigen (OVA) induced a 5.6-fold increase in serum IgE over the PBS control (Fig. 5). Consistent with reports that CTLA4-Ig inhibits parasite-induced (32) or anti–IgD alloantibody-induced (33) IgE responses, CTLA4-Ig decreased serum IgE levels 87% in OVA-treated mice (L6 = 0.115±0.022 μg/ml, and CTLA4-Ig = 0.015±0.006 μg/ml) in our asthma model (Fig. 5).

Lymphocyte analysis in vivo and in vitro. To determine the contribution of costimulatory pathways to lymphocyte activation during OVA challenge, thoracic lymphocytes from each group were analyzed by flow cytometry (Fig. 6). After sensitization and aerosol challenge with OVA, control mice treated with L6 demonstrated an increase in the number of thoracic CD4+ and CD8+ T and B cells. In contrast, treatment with CTLA4-Ig prevented the expansion of the CD4+ and CD8+ T cell subsets, resulting in subset numbers similar to that observed in the Sham-treated group. The expansion of the tho-

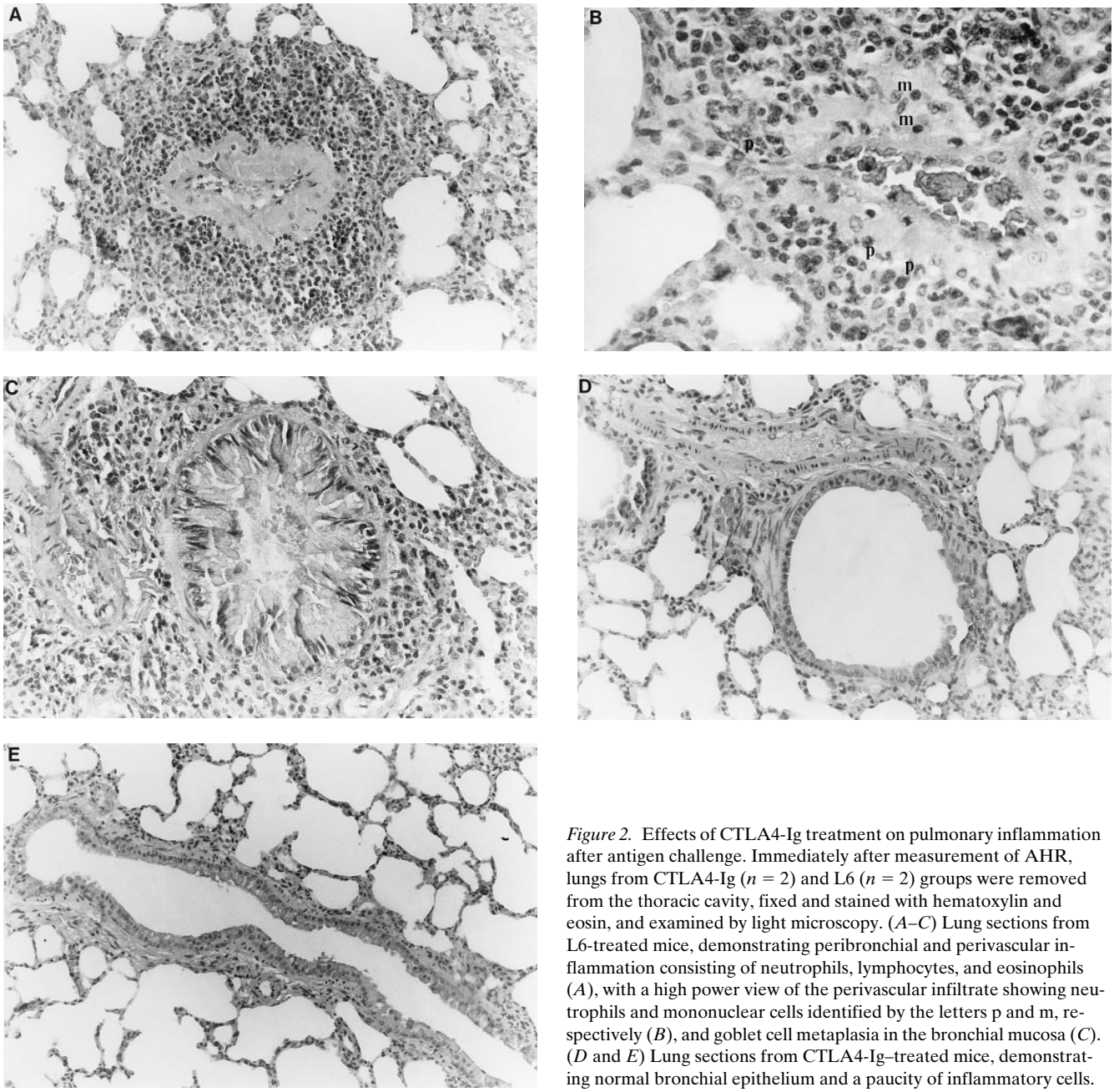

Figure 2. Effects of CTLA4-Ig treatment on pulmonary inflammation after antigen challenge. Immediately after measurement of AHR, lungs from CTLA4-Ig ($n = 2$) and L6 ($n = 2$) groups were removed from the thoracic cavity, fixed and stained with hematoxylin and eosin, and examined by light microscopy. (*A–C*) Lung sections from L6-treated mice, demonstrating peribronchial and perivascular inflammation consisting of neutrophils, lymphocytes, and eosinophils (*A*), with a high power view of the perivascular infiltrate showing neutrophils and mononuclear cells identified by the letters p and m, respectively (*B*), and goblet cell metaplasia in the bronchial mucosa (*C*). (*D* and *E*) Lung sections from CTLA4-Ig–treated mice, demonstrating normal bronchial epithelium and a paucity of inflammatory cells.

