Both CD80 and CD86 co-stimulatory molecules regulate allergic pulmonary inflammation

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Abstract

We examined the roles of CD80 (B7-1) and CD86 (B7-2) in a model of allergic pulmonary inflammation and airway hyper-responsiveness (AHR) by selectively inhibiting either CD80 or CD86. Inhibition of co-stimulation by either CD80 or CD86 affected multiple parameters of the allergic response. Specifically, blockade of either CD80 or CD86 in ovalbumin-sensitized and challenged mice resulted in reduced expression of IL-2Rα (CD25) on CD4+ T lymphocytes, decreased airway eosinophilia, lower serum IgE production and diminished AHR. Importantly, blockade of CD80 and CD86 inhibited production of IL-4 and IL-2, and enhanced IFN-γ production.

Our observations support a role for both CD80- and CD86-mediated co-stimulation in development of allergic pulmonary inflammation.

Introduction

Allergic asthma is a chronic inflammatory disease mediated in part by T cells (1–3). Murine models of allergic airway inflammation mimic characteristic features consistent with human allergic asthma including increased T cell activation, airway eosinophilia, IgE production and enhanced airway responsiveness to methacholine (4–7). Optimal T cell activation requires antigen-specific engagement of the TCR and additional co-stimulatory interactions, the best characterized of which is the CD28 pathway (4,8). CD28, which is expressed on T cells, binds to either the CD80 (B7-1) or CD86 (B7-2) counter-receptors on antigen-presenting cells (APC). Blockade of this co-stimulatory pathway has been shown to prevent the activation of T cells by antigen-primed APC and to induce anergy in T cells in vitro (9,10). It is not known whether CD80 and CD86 play redundant or distinct roles during allergic pulmonary responses. Different kinetics of expression and substantially different intracytoplasmic domains suggest that CD80 and CD86 may have distinct functions. CD86 is constitutively expressed at low levels on monocytes, B cells, dendritic cells and T cells, and is rapidly up-regulated during an immune response. CD80 expression is induced on these same cell types following activation but with slower kinetics and lower levels of expression (11–15).

Inhibition of CD80 and/or CD86 has had different effects, depending on the disease model being studied and when the inhibitor is administered in relationship to disease induction (16–24). For example, in a murine model of contact sensitivity, anti-CD86 mAb decreased disease only if administered before sensitization, while anti-CD80 mAb had no effect (25). In a murine model of experimental allergic encephalomyelitis (EAE), anti-CD80 mAb decreased disease while anti-CD86 mAb exacerbated disease (20). The different outcomes of CD80 and CD86 inhibition may be due to the different kinetics of expression of these molecules or to the nature of the disease itself. In some models CD80 preferentially acts as a co-stimulator for the generation of Th1 cells, while CD86 induces Th2 cells. In a study of EAE, CD86 induced significantly more IL-4 production than CD80, thus potentially dir-
fecting an immune response more towards the Th2 type (11). However, the applicability of these findings to other models is not certain. The effect of blocking either CD80 or CD86 in a given disease may therefore depend on whether that disease is primarily Th1- or Th2-mediated (20), or on whether the CD80 or CD86 molecule is primarily expressed at the time of disease induction. In addition, previous studies have suggested that some reagents (such as anti-CD80 mAb) can exert agonist effects, whereas other reagents (such as anti-CD86 Fab) may be antagonists (21).

We have demonstrated that CD28 co-stimulatory signals are necessary for the development of pulmonary inflammation and airway hyper-responsiveness (AhR) in a murine model of allergic asthma (4). In these studies we found that treatment with CTLA-4-Ig, a soluble fusion protein that binds to both CD80 and CD86, inhibits the development of pulmonary disease in mice sensitized and challenged with the allergen ovalbumin (OVA). However, the specific roles of the CD80 and CD86 counter-receptors remain unknown. We undertook the present study to determine the contribution of CD80 and CD86 to allergen-induced inflammation in our model. We assessed the roles of CD80 and CD86 by utilizing inhibitors that blocked either CD80 or CD86, administered individually or in combination. Employing a murine model of allergic asthma (4,5,7), we demonstrate that both CD80 and CD86 molecules modulate the development of pulmonary allergic inflammation.

