

Differential roles of IL-16 and CD28/B7 costimulation in the generation of T-lymphocyte chemotactic activity in the bronchial mucosa of mild and moderate asthmatic individuals

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Background: IL-16 is an important T-cell chemotactic cytokine in asthmatic airways; its release from allergen-stimulated bronchial mucosa in mild asthma has been shown to be dependent on CD28/B7 costimulation.

Objective: We have extended our previous studies to investigate the role of IL-16 and CD28/B7 costimulation in T-lymphocyte chemotactic activity (TLCA) released from the bronchial mucosa in more severe asthma.

Methods: TLCA was determined in the supernatants of induced sputum and allergen-stimulated bronchial mucosal explants from healthy volunteers and volunteers with mild and moderately severe asthma by means of a Boyden chamber technique. The contribution of IL-16 to the activity was evaluated through use of a neutralizing monoclonal antibody; the contribution of CD28/B7 costimulation to allergen-induced release of TLCA was determined through use of CTLA4-Ig fusion protein and neutralizing monoclonal antibodies to CD80 (B7.1) and CD86 (B7.2).

Results: Induced sputum and unstimulated explants from asthmatic subjects generated significant spontaneous TLCA ($P < .05$). Both mild and moderate asthmatic explants showed significantly elevated *Dermatophagoides pteronyssinus*-induced release of TLCA, but only in mild asthma could sputum and allergen-stimulated explant TLCA be inhibited by anti-IL-16 (median inhibition, 39% and 59%; $P < .05$). In addition, allergen released significant quantities of IL-16 from mild asthmatic explants ($P < .05$) but not from moderate asthmatic explants. Antibodies to the CD28 counter-ligands CD80 and CD86 inhibited allergen-induced release of TLCA in mild asthmatic explants by 94% ($P < .05$) and 62%, but TLCA release

from moderate asthmatic explants was unaffected by CTLA4-Ig.

Conclusion: These results show that TLCA release in moderate asthmatic airways, in contrast to mild asthmatic airways, is not dependent on CD28/B7 costimulation and does not involve IL-16. (*J Allergy Clin Immunol* 2002;110:906-14.)

Key words: Allergen, asthma, bronchial explant, B7, CD28, chemotaxis, costimulation, induced sputum, interleukin 16, T lymphocytes

T lymphocytes are crucial participants in immune reactions, including reactions to inhaled allergens. Studies in asthmatic volunteers have shown an influx of T cells into the airways of allergic asthmatic individuals challenged with allergen.^{1,2} These cells become activated and produce T_H2 cytokines, which in turn amplify the inflammatory response by contributing to further recruitment of T_H2 cells and eosinophils.³ The prolonged T_H2 -dependent inflammation is believed to underlie the pathophysiologic features of asthma and to be an important target for therapy.⁴ It is apparent that the mechanisms through which T cells themselves are attracted into the airways might also provide targets for therapeutic intervention.

IL-16 is an important T-cell chemoattractant.^{5,6} It has been shown to be important in the early accumulation of T cells in the airways 6 hours after local allergen or histamine challenge,^{7,8} and its release from allergen-challenged airways has therefore been assumed to contribute to airway inflammation in allergic asthma. Increased expression of IL-16 has been demonstrated in the bronchial mucosa of atopic asthmatic subjects,⁹ and IL-16 levels are elevated in both the mucosa and bronchoalveolar lavage fluid of asthmatic individuals after segmental challenge.^{10,11} These findings suggest the existence of an allergen-activated mechanism for release of IL-16 in asthmatic airway mucosa.