racic B cell population was also partially blunted by CTLA4-Ig treatment.

To analyze the effect of aerosolized OVA challenge on regional immunity of thoracic lymphocytes, the allergen-specific proliferative response of thoracic and peripheral inguinal lymph nodes was determined (Fig. 7). The results show that the inguinal lymphocytes did not respond in either the L6- or CTLA4-Ig–treated groups. Thoracic lymphocytes from L6 mice, however, specifically responded to OVA. Importantly, CTLA4-Ig blocked the allergen-specific response by the thoracic lymphocytes. Thus, CTLA4-Ig can effectively inhibit the response to aerosolized antigen by thoracic T cells. The results of the lymph node proliferation assay indicate that the thoracic, but not peripheral, lymph nodes are reactive to OVA antigen; suggesting that we have induced regional immunity by administration of aerosolized allergen. Concomitant with a significant decrease in AHR, CTLA4-Ig significantly diminishes the proliferation of thoracic T cells demonstrated by the reduction of pulmonary parenchymal and airway inflammation.

Discussion

Blockade of the CD28:B7 costimulatory pathway has been shown to exert a beneficial effect on several clinical conditions relevant to human disease. For example, CTLA4-Ig prolongs graft survival and ameliorates autoimmune disease (14–16). In asthma, although activated T cells play a role in the initiation and maintenance of airways inflammation, the importance of T cell costimulation in bronchial hyperresponsiveness has not been characterized. Therefore, we tested the hypothesis that