Methods

Animals

Male BALB/c mice were obtained from Jackson Laboratories (Bar Harbor, ME) and were 5 weeks old at the beginning of the protocol. They were maintained according to the guidelines of the Committee on Animals at Harvard Medical School and the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. Mice were allowed free access to water and commercially pelleted mouse feed.

Antigen sensitization and challenge

The protocol for induction of AHR via antigen sensitization and aerosol challenge has been previously described (4,5,7). On days 0 and 7, mice were sensitized via i.p. injection with chicken ovalbumin (OVA; grade III; Sigma, St Louis, MO) and aluminum hydroxide (J. T. Baker Chemical, Phillipsburg, NJ) in PBS (Sigma). On days 14–20, mice underwent aerosolized antigen challenge: they were placed in a plastic chamber and received 6% OVA via an ultrasonic nebulizer (5000; DeVilbiss, Somerset, PA) for 20 min per day.

Antibody treatment

BALB/c mice were treated with the mAb or fusion proteins described below via i.p. injection every other day during the antigen challenge phase of the protocol. The following reagents were used: control hamster Ig; the anti-CD80 mAb 16-10A1 (28); control rat Ig; the anti-CD86 mAb GL-1 (15); CTLA-4-Ig, which binds to murine CD80 and CD86 with equal affinity and inhibits murine T cell responses in vitro and in vivo (4,29); Y100F, a mutant form of CTLA-4-Ig which selectively binds CD80 (13); and L6, an IgG monoclonal antitumor antibody which controls for the Ig portion of the fusion proteins (4). Antibodies were purified from hybridoma cells; hamster and rat Ig were purified from serum (Sigma) as previously described (30). Supernatants were purified by HPLC (BioCad Sprint Perfusion Chromatography System; Perceptive-Bio Systems, Framingham, MA) using Protein A and G columns. Purified protein samples were analyzed on a 12.5% polyacrylamide gel in the presence of 0.1% SDS and stained with Coomassie brilliant blue. Fab were prepared as described (31). The anti-CD80 mAb, anti-CD86 mAb and anti-CD80 Fab treatment groups with their respective controls received a total of 400 µg of antibody, with the first of four doses administered via tail vein injection. The CTLA-4-Ig and Y100F treatment groups received a total of 500 µg of fusion protein i.p. The anti-CD80 Fab and anti-CD86 mAb combination treatment group and its controls received 100 µg of each antibody with each of four injections.

Determination of lung resistance and airway reactivity

On day 21, i.e. 1 day after the final aerosol challenge, mice were studied for the determination of lung resistance and airway reactivity as previously described (4,5,7). Mice were anesthetized with i.p. injections of pentobarbital sodium (Anthony Products, Arcadia, CA). The metal portion of a 19 gauge tubing adapter was then inserted into the trachea and secured in place. An internal jugular vein was cannulated with a Silastic catheter attached to a 0.1 ml microsyringe (Hamilton, Reno, NV) and used to administer methacholine (acetyl-β-methylcholine chloride; Sigma). Pulmonary resistance and dynamic compliance were determined as previously described (32). Dose–response curves to methacholine were obtained by administering increasing doses of methacholine (33–330 µg/kg).

Sample collection

After determination of lung resistance and airway reactivity, mice were removed from the plethysmography chamber and killed by cardiac puncture. Lungs were removed, inflated with OCT compound (Miles, Elkhart, IN) immersed in buffered formalin fixative, stained with hematoxylin & 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 gently withdrawing lavage solution (1.0 ml PBS with 0.6 M EDTA) 3 times via the tracheal cannula. Cytospins were prepared (Shandon Scientific, Cheshire, UK), fixed (leukostat fixative solution; Fisher Diagnostics, Y (Leukostat solutions I and II) and were 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 for each sample. The investigator counting the cells was blinded to the treatment groups.