We have previously described a bronchial explant model that enables airway mucosal tissue, obtained by bronchoscopic biopsy, to be challenged with allergen *ex vivo* and the supernatants to be analyzed for chemotactic activity and cytokine levels.¹² Through use of this model, IL-16 was found to contribute significantly to the T-cell chemotactic activity released from allergen-stimulated

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Abbreviations used

CTLA4-Ig: Cytotoxic T-lymphocyte antigen 4-immunoglobulin fusion protein

Der p: *Dermatophagoides pteronyssinus*

biopsy specimens from subjects with mild asthma who at the time of study required inhaled bronchodilators alone. Significant release of IL-16 could be induced through use of allergen, and this was inhibited significantly by blockade of the CD28/B7 costimulatory pathway with cytotoxic T-lymphocyte antigen 4-immunoglobulin (CTLA4-Ig) fusion protein.¹² We have also made a preliminary report on the presence of T-cell chemotactic activity in sputum induced in asthmatic individuals by inhalation of hypertonic saline solution,¹³ a method that is widely used to sample airways in both healthy and diseased subjects.¹⁴

In the present investigation, we have sought to extend our previous observations to study asthmatic subjects with moderate persistent disease who require inhaled corticosteroids for disease control to determine whether the release of chemotactic activity from the bronchial mucosa is related to disease severity and whether this is accounted for by IL-16. We have also explored further the dependence of T-cell chemotactic activity release on CD28 costimulation mechanisms, seeking to identify any preferential usage of CD86 (B7.2) as opposed to CD80 (B7.1) molecules (both ligands for CD28). Finally, in combining the use of the bronchial explant model and induced sputum, we have compared the activities in 2 airway compartments—bronchial mucosa and lumen—that reflect airway inflammation in asthma.

METHODS

Materials

Recombinant human IL-16 and neutralizing antihuman IL-16 monoclonal antibody (clone 14.1) were produced in the laboratories of 2 of the authors (W.W.C. and D.M.C.). CTLA4-Ig fusion protein was produced as previously described.¹⁵

Dermatophagoides pteronyssinus extracts were obtained from ALK-Abelló A/S (Hørsholm, Denmark) as standardized preparations of 500,000 SQ units. AIM V serum-free culture medium, HEPES buffer, L-glutamine, sodium pyruvate, and 2-ME were from Gibco Life Technologies Ltd (Paisley, United Kingdom). IL-16 ELISA kits were purchased from Biosource International (Nivelles, Belgium).

Forty-eight well microchemotaxis chambers were produced by Neuro Probe, Inc (Gaithersburg, Md). Chemotaxis filter membranes were supplied by Osmonics, Inc (Livermore, Calif). May-Grünwald and Giemsa stain solutions were purchased from Raymond A. Lamb, Ltd (Eastbourne, United Kingdom).

Subjects

Thirty-six nonsmoking subjects were recruited (Table I): 12 healthy controls, 12 individuals with mild intermittent asthma (treated with inhaled β_2 -agonist as required to relieve symptoms only), and 12 individuals with moderate persistent asthma (treated with regular inhaled corticosteroids and inhaled bronchodilators as required). Asthma severity was defined immediately before subjects' inclusion in the study according to the criteria of the American Thoracic Society.¹⁶ All asthmatic subjects were atopic and exhibited sensitivity to house dust mite, *D pteronyssinus* (Der p). The subjects

with moderate asthma were asked to refrain from using their corticosteroid inhalers for 5 days before bronchoscopy to minimize pharmacologic actions of steroid residues in the sampled tissues.

All human studies were approved by the Southampton and South-West Hampshire Local Research Ethics Committee.

Sputum induction and processing

Each subject undergoing bronchoscopy also provided a sputum sample at least 3 days before the procedure. Sputum induction and processing were carried out as described previously.¹⁷

Bronchial explant culture

Bronchoscopy and endobronchial biopsy were conducted according to the National Heart, Lung, and Blood Institute guidelines, as previously reported.¹⁸ Four to 12 subcarinal bronchial biopsy specimens were taken and cultured for 24 hours, as described.¹⁹ Control explants were incubated in medium alone; other tissues were incubated in medium containing 2000 SQ units/mL of standardized, LPS-free Der p allergen extract in the absence or presence of (a) human CTLA4-Ig fusion protein or (b) neutralizing monoclonal antibodies to CD80 or CD86. At the end of the incubation period, the culture medium was aspirated and centrifuged to precipitate shed cells, mucus, and cell detritus. Supernatants were stored at -80°C until required.

