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Costimulation Through CD86 Is Involved in Airway Antigen-Presenting Cell and T Cell Responses to Allergen in Atopic Asthmatics

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Atopic allergic asthma is characterized by activation of Th2-type T cells in the bronchial mucosa. Previous reports have suggested an important role for costimulation through the CD28/CTLA4-CD80/CD86 pathway in allergen activation of T cells in animal models of inhaled allergen challenge. However, human allergen-specific lines and clones were reported to be costimulation independent. We therefore examined CD80 and CD86 dependence of allergen-induced T cell proliferation and cytokine production in peripheral blood and bronchoalveolar lavage from atopic asthmatic subjects and controls. Both allergen-induced proliferation and IL-5 production from PBMC were inhibited by CTLA4-Ig fusion protein and anti-CD86, but not anti-CD80 mAbs. When allergen-specific CD4+ T cell lines from peripheral blood were examined, proliferation and cytokine production were found to be independent of CD80 or CD86 costimulation. However, when cells obtained directly from the airways were examined, allergen-induced proliferation of bronchoalveolar lavage T cells from atopic asthmatic subjects was inhibited by anti-CD86 but not anti-CD80. In addition, bronchoalveolar lavage-adherent cells from asthmatic, but not control subjects showed APC activity to autologous T cells. This was also inhibited by anti-CD86 but not anti-CD80. Thus allergen-induced T cell activation and IL-5 production in the airway in asthmatic subjects is susceptible to blockade by agents interfering with costimulation via CD86, and this may hold therapeutic potential in asthma.


Roles for both CD80 and CD86 costimulation pathways in airway hyperresponsiveness and Ag-induced inflammatory airway eosinophilia in mice have been suggested (19–21). In addition, some reports suggest that CD86, but not CD80, costimulation can preferentially activate IL-4 expression and Th2 development (22, 23). However, recent data suggest that although human PBMC memory responses, including those to allergen, are dependent on CD86 costimulation, recently activated T cells and human Th clones are not susceptible to inhibition by anti-CD86 Abs (24, 25). We therefore examined the ability of CTLA4-Ig, a soluble fusion protein of CTLA4 and human γ-1 constant region that binds B7 molecules with high affinity, or Abs to CD86 and CD80, to inhibit allergen-induced peripheral blood T cell proliferation and IL-5 production and the dependence on CD80 and CD86 of airway APC and airway T cell responses to allergen.

Materials and Methods

Patients and clinical evaluation

Eight atopic asthmatic subjects (mean age, 25.8; range, 22–36; four male) and seven nonatopic nonasthmatic control subjects (mean age, 25.3; range, 20–40; five male) were studied. Asthmatics had a history compatible with asthma and demonstrated reversible airflow obstruction of at least 15% and histamine PC_{20}^{\text{a}} of <4 mg/ml, and all had a positive skin prick test to house dust mite extract (Aluprick, ALK Horsholm, Denmark). Atopy was defined as previously (5) (at least one positive skin prick test to a common aeroallergen). Volunteers were assessed at an initial visit with a questionnaire on asthma symptoms, allergen skin prick tests, measurement of serum IgE concentration, and spirometry. Bronchial responsiveness to histamine was measured using a Wright nebulizer by the method of Cockroft et al. (26). Asthmatic subjects completed a 2-week peak expiratory flow record to document variability in airflow obstruction and discontinued inhaled...
corticosteroids for at least 2 weeks before bronchoscopy. The asthmatic subjects had a mean serum IgE concentration of 324 IU/ml (range, 62–1009), mean FEV1 of 90.5% predicted (range, 63–102%), and geometric mean PC20 of histamine of 1.74 mg/ml (range, 0.4–3.6). None had received oral corticosteroids in the 2 months before the study, and permitted medication was inhaled β2-agonists as required only. Control subjects all had no history of respiratory disease or allergy, negative skin prick tests, and normal IgE (mean, 27; range, 4–100) and had mean FEV1 of 100% predicted (range, 88–119). All had histamine PC20 >16 mg/ml. None of the study volunteers had smoked in the preceding year, and none had more than 5 pack years in the past. All study volunteers had received Calmette-Guérin bacillus vaccination against tuberculosis. The study was approved by the ethics committee of the Royal Brompton Hospital, London, U.K.