Lymphocyte isolation and quantification

As previously described (4,5,7), thoracic lymph nodes were obtained by dissection and placed in RPMI 1640 medium at 4°C (Whittaker, Walkersville, MD). Single-cell suspensions...
were prepared by gently pressing the lymph nodes through a stainless steel screen mesh with a rubber plunger. Cell suspensions were centrifuged at 1800 r.p.m. for 5 min at 4°C, the supernatant was discarded and the cells were resuspended in PBS with 3% bovine calf serum (Irvine, Santa Anna, CA). Lymphocytes were counted with a hemocytometer.

Flow cytometry

The following mAb were used for flow cytometry. FITC-conjugated anti-CD4 (GK 1.5), phycocerythrin (PE)-conjugated anti-CD4 (GK 1.5) and biotinylated anti-IL-2Rα chain (7D4) were obtained from PharMingen (San Diego, CA). Streptavidin–Red 613 was obtained from Gibco (Grand Island, NY). The mAb specific for CD8 was purified from 53.6 hybridoma supernatant and conjugated to Cy5 (33). Thoracic lymphocytes were analyzed by flow cytometry as previously described (33). Cells were washed, counted and pooled as needed to obtain 5×10^5 cells/well. Cell samples were suspended in wash buffer and incubated for 30 min at 4°C with saturating concentrations of fluorochrome-labeled mAb. Samples were washed twice, resuspended in wash buffer, fixed with 0.5% paraformaldehyde and stored in the dark at 4°C until analysis. 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 Coulter Elite software. Populations were counted based on discrete histogram populations. Percent of total lymphocytes expressing CD4 and IL-2Rα were calculated with Coulter Elite software.

Serum IgE levels

Serum IgE levels were measured by an ELISA assay using serum obtained from peripheral blood by cardiac puncture. Briefly, microtiter plates were prepared by coating with antibody (PharMingen), incubating overnight at 4°C, washing 3 times with PBS/0.05% Tween (washing solution), blocking with 3% BSA/PBS and washing again. Serum was added to wells and the samples were incubated overnight at 4°C. Plates were washed and then the secondary antibody (antimouse κ light chain; PharMingen) was added. After incubation at room temperature for 1 h, samples were washed and treated with avidin–peroxidase. The plates were incubated, washed, treated with 2,2’-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) and read at 450 nm.

Cytokine analysis

Lung lymphocytes were purified from whole lung isolates via nylon wool purification followed by enrichment over a single-step Ficoll gradient. Cells were suspended in cell culture media (RPMI 1640, 8% FCS, 1% penicillin/streptomycin and 1% glutamine) and stimulated with concanavalin A for 24 h at 37°C. 5% CO2. Supernatant was harvested and analyzed via ELISA (Endogen, Cambridge, MA) for IL-4 and IFN-γ production.

Statistical analysis

All data are reported as mean ± SEM. Data were analyzed using the JMP 3.0 statistical package (SAS Institute, Cary, NC). Parametric data were analyzed using the Tukey–Kramer test; non-parametric data were analyzed using the Wilcoxon/Kruskal–Wallis rank-sum test. A P value <0.05 was considered significant.

Results

Both CD80 and CD86 mediate lymphocyte activation in vivo

In human asthmatics, IL-2Rα (CD25) expression on CD4+ lymphocytes is elevated in both peripheral blood and BAL fluid, and has been shown to correlate with several indices of disease severity. We have previously shown that allergen challenge with OVA produces up-regulation of CD25 expression on CD4+ thoracic lymphocytes (7). To determine the contribution of the CD80 and CD86 molecules to pulmonary lymphocyte activation, we examined thoracic lymphocytes from allergen-sensitized and challenged mice that were treated with modulators of the co-stimulatory counter-receptors (Fig. 1). OVA-sensitized and aerosol challenged mice had increased expression of CD25 on CD4+ T cells, correlating with an increased level of activation (Fig 1). As expected, this up-regulation of CD25 was decreased by treatment with CTLA-4–Ig (Fig. 1). Interestingly, treatment with either anti-CD80 mAb or anti-CD86 mAb also led to a decreased expression of CD25 on CD4+ T cells (Fig. 1). Given prior reports suggesting that anti-CD80 mAb may function as an agonist (21), we also analyzed effects of administering the anti-CD80 Fab. Treatment with anti-CD80 Fab had a greater effect on decreasing the proportion of activated CD4+ lymphocytes than did administration of the intact antibody (Fig. 1). Thus, blockade of either CD80 or CD86 or both molecules achieved a reduction in activated pulmonary CD4+ lymphocytes, supporting the notion that the two molecules have complementary roles in mediating co-stimulation of T cells.