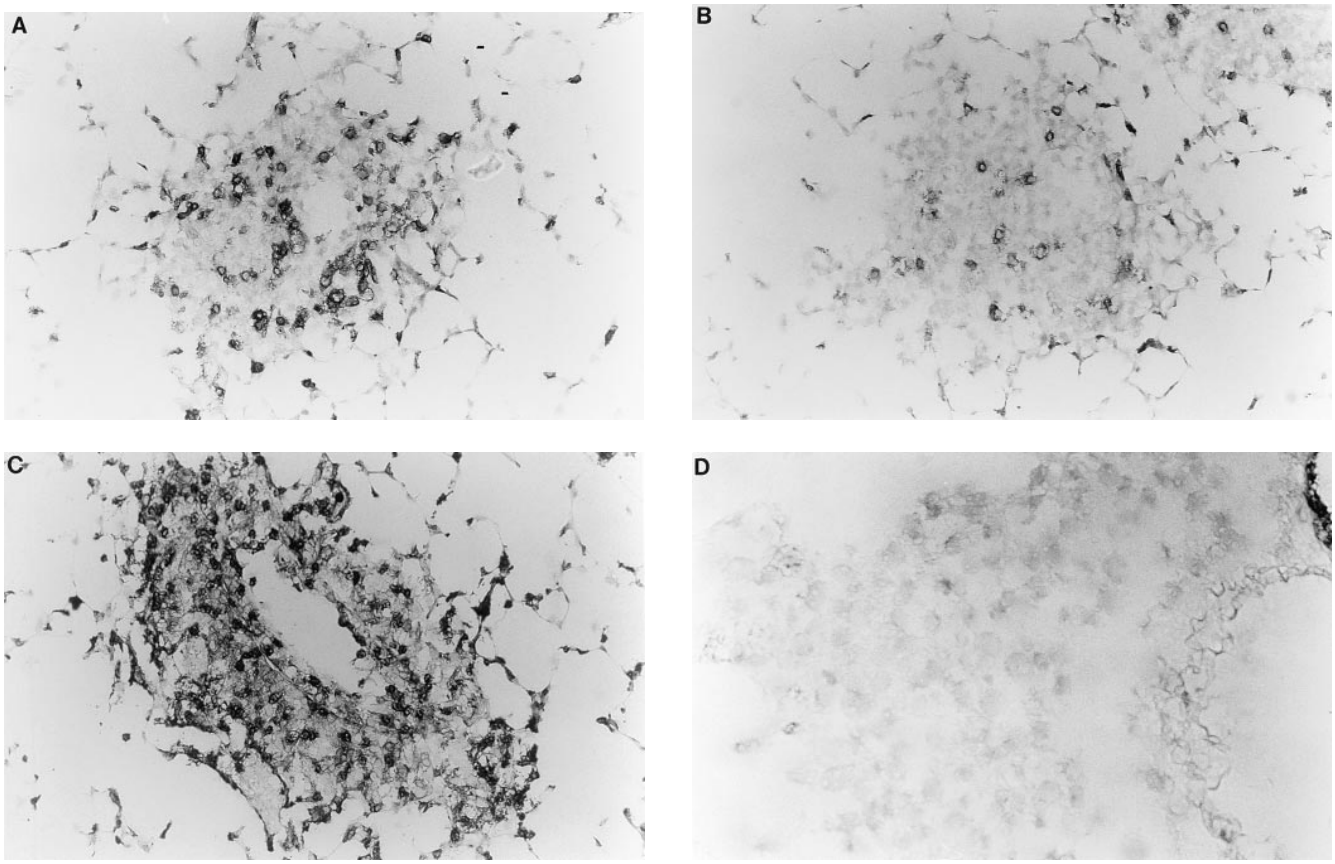

Figure 3. Characterization of the cells within the OVA-induced pulmonary infiltrate. Cryostat sections of lung specimens from OVA-treated mice who had received L6 were analyzed by immunohistochemistry with immunostaining for CD4, CD8, CD11b, and control rat Ig (*A–D*), respectively. CD4+ and CD8+ T lymphocytes, and CD11b monocyte/macrophages are detected, while control has minimal background staining.

inhibition of the CD28:B7 costimulatory pathway would abrogate AHR. Our results show that blockade of costimulation with CTLA4-Ig inhibits AHR, inflammatory infiltration, expansion of thoracic lymphocytes, and allergen-specific responsiveness of thoracic T cells, all features of asthma.

It is well established that costimulatory signals delivered to T cells through CD28 are necessary for lymphocyte activation under most conditions (7), and conversely, blocking CD28 signaling with CTLA4-Ig has been shown to produce prolonged T cell unresponsiveness termed anergy (13). Our data extend these observations by linking T cell activation through CD28 costimulation to the physiologic phenotype of airway hyperresponsiveness. Multiple pathways have been implicated in the pathogenesis of AHR; however, our results indicate that costimulation of T cells is a necessary early event in the induction of AHR in our model. These findings also demonstrate that the inflammatory cascade in asthma, which culminates in lymphocyte, eosinophil, and neutrophil recruitment and activation

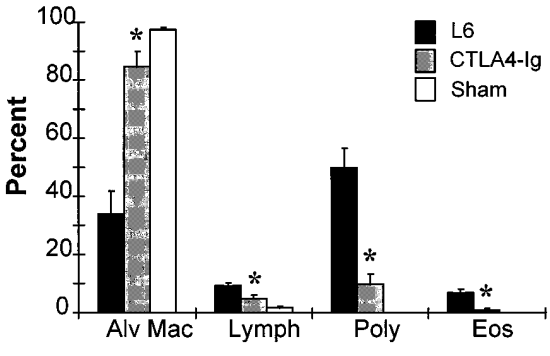

Figure 4. Relative proportions of inflammatory cells in BAL fluid. $^*P < 0.05$ for CTLA4-Ig ($n = 9$) vs. L6 ($n = 10$) groups. Sham mice ($n = 12$) differed significantly from both CTLA4-Ig and L6 mice in proportions of alveolar macrophages (*Alv Mac*), lymphocytes (*Lymph*), neutrophils (*Poly*), and differed from L6 mice only with respect to eosinophils (*Eos*).

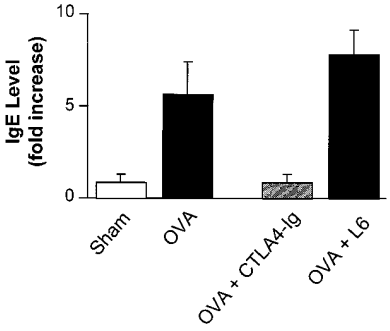

Figure 5. CTLA4-Ig decreases OVA-induced increase in serum IgE. Peripheral blood was obtained by cardiac puncture and serum IgE analyzed by ELISA as described (23). The Sham, OVA, CTLA4-Ig–, and L6-treated groups are shown as indicated from two separate experiments, and the controls have been adusted as the baseline±SEM. (L6 = 0.115±0.022 μg/ml, CTLA4-Ig = 0.015±0.006 μg/ml.)