Fiberoptic bronchoscopy was performed as described (5). In brief, all subjects were premedicated with nebulized albuterol, 2.5 mg, and sedated with i.v. midazolam. BAL was collected from the right middle lobe following instillation of 180–240 ml of sterile 0.9% saline in 60-ml aliquots.

Cell preparation

PBMC were isolated from heparinized blood samples by density gradient centrifugation over Ficoll-Paque (Pharmacia, Uppsala, Sweden). Cells were washed twice in HEPES-buffered RPMI 1640 (Sigma, Poole, U.K.) and resuspended in RPMI 1640 (Sigma) supplemented with 5% human AB serum (Sigma), 100 U/ml penicillin, 100 μg/ml streptomycin (Life Technologies, Paisley, U.K.), and 2 mM L-glutamine (Life Technologies) (complete RPMI). BAL cells were isolated by passing BAL through two layers of sterile gauze to remove mucus and washed twice in complete RPMI 1640 medium (Sigma). T lymphocytes were then purified by passage of BAL cells through a T cell enrichment column containing anti-human Ig-coated glass beads (R&D Systems, Abingdon, U.K.). This gave T cell purity of 85–90% for both blood and BAL, with <0.5% monocytes or B cells as judged by flow cytometric analysis with anti-CD3, anti-CD14, and anti-CD20 mAbs, respectively. To isolate CD4+ CD45RA−, and CD45RO+ cells from peripheral blood, depletion columns were used, with the same human Ig-coated glass bead separation columns after cells had been incubated with a mixture of Abs to remove CD8 and CD45RA/O cells, according to the manufacturer’s instructions (R&D Systems). Resulting cell populations were >97% pure, as judged by flow cytometry. Adherent cells (from BAL or PBMC) were isolated by culture on 90-mm plastic petri dishes for 2 h at 37°C with 5% CO2 in air. Nonadherent cells were then removed by three vigorous washes with RPMI 1640 medium containing 1 U/ml preservative-free heparin (Evans Medical, Leatherhead, U.K.). Adherent cells were recovered by incubation at 37°C for 15 min in PBS containing 10 mM EDTA (British Drug Houses, Poole, U.K.) followed by harvesting using a rubber cell scraper. Cells were resuspended and counted before assay. BAL-adherent cells contained no detectable B cells by immunocytochemical staining of cytotoxicity preparation with anti-CD20 Ab.

mAbs and cytokines

Anti-CD80 (BB1; IgM) was a kind gift of Dr Edward Clark, University of Birmingham, Birmingham, U.K. Anti-CD86 (BU63; IgG1) was a kind gift of Professor I. McClennan and Dr. D. Harding, Birmingham University, Birmingham, U.K. Anti-CD80 (BU63; IgG1) and anti-CD86 (BU63; IgG1) were from Caltag (San Francisco, CA). IL-2 was from R&D Systems. Anti-CD3, anti-CD14, anti-CD20, anti-CD80 (BB1; IgM), and control IgG2b control mAb (TNP) tri-nitrol phenol was purchased from PharMingen (San Diego, CA). Anti-CD86 (BU63; IgG1) was a kind gift of Dr Edward Clark, University of Birmingham, Birmingham, U.K. Phycoerythrin-labeled goat anti-mouse Ig and goat anti-mouse IgM, anti-CD45RA, and anti-CD45RO were from Becton Dickinson, Cowley, Oxford, U.K. Phycoerythrin-conjugated anti-CD3, anti-CD14, anti-CD20, anti-CD80 (BB1; IgG1), and anti-CD45RA were from Becton Dickinson, Cowley, Oxford, U.K. Abs directed against tumor-associated Ags (IgM and IgG1, respectively) were used as isotype controls and were a kind gift of Professor M. Ritter, Royal Postgraduate Medical School, London, U.K. An IgG2b control mAb (TNP) tri-nitrol phenol was purchased from PharMingen. CTLA-4-Ig fusion protein was a kind gift of Dr J. Ledbetter, Bristol-Myers Squibb Pharmaceutical Research Institute, Seattle, WA, and control fusion protein was a kind gift from Dr. M. Dallman, Imperial College, London, U.K. Phycoerythrin-conjugated anti-CD3, anti-CD14, anti-CD20, anti-CD45RA, and anti-CD45RO were from Becton Dickinson, Cowley, U.K. Phycoerythrin-labeled goat anti-mouse Ig and goat anti-mouse IgM were from Caltag (San Francisco, CA). IL-2 was from R&D Systems.