Both CD80 and CD86 contribute to the development of AHR

Increased airway reactivity to methacholine is a defining feature of asthma, and reflects a combination of increased smooth muscle sensitivity due to inflammatory mediators and airway narrowing due to inflammation. To investigate the co-stimulatory roles of CD80 and CD86 in the development of AHR, pulmonary resistance and airway reactivity to i.v. methacholine were determined using plethysmography. In our model, exposure of previously sensitized mice to an aerosolized form of OVA leads to increased AHR when our model, exposure of previously sensitized mice to an aerosolized form of OVA leads to increased AHR when compared to mice sensitized and challenged with PBS alone (4,5,7).

Treatment with anti-CD80 mAb did not prevent mice from developing AHR (Fig. 2A). Given our awareness of the possible agonist properties of the anti-CD80 mAb, we employed Y100F, a mutated form of CTLA-4–Ig which is a selective agonist of CD80 (11). A fusion protein, Y100F does not show evidence of Fc-mediated agonist effects. Treatment with Y100F led to a significant decrease in AHR (Fig. 2A), suggesting that CD80 plays a role in promoting the development of AHR in our model. Consistent with previous reports...
CD80 and CD86 regulate allergic pulmonary inflammation (23), we found that treatment with anti-CD86 mAb decreased allergen-induced AHR (Fig. 2A), suggesting that AHR is at least partially dependent on CD86 interactions. While treatment with a combination of anti-CD80 mAb + anti-CD86 mAb (Fig. 2B) had no effect on AHR, when a combination of Y100F plus anti-CD86 mAb was used to block both CD80 and CD86, AHR significantly decreased, similar to the effect achieved with CTLA-4–Ig treatment (Fig. 2B). The serum levels of CTLA-4–Ig and Y100F were found to be similar in this experiment, ranging from 26 to 43 and 43 to 55 μg/ml respectively. Taken together, these results suggest that both CD80 and CD86 support the development of AHR in our model.

**CD80 and CD86 modulate pulmonary inflammation**

Allergen sensitization and challenge results in a subsequent lung inflammatory response characterized by the presence of large numbers of eosinophils in the BAL fluid, lung and airway tissue (4). We therefore assessed the effect of co-stimulatory blockade on allergen-induced pulmonary inflammation by examining the cellular component of BAL fluids. While administration of anti-CD80 mAb did not have a significant effect, blockade of CD80 with Y100F decreased eosinophil infiltration into the airways of allergen-sensitized and challenged mice (Fig. 3). Administration of anti-CD86 mAb also decreased the expansion of the eosinophil subset in the BAL, as did treatment with CTLA-4–Ig (Fig. 3). Similar to our results, Y100F treatment diminished airway eosinophilia in another model (13). Also consistent with our data, prior work also demonstrated decreased eosinophilia with anti-CD86 mAb treatment (23). Taken together these data suggest that recruitment of eosinophils to the lung is mediated by both CD80 and CD86.

Pulmonary inflammation was also assessed by histological analysis of lung sections. Usual changes induced by allergen sensitization and challenge are consistent with pathological features of human asthma including cellular peribronchial and perivascular infiltrates and hyperplasia of the mucus-secreting goblet cells lining the bronchial epithelium. These features are unchanged after treatment with control Ig (Fig. 4A). Slightly attenuated inflammation was observed in mice treated with either anti-CD86 mAb or Y100F (Fig. 4B and C). In these mice, there were fewer inflammatory cells in the peribronchial and perivascular areas. In contrast, treatment with an inhibitor of both CD80 and CD86, CTLA-4–Ig, produced a marked decrease in both cellular infiltrates and inflammatory changes within the bronchial mucosa (Fig. 4D).