T-lymphocyte chemotaxis

T lymphocytes were isolated from peripheral blood of atopic, corticosteroid-free, mildly asthmatic donors, as described previously.¹² Cells were resuspended in RPMI 1640 medium supplemented with 2-mmol/L L-glutamine, 10-mmol/L HEPES, and 0.4% (w/v) RPMI-BSA and preincubated for at least 45 minutes at 37°C and 5% CO_2 before use.

T-lymphocyte chemotactic activity of sputum or explant culture supernatants was determined in 48-well modified Boyden chambers, as described.¹² To minimize bias resulting from assaying samples from different groups against cells from different donors, 1 sample from each group was assayed in each experiment. A positive control (10 ng/mL IL-16 or 1 $\mu\text{mol/L}$ platelet-activating factor) was included in each experiment; results were used only if a chemotactic response to the control was obtained.

Data are presented as chemotactic indices, calculated as the number of cells migrating in response to the sample of sputum supernatant $\div$ the number of cells migrating in response to RPMI-BSA medium.

IL-16 measurement

Immunoreactive IL-16 was quantified in explant culture supernatants through use of a specific ELISA, as described.⁷ "Spiking" of culture supernatants with recombinant human IL-16 revealed a very good recovery efficiency (mean, 100%; coefficient of variation, $\pm 18\%$). In contrast, sputum supernatants exhibited a mean recovery efficiency of 79% with a coefficient of variation of $\pm 64\%$. Because of this high degree of variability, quantification of IL-16 in sputum was regarded as unreliable and data are not further reported.

Statistical analysis

All statistical analyses were performed through use of InStat version 3.02 for Windows 95/98 (GraphPad Software, San Diego, Calif). Because of nonnormal distribution of data, nonparametric methods were used throughout. Chemotactic indices were compared with a hypothetical median of 1.0; IL-16 concentrations were compared with a hypothetical median of 0 through use of a 1-sample Wilcoxon rank-sum test. Comparisons among groups (healthy controls, subjects with mild asthma, subjects with moderate asthma)

TABLE I. Subject characteristics

	Healthy control	Mild asthma	Moderate asthma
Age (y)	29 (11)	31 (6)	34 (12)
Sex: male/female	3/9	7/5	4/8
Serum IgE (IU/mL)	7.6 (<5 to 33)	123 (33 to 407)*	103 (<5 to 1520)*
Der p skin prick wheal area (mm ²)	0	45.8 (42.5)	54.6 (95.8)
FEV ₁ (% predicted)	102 (11.8)	93.8 (8.56)†	89.5 (14.5)†
PC ₂₀ histamine, cumulative (mg/mL)	>16	5.7 (0.8-16)	1.3 (0.1-9.3)‡

Data are given either as arithmetic means and SDs (age and FEV₁) or as geometric means and ranges (IgE and PC₂₀) from 12 subjects per group.

* $P < .001$ compared with healthy control group.

† $P < .05$ compared with a hypothetical mean of 100%.

‡ $P < .05$ compared with mild asthmatic group.

were performed through use of the Kruskal-Wallis test; pairwise comparisons between groups were performed with a post-hoc Dunn's multiple comparisons test. Paired sets of data from untreated and treated tissues were compared through use of the Wilcoxon matched-pairs signed-rank test. When the same data set was used in more than 1 comparison, as was the case for experiments with anti-CD80 and anti-CD86 monoclonal antibodies, the Friedman test was used with a post-hoc Dunn's multiple comparison test. In all cases, P values less than or equal to .05 were defined as significant.

RESULTS

T-cell chemotactic activity in induced sputum

The moderate asthmatic group had significant sputum eosinophilia ($P < .05$ in comparison with healthy controls), but neither subjects with mild asthma nor subjects with moderate asthma had elevated sputum lymphocyte counts. Other cell counts did not differ between the subject groups.

Sputum supernatants from healthy control subjects exhibited no significant T-cell chemotactic activity (median chemotactic index, 1.5 [range, 0.7-2.6]). In contrast, sputum samples from both subjects with mild persistent asthma and subjects with moderate persistent asthma caused significant chemotaxis, their median chemotactic indices being 3.3 (1.4-6.2) and 2.2 (1.2-3.9), respectively. The chemotactic activity of mild asthmatic sputum was significantly higher than that of healthy control ($P < .05$), whereas the activity of moderate asthmatic sputum did not show this significant difference (data not shown).