Proliferation studies and cytokine generation

T cells were incubated at 106 cells/ml in 200-μl volumes with 10 μg/ml Dermatophagoides pteronyssinus (ALK), or 10 μg/ml Mycobacterium tuberculosis (PPD) (Evans Medical, Leatherhead, U.K.). To measure allergen-specific proliferation of BAL T cells, 106 irradiated (3000 rads) autologous PBMC were added as APCs to 2 × 106 BAL lymphocytes, in the presence or absence of 10 μg/ml D. pteronyssinus extract (ALK). To assess BAL or peripheral blood-adherent cells as APC, these were added to isolated peripheral blood T cells in 96-well plates at a ratio of 2 × 106 APC to 1 × 106 T cells in quadruplicate cultures. Proliferation was measured on day 7 by adding 0.5 μCi of [methyl-3H]thymidine (Amersham, Arlington Heights, U.S.) for the last 16 h of culture, and measuring label incorporation. Optimal cell ratios were determined for each of the different assays in preliminary experiments (data not shown). To assess dependence on the different assays of CD80 or CD86, mAbs to CD80 (BB1) (1.0–10 μg/ml) or CD86 (BU63 or IT2), 0.01–0.5 μg/ml, or CTLA4-Ig, 0.1–10 μg/ml, were added to quadruplicate wells. For cytokine generation, PBMC were stimulated with allergen at 5 × 106 cells/ml, and supernatants were collected on day 6. In some experiments, PBMC cultures were supplemented with 0.1, 1.0, or 10 ng/ml IL-2.

Cell lines

To generate allergen-specific T cell lines, PBMC were cultured with 10 μg/ml allergen at 106 cells/ml for 7 days. Cultures were then supplemented with fresh medium containing 20 U/ml recombinant human IL-2 (R&D Systems) and expanded for a further 7 days. Lymphoblasts were then washed and restimulated with irradiated autologous PBMC (3000 rads) and allergen and expanded in IL-2 (20 U/ml) for a further 7 days. After a further restimulation cycle, lymphoblasts were stimulated with APC and allergen, to measure allergen-induced proliferation or to generate cytokines. Briefly, 5 × 106 cells were incubated with an equal number of irradiated PBMC, with or without allergen in quadruplicate 200-μl cultures. After incubation for 48 h, 0.5 μCi of [methyl-3H]thymidine was added to each well, and label incorporation was assayed 16 h later. For cytokine generation, T cell lines were resuspended with irradiated PBMC (both at 0.5 × 106 cells/ml), and allergen and supernatants were collected 48 h later.

Mixed lymphocyte reaction

PBMC were isolated from two unrelated donors by density gradient centrifugation, as above, and resuspended in complete RPMI 1640 medium (containing 5% human AB serum). Cells were cultured alone or together at 5 × 106 cells/well (each donor), in the presence or absence of Abs, BB-1 (CD80; 5.0 and 10.0 μg/ml) or BU63 (CD86; 1.0 and 0.1 μg/ml), for 6 days before the addition of 1 μCi of [methyl-3H]thymidine for the last 8 h of culture. Proliferation was measured by thymidine uptake measured by scintillation spectroscopy.

Cytokine assays

IL-5 concentrations in culture supernatants were assayed by a specific sandwich ELISA sensitive above 6.5 pg/ml, as previously described (27). IL-4, IL-13, and IFN-γ were also measured by ELISA (CMB, Amsterdam, The Netherlands), with sensitivities of 1, 0.5, and 2 pg/ml, respectively.

Flow cytometry

BAL cells were centrifuged at 500 × g for 7 min and then washed once in RPMI 1640 medium (Life Technologies). Cells were resuspended in medium at 106 cells/ml, and an aliquot was taken for cytokine preparation. These were stained with May-Grünwald-Giemsa (Sigma) for differential cell counts. Aliquots of BAL cells and PBMC (5 × 106 cells in 100 μl, isolated as below) were incubated with control Abs, BU63, IT2, or BB1 to a final concentration of 5 μg/ml on ice for 15 min, washed twice in PBS with 1% BSA and 0.1% sodium azide (Sigma) (PAB), and then incubated with phycoerythrin-conjugated goat anti-mouse Abs. Following a further two washes in PAB, cells were fixed in 2% formaldehyde in Isoton (Coulter, Luton, U.K.) and then analyzed on a EPICS Elite flow cytometer (Coulter). Alveolar macrophages and monocytes were identified on the basis of forward and side light scatter characteristics, and expression of CD80 or CD86 was analyzed in terms of specific mean fluorescence (relative to isotype control Ab).