Measurements of IgE levels in the serum (Fig. 5) further suggest that co-stimulation through both CD80 and CD86 is

![Fig. 1. Disruption of co-stimulation through CD80 or CD86 leads to decreased CD25 expression on CD4+ lymphocytes. Analysis of lymphocyte activation by flow cytometry. Thoracic lymphocytes were obtained by lymph node dissection from OVA-sensitized and challenged mice treated with control rat Ig (n = 14), anti-CD80 mAb (n = 12), anti-CD80 Fab (n = 5), anti-CD86 mAb (n = 13) or CTLA-4–Ig (n = 14), incubated with mAb-fluorochrome conjugates and analyzed via flow cytometry as previously described (4,7). PBS mice (n = 12) were sensitized and challenged with PBS. Lymphocytes were gated according to size in a forward and side density contour plot. CD4+ and CD8+ subsets were determined by gating on discrete populations in a density contour plot. CD25+ expression was determined by gating on a histogram plot. Percent of total lymphocytes expressing CD4 and CD25 was calculated with Coulter Elite software. Data given as mean ± SE and are representative of four separate experiments.](image-url)
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Fig. 2. (A) Abrogation of AHR with disruption of either the CD80 or CD86 co-stimulatory pathway. Pulmonary resistance, expressed as a percent of baseline resistance ± SEM, was measured in living, mechanically ventilated mice, sensitized and challenged with OVA, and treated with control L6 (n = 12), anti-CD80 mAb (n = 13), Y100F (n = 6) or anti-CD86 mAb (n = 17). (B) Abrogation of AHR with disruption of both CD80 and CD86. As in (A), mice were treated with control L6 (n = 12), anti-CD80 mAb + anti-CD86 mAb (n = 6), Y100F + anti-CD86 mAb (n = 6) or CTLA-4-Ig (n = 13). *Values for treatment groups that differ significantly from the control group (P < 0.05); **values for treatment groups that differ significantly from the control group (P < 0.01).

Effects of B7 co-stimulation on Th2 cytokines

As signaling through the CD28 receptors, CD80 and CD86, may affect the profile of cytokine expression, we investigated the role of CD80 and CD86 in the induction of a pulmonary Th2 response. In vivo blockade of CD80 and CD86 using CTLA-4-Ig in allergen-sensitized and challenged mice inhibited the production of IL-4 from two sites: in vivo BAL fluid (OVA + control L6 = 0.334 ± 0.0006 ng/ml, OVA + CTLA-4-Ig = 0.184 ± 0.02 ng/ml) and from lung lymphocytes activated in vitro (Fig. 6). Further, CTLA-4-Ig treatment decreased IL-2 from in vivo BAL fluid (OVA + control L6 = 10.98 ± 1.86 U/ml, OVA + CTLA-4-Ig = 4.32 ± 0.65 U/ml). In contrast, CTLA-4-Ig treatment enhanced production of IFN-γ in vitro (Fig. 6). Treatment of OVA-sensitized and challenged mice with a combination of anti-CD80 Fab and anti-CD86 mAb also diminished the production of IL-4 from activated lung lymphocytes (OVA + control = 0.16 ± 0.04 ng/ml, OVA + anti-CD80 Fab + anti-CD86 mAb = 0.08 ± 0.06 ng/ml) and increased the production of IFN-γ in BAL (OVA + control L6 = 40.7 ± 8 U/ml, OVA + anti-CD80 Fab + anti-CD86 mAb =
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Fig. 3. Blockade of either B7 co-stimulatory pathway sufficient to diminish airway eosinophilia. Eosinophils (percent of total cells) in BAL fluid shown for OVA-sensitized and challenged mice treated with control L6 + rat Ig (OVA + control = 72 ± 3%; n = 10), anti-CD80 mAb (OVA + anti-CD80 mAb = 44 ± 10%; n = 6), Y100F (OVA + Y100F = 2 ± 1%; n = 4) or anti-CD86 mAb (OVA + anti-CD86 mAb = 18 ± 6; n = 19). PBS mice sensitized and challenged with PBS (PBS = 0.3 ± 0.3%; n = 6). Data are expressed as mean ± SEM. *Values for treatment groups that differ significantly from the control group (P < 0.05).