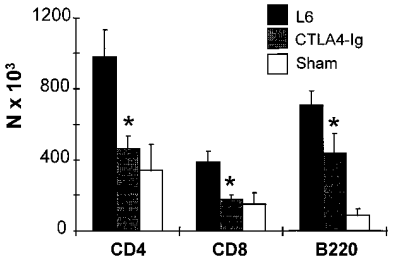

Figure 6. Analysis of lymphocyte subsets. Thoracic lymphocytes were obtained by lymph node dissection from L6- ($n = 14$), CTLA4-Ig– ($n = 14$), and Sham- ($n = 7$) treated mice, incubated with monoclonal antibody–fluorochrome conjugates, and analyzed via flow cytometry. Data are expressed ±SEM. Proportions of lymphocytes expressing CD4, CD8, or B220 (a B lymphocyte marker). $*P < 0.01$ for Sham vs. both L6 and CTLA4-Ig.

in the airways, is largely dependent upon CD28:B7 costimulatory signals.

In our model, T cells develop antigen-specific responses to OVA. Interestingly, OVA responses are restricted to the thoracic, but not peripheral, lymphocytes. Systemic administration of CTLA4-Ig blocks the activation of thoracic T cells. By preventing the regional lymphocyte activation that normally occurs when sensitized animals are exposed to aerosolized antigen, CTLA4-Ig treatment markedly attenuates subsequent pulmonary inflammation and airway reactivity.

When we administered CTLA4-Ig during systemic sensitization with OVA, we also found a significant decrease in AHR and pulmonary inflammation (P. Finn, unpublished observations). We focused our analysis, in particular, on the effects of administration of CTLA4-Ig during the aerosol challenge phase, since the natural history of asthma is characterized by exposure and sensitization to antigen months or years before the onset of airway symptoms. Therefore, relevant treatments must be effective during aerosol exposure. We previously demonstrated in our model that increased airway reactivity in response to OVA aerosol challenge is dependent upon prior OVA sensitization, and AHR will not occur after aerosol challenge with OVA in unprimed mice (17). When CTLA4-Ig is administered during aerosol challenge, a significant decrease

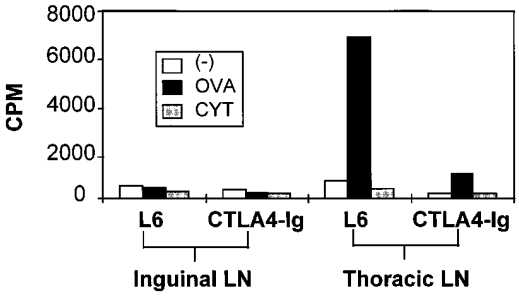

Figure 7. Effects of CTLA4-Ig treatment on lymphocyte proliferation in OVA-treated mice. Lymphocytes from thoracic and peripheral (*inguinal*) lymph nodes (*LN*) of L6- ($n = 8$) and CTLA4-Ig– ($n = 8$) treated mice were obtained by dissection, and incubated with media (−), antigen (*OVA*), irrelevant antigen cytochrome c (*CYT*), or the mitogen Concanavalin A (*ConA*, not shown) and [^{3}H]thymidine for 8–12 h. The cpm for ConA stimulation of thoracic LN was L6 = 118,011, CTLA4-Ig = 108,774. The cpm for inguinal LN was L6 = 204,002, CTLA4-Ig = 185,997. Shown is a representative experiment of at least three independent experiments.

in AHR and pulmonary inflammation is observed, suggesting that CTLA4-Ig prevents AHR by inhibiting the secondary immune response in animals already sensitized to OVA. CTLA4-Ig may be inhibiting the reactivation of previously sensitized T cells, or inhibiting the recruitment and activation of additional thoracic T cells during the challenge phase. Given that allergic asthma represents a secondary response, these findings may have important therapeutic implications. While earlier investigations using xenogeneic transplantation models have found that CTLA4-Ig is only partially effective in inhibiting secondary immune responses (13), our data indicate that secondary immune responses were markedly attenuated. The mechanism(s) by which CTLA4-Ig inhibits immunity during the aerosol challenge phase remain to be determined.

Our results provide comprehensive evidence that CD28:B7 costimulatory signals are necessary for the development of pulmonary inflammation and AHR in response to aerosolized antigen challenge. When administered during aerosol challenge, CTLA4-Ig effectively inhibits the immunologic, pathologic, and physiologic features characteristic of asthma in a murine model.

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