Statistical analysis

Comparison of fluorescence intensity and T cell proliferation (cpm) between groups was by Student’s t test. Data were analyzed using MINITAB 9 Release 9.2 (Minitab, State College, PA). Differences were considered significant if p < 0.05.

Results

Both atopic and nonatopic individuals showed proliferative responses of PBMC to allergen and PPD, in accordance with previous reports (28). To examine the costimulator dependence of this proliferation CTLA-4-Ig or mAbs to CD80 or CD86 were titrated into cultures. As shown in Fig. 1, both CTLA4-Ig and anti-CD86 inhibited allergen-induced proliferation in a dose-dependent manner, whereas anti-CD80 Ab had no effect. Control fusion protein
did not inhibit proliferation (data not shown). Allergen-stimulated proliferation of PBMC was also inhibited by anti-CD86 in nonatopic donors, as was PBMC proliferation to PPD (data not shown).

To confirm biologic activity of the Abs at the concentrations used, both anti-CD80 (BB-1) and anti-CD86 (BU63) were shown to inhibit proliferation in a two-way mixed lymphocyte reaction (Fig. 1).

**Allergen-induced cytokine production from PBMC is inhibited by CTLA4-Ig and anti-CD86 Abs**

As previously reported (28), culture of PBMC from atopic individuals with allergen resulted in IL-5 production. This was inhibited by both CTLA4-Ig and anti-CD86 mAbs to a similar extent, whereas anti-CD80 or control fusion protein had no effect (Fig. 2). Titration of CTLA4-Ig showed that maximal inhibition of allergen-induced PBMC IL-5 production was 89.5% (±2.1 SEM) and 81.1 (±6.2) % for anti-CD86. In addition, allergen induced IL-13 production (mean 651 pg/ml, SEM 332 pg/ml) and IFN-γ production (mean 870 ± 402 pg/ml) from PBMC from atopic donors, and this was also inhibited by anti-CD86 (mean, 71.3 ± 7.3% for IL-13 and 94.4 ± 3.9% for IFN-γ), but not anti-CD80 mAbs. IL-4 production was not detected in these cultures.

Both CD45RA+ and CD45RO+ CD4+ T cells contribute to proliferative and IL-5 responses of PBMC to allergen

When CD45RA+ and CD45RO+ CD4+ T cells were separated from peripheral blood and cultured separately with irradiated PBMC and allergen, proliferation and IL-5 production was seen from both subsets (Table I).
Table I. Allergen-induced proliferative responses and IL-5 production result from both CD45RA⁺ and CD45RO⁺ T cell populations

<table>
<thead>
<tr>
<th></th>
<th>CD45RA⁺ T Cells</th>
<th>CD45RO⁺ T Cells</th>
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<tbody>
<tr>
<td>%CD45RA⁺</td>
<td>97.0</td>
<td>2.4</td>
</tr>
<tr>
<td>%CD45RO⁺</td>
<td>0.1</td>
<td>97.3</td>
</tr>
<tr>
<td>Proliferation (cpm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medium</td>
<td>880 (565)</td>
<td>1,255 (741)</td>
</tr>
<tr>
<td>Allergen</td>
<td>12,115 (3070)</td>
<td>28,454 (2968)</td>
</tr>
<tr>
<td>IL-5 (pg/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medium</td>
<td>349 (10.7)</td>
<td>185 (3.7)</td>
</tr>
<tr>
<td>Allergen</td>
<td>2,316 (50.9)</td>
<td>7,373 (205)</td>
</tr>
</tbody>
</table>

* Results are after 6 days of culture of 4 × 10⁶ T cells with 2 × 10⁶ irradiated PBMC (as APC) and allergen (house dust mite extract at 10 μg/ml) and are from triplicate wells with standard deviations in parentheses. Data are representative of three separate atopic donors.