Role of IL-16 in sputum T-cell chemotactic activity

Preincubation with anti-IL-16 mAb 14.1 (10 μ g/mL, a concentration that fully neutralized the chemotactic activity of 10 ng/mL recombinant human IL-16) caused significant inhibition of the T-cell chemotactic activity of sputum samples from subjects with mild asthma (median, 38.5 % [3.85% to 87.5%] inhibition; $n = 11$; $P = .001$) but had no effect on moderate asthmatic sputum chemotactic activity (8.01% [-26.3% to 27.3%] inhibition; $n = 12$; $P = .577$). The residual activity in the anti-IL-16-treated mild asthmatic sputum was not significantly different from that of healthy control sputum ($P = .689$; data not shown).

T-cell chemotactic activity in bronchial explant supernatants

In preliminary experiments, supernatants of allergen-stimulated asthmatic bronchial explants were found to exhibit greater chemotactic activity at 1:10 dilution than when undiluted (data not shown). This might result either from dilution of inhibitory factors in the fluid or from the fact that chemoattractants present in the fluid have characteristic bell-shaped concentration-response curves. To maximize the measured signal in subsequent experiments, all samples were assayed at 1:10 dilution.

Supernatants of unstimulated bronchial explants from healthy control subjects exhibited no significant chemotactic activity (median chemotactic index, 0.85 [0.40-2.4]; $n = 12$; $P = .266$), 9 of 12 having chemotactic index values that were less than 1. Supernatants of unstimulated mild (1.1 [0.90-4.8]; $n = 12$) and moderate persistent asthmatic bronchial explants (1.4 [1.0-2.2]; $n = 12$) displayed significant chemotactic activity ($P = .020$ and $P = .002$, respectively). The chemotactic activity of supernatants of unstimulated explants from subjects with moderate persistent asthma was significantly higher than that of healthy controls (Fig 1, A).

After treatment with specific allergen (Der p; 2000 SQ units/mL), chemotactic activity in the supernatants of healthy control bronchial explants showed a decrease in comparison with medium-treated control tissues (Fig 1, A), indicating the possible production of inhibitory factors in healthy airways. In contrast, mild asthmatic explants exhibited a significant increase in T-cell chemotactic activity after allergen stimulation (Fig 1, A). Whereas the allergen-induced increase in chemotactic activity release from subjects with moderate asthma did not achieve statistical significance, the median difference (activity released from allergen-stimulated explants minus activity released from control explants) was significantly higher in both asthmatic groups than in healthy controls (Fig 1, A).

Role of IL-16 in explant T-cell chemotactic activity

Unstimulated bronchial explants from all 3 groups released significant quantities of immunoreactive IL-16