Fig. 4. Pulmonary inflammation after antigen challenge in mice treated with inhibitors of CD80 and CD86. Immediately after measurement of AHR, lungs from treated and control mice were removed from the thoracic cavity, fixed and stained with hematoxylin & eosin, and examined by light microscopy. Characteristic inflammatory changes representative of allergen-sensitized mice, including peribronchial and perivascular inflammation consisting of eosinophils, neutrophils and lymphocytes, are observed in lung sections from L6 control mice (A). Slightly attenuated inflammatory infiltrate observed in lung sections from mice treated with anti-CD86 mAb (B) or Y100F (C). Lungs from mice treated with CTLA-4-Ig, demonstrating normal bronchial epithelium and a paucity of inflammatory cells (D).

Discussion

T cell activation requires the cognate interaction of T cells with co-stimulatory molecules expressed on APC. Activated CD4+ T cells have been shown to play a central role in the pathogenesis of many diseases, including asthma. We investigated the roles of CD80 and CD86 in the generation of allergen-induced pulmonary responses, specifically as these molecules impact T cell activation. Using flow cytometry we have previously shown that allergen challenge results in activation of thoracic lymphocytes as manifested by increased CD25 expression on T cells (7). In the present study we used multiple inhibitors to examine the effect of blocking CD80 and CD86 on T cell activation. Although there may be quantitative differences in the roles of CD80 and CD86, in vivo inhibition of either CD80 or CD86 leads to a decrease of activated T cells after allergen exposure, supporting the notion that both CD80 and CD86 contribute to T cell activation. Thus, our findings demonstrate a role for CD80 and CD86 co-stimulation supporting T cell activation in an in vivo model of pulmonary inflammation and AHR.

We then examined the individual roles of CD80 and CD86 on other parameters of allergen induced pulmonary responses. We demonstrated that both CD80 and CD86 contribute to the development of pulmonary inflammation and AHR. We also observed the effect of blocking CD80 and CD86 on airway eosinophilia and found that administration of a CD80 inhibitor (Y100F) or anti-CD86 mAb prevented increased BAL eosinophilia after allergen exposure. These results suggest that recruitment of eosinophils to the lung is influenced by both CD80 and CD86. While blockade of CD80 and CD86 may not equally affect all the allergic parameters, our observations suggest that co-stimulation through both molecules may play a role in maintaining a pulmonary inflammatory response.

Other investigators, using individual anti-CD80 and anti-CD86 mAb, suggest that CD86, but not CD80, plays a role in the generation of allergen-induced pulmonary responses (23). Some factors that may account for the different results include different protocols, mouse strains, doses of inhibitors and methods used to quantify AHR (23). However, we favor the notion that possible agonist effects of the anti-CD80 mAb may have confounded the interpretation of results by other groups since these other studies did not use either Y100F or anti-CD80 Fab (23). Consistent with this interpretation, one study demonstrated that Y100F was more effective than anti-CD80 mAb at decreasing IL-4 production after allergen exposure (24). Our results show that either anti-CD80 Fab or Y100F, but not anti-CD86 mAb, can effectively decrease pulmonary allergic responses. Consistent with our results, other reports have indicated that inhibition of CD80 with Y100F decreases allergen-induced pulmonary eosinophilia (13). We found that Y100F treatment also suppressed allergen-induced IgE production, an observation which suggests that at the time point examined in our model, antibody isotype switching is dependent on co-stimulatory signals delivered...
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Fig. 5. Blockade of both CD80 and CD86 leads to inhibition of serum IgE. Allergen-sensitized and challenged mice were treated with control rat Ig (OVA + control = 304 ± 56 ng/ml), Y100F (OVA + Y100F = 134 ± 48 ng/ml), anti-CD86 mAb (OVA + anti-B7-2 mAb = 58 ± 24 ng/ml) or CTLA-4–Ig (OVA + CTLA-4–Ig = 26 ± 31 ng/ml) during the challenge phase of the protocol. PBS mice were sensitized and challenged with PBS (PBS = 36 ± 15 ng/ml). Data are expressed as mean ± SEM of IgE for n = 6–8 mice per group. *Values for treatment groups that differ significantly from the control group (P < 0.05).