Anti-CD86 inhibition of allergen-induced IL-5 production by PBMC from atopic donors is not IL-2 dependent

In some in vitro systems, T cell IL-5 production is IL-2 dependent (29). Since blockade of CD86 costimulation inhibits IL-2 synthesis (10), it was possible that the effects we observed on allergen-induced IL-5 production were an indirect result of inhibition allergen-induced IL-2 production. To test this, we attempted to restore proliferation and IL-5 production by adding exogenous IL-2 to allergen-stimulated PBMC cultured in the presence of anti-CD86 Abs. As shown in Fig. 3, although titration of exogenous IL-2 restored T cell proliferation to allergen, IL-2 did not overcome anti-CD86 inhibition of allergen-induced IL-5 production.

Proliferation and cytokine production by allergen-specific T-cell lines is not inhibited by anti-CD86 Abs.

To examine the CD86 dependence of recently activated allergen-specific T cells, T cell lines prepared from atopic subjects were used 7 days after their last Ag restimulation. Allergen-induced proliferation, IL-4 and IL-5 production from these CD4⁺ T cell lines were not affected by mAbs to CD86 (Fig. 4) or CD80 (data not shown).

BAL-adherent cells from asthmatic subjects present allergen in a CD86-dependent manner

Resting monocytes express low levels of CD80 and CD86, and CD86 is rapidly up-regulated following activation, with later induction of CD80 (8, 9). For this reason, we examined expression of CD80 and CD86 on freshly isolated alveolar macrophages from atopic asthmatic subjects and control individuals. Although alveolar macrophages from healthy control subjects have been characterized as poor APC (30) and suppress T cell responses to other APC (31), previous reports suggest that BAL cells from asthmatic subjects have APC activity (32, 33). We therefore examined the ability of BAL-adherent cells from asthmatic and control subjects to stimulate allergen- and PPD-induced proliferation of autologous peripheral blood T cells and the CD80 and CD86 dependence of such proliferation. Alveolar macrophages from both asthmatic and control subjects expressed CD86 and, to a lesser extent CD80 (Table II). BAL-adherent cells from asthmatics induced proliferation of autologous T cells to both allergen and PPD, to an extent comparable with peripheral blood-adherent cells (no significant difference in counts per minute), whereas BAL cells from nonasthmatic control subjects were poor APC (p < 0.05 for proliferation with blood cells compared with BAL) (Fig. 5). T cell proliferation to allergen or PPD, presented by airway APC, was inhibited by anti-CD86, but not CD80 (Fig. 5). In addition, proliferation of T cells from nonasthmatic subjects to allergen and PPD was inhibited by anti-CD86 (Fig. 5).

Allergen-induced airway T cell responses in asthmatic subjects are inhibited by anti-CD86

To examine the CD86 dependence of airway T cells, BAL T lymphocytes were isolated from asthmatic subjects and cultured with allergen presented by autologous irradiated PBMC. As shown in Fig. 6, house dust mite allergen-induced proliferation of BAL T cells from asthmatic subjects was inhibited by anti-CD86 mAbs.

Discussion

There is now considerable evidence for the importance of Th2-type T cells in orchestrating the eosinophilic airway inflammation that characterizes asthma (1, 5, 34). Recent studies of animal models of Ag-induced airway hyperresponsiveness suggest a role for CD86-CD28 T cell costimulation in activation of Th2 cells leading to airway eosinophil infiltration and local IgE synthesis (19–21). We show in this study that PBMC proliferation and IL-5 and IL-13 production to allergen stimulation is inhibited by CTLA4-Ig fusion protein and by Abs to CD86, but not CD80. However, recently activated allergen-specific T cell lines were not dependent on CD86 costimulation for either proliferation or cytokine production, raising the possibility that airway Th cells, which have previously been poorly characterized, may respond similarly.
been shown to be predominantly of memory phenotype, might not be inhibited by anti-CD86. We therefore examined both APCs and T cells from BAL from asthmatic subjects. Here we show, for the first time, that Ag presentation by airway APC and allergen-induced proliferation from airway T cells is CD86 dependent.