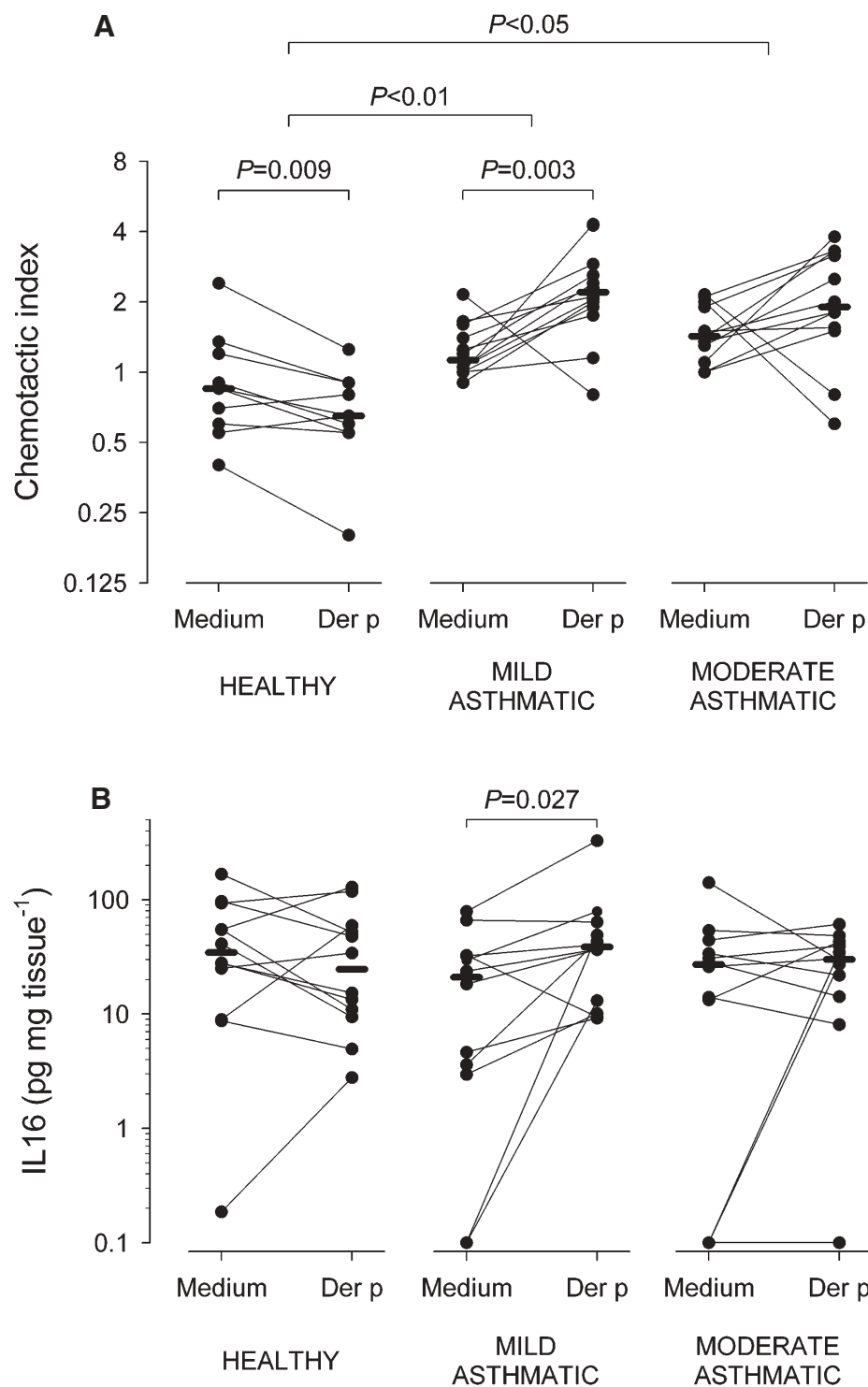


FIG 1. A, Allergen-induced release of T-cell chemotactic activity from bronchial explants. Bronchial biopsy specimens from healthy control (left), mildly asthmatic (center), and moderately asthmatic (right) subjects were cultured for 24 hours in the absence (Medium) or presence of *D pteronyssinus* extract (Der p; 2000 U/mL). Culture supernatants were used in modified Boyden chamber chemotaxis assays at 1:10 dilution (see Methods section). For comparison, recombinant human IL-16 (10 ng/mL) exhibited a geometric mean chemotactic index of 2.1 (95% CI, 1.8-2.4; n = 17). **B**, Allergen-induced release of IL-16 from bronchial explants. Experiments were performed as described for Fig 1, A. Culture supernatants were assayed for immunoreactive IL-16 by ELISA. Data are individual values from 12 subjects per group. Horizontal bars represent median values.