Through both CD80 and CD86. Further, investigation in a different model of allergic inflammation indicated that interruption of both CD80 and CD86, and not either one alone, is necessary to prevent a Th2 response (24).

Interestingly, several parameters were not equally inhibited by in vivo blockade of either CD80 or CD86. For example, CD80 blockade had a greater effect on eosinophilia, whereas CD86 blockade had a greater effect on production of serum IgE. Also, our results and those of others suggesting that the anti-CD80 mAb may exert agonist effects suggest that CD80 may have unique functions. It is possible, too, that some functions of CD80 and CD86 are distinct, whereas other functions are overlapping and potentially redundant. Characterization of CD80- and CD86-deficient mice has shown that while CD80 is more important in initiating antibody responses in the absence of adjuvant, stimulation of CD80 by adjuvant in CD86-deficient mice can induce CD80 to compensate for the absence of CD86 (34), thus demonstrating that both CD80 and CD86 have the capacity to play a central role in Ig class switching. Also, it is possible that CD80 and CD86 are functionally interchangeable, but have different pathogenic effects due to different kinetics of expression. It has been suggested that the expression of CD80 may be enhanced relative to CD86 during the acute and chronic stages of an inflammatory disease process (21). Miller et al. propose that anti-CD86 mAb would provide effective blocking of T cell-dependent responses during their initiation while CD80 expression is limited and that CD80 becomes the functionally dominant co-stimulatory molecule in terms of maintaining disease pathogenesis. Since allergic asthma comprises a secondary immune response in a previously sensitized individual, we analyzed the effects of administration of anti-CD80 and anti-CD86 treatment during the aerosol challenge phase, after sensitization to OVA. In the setting of chronic disease it may be necessary to both block binding of CD86 to prevent the activation of newly recruited naïve T cells and to inhibit the delivery of perpetuating co-stimulatory signals through CD80. Further investigation will be necessary to elucidate the

Fig. 6. Inhibition of Th2 cytokine production by blocking co-stimulation through both CD80 and CD86. Mice were treated with either OVA + CTLA-4–Ig (n = 10) or OVA + control L6 (n = 10). Lung lymphocytes were pooled and stimulated with concanavalin A for 24 h in vitro and cytokine levels determined by ELISA. Data are expressed as mean ± SEM of duplicate cultures and shown in ng/ml (IL-4) or U/ml (IFN-γ).
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mechanism of action of both CD80 and CD86 and their ligands, whose signal transduction pathways undoubtedly intercalate in complex ways to modulate T and B cell responses.

It has been proposed that a deviation towards the T<sub>1</sub> phenotype might confer protective effects against developing atopic asthma (35). In our model, although disruption of either CD80 or CD86 alone had effects on multiple outcomes of allergic reactivity, disruption of both co-stimulatory pathways led to decreased production of T<sub>1</sub> cytokines with a concomitant increase in a T<sub>1</sub> cytokine (IFN-γ) and to resolution of the cellular inflammation seen on histological lung sections. This observation suggests that CD80 and CD86 may mediate their effects by both diminishing the level of activated T cells and by deviating the cytokine profile of the activated CD4<sup>+</sup> T cells. Taken together, our data support a role for both CD80 and CD86 in allergic pulmonary inflammation. Employing co-stimulatory blockade as a potential therapeutic modality may therefore require consideration of the use of a combination of agents directed at the CD80 and CD86 molecules.

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Abbreviations

AHR airway hyper-responsiveness
APC antigen-presenting cell
BAL bronchoalveolar lavage
EAE experimental autoimmune encephalomyelitis
OVA ovalbumin
PE phycoerythrin

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