The role of CD28/CTLA4-CD80/CD86 costimulatory signals in the development and maintenance of Th1 and Th2 appears to depend on the system studied (9, 22, 35–37). This may result from differential engagement of the different ligand pairs and the kinetics of expression of the molecules in different systems. Although studies examining repetitive stimulation of human peripheral blood naive T cells suggest a preferential role for CD86 in primary development of IL-4-producing cells (23, 38), other studies suggest that CD80 and CD86 provide similar costimulatory signals for T cell proliferation and cytokine production (35, 37). CTLA4-Ig treatment reduced pathology in a number of animal models of autoimmune disease (39, 40). CTLA4-Ig blocked aerosol Ag-induced bronchial hyperresponsiveness, IgE synthesis, and eosinophilia in at least three different mouse strains (19–21). Anti-CD86 Abs introduced into the airway in one mouse Ag challenge model inhibited eosinophil infiltration, IgE, and bronchial hyperresponsiveness, whereas anti-CD80 had little effect (21). In contrast, Harris et al. (41) suggested CD80 dependence of airway eosinophilia in a similar model. We studied allergen-induced T cell responses in both peripheral blood and airway T cells and airway APC from patients with chronic stable asthma, because this situation is most amenable to therapy. We show blockade of allergen and PPD responses by anti-CD86 but not anti-CD80, and the lack of effect of anti-CD80 was supported by the similar degree of inhibition of allergen-induced proliferation and cytokine production by CTLA4-Ig and CD86. This presumably reflects the predominant expression of CD86 by both PBMC and BAL cells. Thus, CD86 would seem most relevant to chronic asthma, although we cannot rule out a role of CD80 in T cell activation in acute exacerbations of disease. To confirm that the Ab to anti-CD80 was indeed biologically active at the concentrations used, we showed inhibition of mixed lymphocyte reaction by both anti-CD80 and anti-CD86.

In contrast to PBMC, proliferation and cytokine production by recently activated allergen-specific T cell lines was not inhibited by anti-B7 Abs. These findings are in agreement with the recent demonstration by Bashian et al. (24) that human Th1 and Th2 clones were not susceptible to inhibition by CD80 or CD86, and the report by Yi-qun et al. (25) that T cell clones and in vitro preactivated T cells could be restimulated in the absence of CD80 or CD86 costimulation. However, both groups also found that peripheral blood memory cells were dependent on CD28 costimulation. It was therefore important to determine whether airway memory T cells, presumably frequently exposed to environmental allergen stimulation, are CD86 dependent or, like clones and T cell lines, have lost costimulator dependence. The current results suggest that in chronic asthma, allergen-specific airway cell proliferation is costimulator dependent. This is in agreement with animal models where administration of CTLA4-Ig or anti-CD86 after initial priming still lead to inhibition of subsequent Th2 activation after aerosol challenge (19–21). Because of the limitation of cell numbers, we did not test the effect of anti-CD80 on proliferation of BAL T cells; therefore, it remains possible that costimulation through this molecule plays a role in airway T cell activation. We were not able to detect cytokine production from the limited number of BAL T cells obtained, and it will require studies of the effect of CTLA4-Ig or CD86 blockade on allergen challenge or chronic asthma patients to confirm the importance of this potential therapeutic target.

Table II. Expression of CD80 (BB1) and CD86 (IT2.2 and BU63) by alveolar macrophages and peripheral blood monocytes from atopic asthmatic (n = 8) and nonatopic control subjects (n = 9)*

<table>
<thead>
<tr>
<th></th>
<th>CD80 (BB1)</th>
<th>CD86</th>
</tr>
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<tbody>
<tr>
<td>Alveolar macrophages</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asthmatics</td>
<td>2.1 ± 1.0</td>
<td>11.6 ± 1.4</td>
</tr>
<tr>
<td>Control subjects</td>
<td>1.3 ± 0.3</td>
<td>14.1 ± 3.7</td>
</tr>
<tr>
<td>Blood monocytes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asthmatics</td>
<td>0.1 ± 0.0</td>
<td>3.2 ± 0.9</td>
</tr>
<tr>
<td>Control subjects</td>
<td>0.2 ± 0.2</td>
<td>2.5 ± 0.5</td>
</tr>
</tbody>
</table>

*Cell populations were identified on the basis of forward and side light scatter, and specific mean fluorescence is shown relative to isotype control Abs (IgM for BB1, IgG1 for BU63, and IgG2b for IT2.2). Values are means ± SEM. There were no significant differences in specific mean fluorescence between asthmatic and control subjects for either alveolar macrophages or monocytes.
isotype control Ab added at 1 \times 10^5 cells/well in 96-well plates) with autologous peripheral blood T cells (separated on T cell columns and used at 1 \times 10^3 and 2). Exogenous IL-2 did not restore allergen-induced IL-5 production by anti-CD86 was an indirect result from diminished IL-2. We performed cultures in the presence of allergen and anti-CD86 Ab, which inhibits both proliferation and IL-5 production (Figs. 1 and 2). Exogenous IL-2 did not restore allergen-induced IL-5 production in cultures inhibited by anti-CD86. This raises the possibility that inhibition of IL-5 production by anti-CD86 is a direct effect on the T cell, rather than an indirect consequence of inhibition of proliferation secondary to reduced IL-2 production.

The BAL-adherent cell population studied here was predominately alveolar macrophages. However, APC activity likely also included airway dendritic cells, such as the CD1a^+ population described by Van Haarst et al. (48). There was no increase in CD1a^+ cell numbers in BAL from asthmatics when compared with control subjects (data not shown). However, we did not fully phenotype the adherent population studied and cannot exclude a difference in airway macrophage subpopulations between asthmatics and control subjects. B cells were not present in the BAL-adherent cell population studied here, although they may have potential as APCs in the airway mucosa. Chelen et al. (49) reported a relative deficiency in B7 expression by alveolar macrophages and showed that these cells did not increase B7 expression on activation. We studied BAL cells freshly isolated from asthmatics and control subjects, but did not examine the effects of in vitro activation. Since alveolar macrophages lack CD14, we were not able to use a specific phenotypic marker for these cells, but there was a clear separation of this population from other BAL cells by forward and side scatter at flow cytometry (50). It is possible that in vitro regulation of CD86 expression differed between the two groups studied, or that the relative signals from CD28 and CTLA4 differ between T cells from asthmatic subjects and controls, and that the balance between activating and inhibitory T cell molecules leads to differing responses to CD86 costimulation. Both CD45RA^+ and CD45RO^+ T cells from peripheral blood showed proliferation and IL-5 production to allergen, and, in agreement with previous reports (50, 51), >90% of the T cells in BAL were of CD45RO phenotype (data not shown), so that the inhibition we observed likely includes interruption of costimulation of a memory T cell response.
Cells other than T cells are thought to contribute to airway inflammation in asthma (52). In this regard, in studies of mice infected with Heligmosomooides polygyrus fected with inflammation in asthma (52). In this regard, in studies of mice infected with Heligmosomooides polygyrus fedected with inflammation in asthma (52). In this regard, in studies of mice infected with Heligmosomooides polygyrus fedected with inflammation in asthma (52). In this regard, in studies of mice infected with Heligmosomooides polygyrus fedected with inflammation in asthma (52). In this regard, in studies of mice infected with Heligmosomooides polygyrus fedected with inflammation in asthma (52). In this regard, in studies of mice infected with Heligmosomooides polygyrus fedected with inflammation in asthma (52). In this regard, in studies of mice infected with Heligmosomooides polygyrus fedected with inflammation in asthma (52). In this regard, in studies of mice infected with Heligmosomooides polygyrus fedected with inflammation in asthma (52). In this regard, in studies of mice infected with Heligmosomooides polygyrus fedected with inflammation in asthma (52). In this regard, in studies of mice infected with Heligmosomooides polygyrus fedected with inflammation in asthma (52). In this regard, in studies of mice infected with Heligmosomooides polygyrus fedected with inflammation in asthma (52). In this regard, in studies of mice infected with Heligmosomooides polygyrus fedected with inflammation in asthma (52). In this regard, in studies of mice infected with Heligmosomooides polygyrus fedected with inflammation in asthma (52). In this regard, in studies of mice infected with Heligmosomooides polygyrus fedected with inflammation in asthma (52). In this regard, in studies of mice infected with Heligmosomooides polygyrus fedected with inflammation in asthma (52). In this regard, in studies of mice infected with Heligmosomooides polygyrus fedected with inflammation in asthma (52). In this regard, in studies of mice infected with Heligmosomooides polygyrus fedected with inflammation in asthma (52). In this regard, in studies of mice infected with Heligmosomooides polygyrus fedected with inflammation in asthma (52). In this regard, in studies of mice infected with Heligmosomooides polygyrus fedected with inflammation in asthma (52). In this regard, in studies of mice infected with Heligmosomooides polygyrus fedected with inflammation in asthma (52). In this regard, in studies of mice infected with Heligmosomooides polygyrus fedected with inflammation in asthma (52).

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