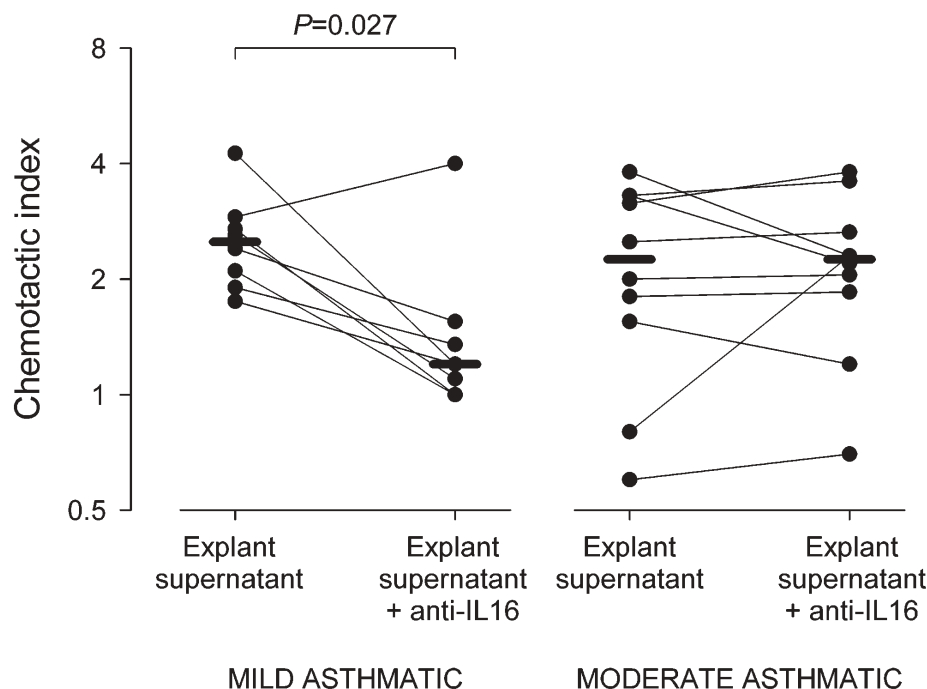


FIG 2. The effect of anti-IL-16 on T-cell chemotactic activity released from allergen-stimulated asthmatic bronchial explants. Explant supernatants were assayed for chemotactic activity as described for Fig 1 after 30 minutes' incubation with or without anti-human IL-16 mAb 14.1 (10 μ g/mL). An isotype-matched control monoclonal antibody had no effect on chemotactic activity (see text). Data are individual measurements from 8 mild asthmatic (left) and 10 moderate asthmatic (right) samples. Horizontal bars represent median values.

into the medium (median [range] per milligram of tissue: healthy control, 35 [0.20-170] pg; mild asthma, 21 [0.10-79] pg; moderate asthma: 27 [0.10-140] pg; all $P < .01$), with no significant difference among the groups. Der p stimulation induced a small (median, 39%) but statistically significant increase in IL-16 release from mild asthmatic explants but not from healthy control or moderate asthmatic explants (Fig 1, B).

Preincubation with anti-IL-16 monoclonal antibody led to a substantial inhibition of T-cell chemotactic activity of supernatants of allergen-stimulated bronchial explants from subjects with mild asthma (median, 59% decrease) but had no effect in subjects with moderate asthma (Fig 2). Isotype-matched control monoclonal antibodies had no significant effect on the activity in culture supernatants (median, 5.5% increase; $n = 6$; $P = .130$).

Dependence of bronchial explant responses on CD28/B7 costimulation

Allergen-induced release of T-cell chemotactic activity from mild asthmatic bronchial biopsy explants was inhibited significantly when monoclonal antibodies against either CD80 or CD86 were added to cultures (Fig 3, A), the magnitude of inhibition (median, 94% and

62%, respectively) being comparable to that observed previously for CTLA4-Ig (80%).¹² In contrast to what was seen in our previous study with mild asthmatic explants,¹² the presence in culture of the high-affinity, nonsignaling CD28 analog, CTLA4-Ig, had no effect on the allergen-induced release of chemotactic activity from moderate asthmatic bronchial explants (median inhibition, 5.6%; Fig 3, A); CTLA4-Ig also failed to affect the allergen-induced release of IL-16 from moderate asthmatic bronchial explants (Fig 3, B).

The release of IL-16 from allergen-stimulated mild asthmatic explants was markedly inhibited by anti-CD86; anti-CD80, however, had no significant effect on allergen-induced IL-16 release (Fig 3, B). Having failed to show any effect of CTLA4-Ig in moderate asthmatic explants, we did not conduct further experiments with anti-CD80 and CD86 antibodies in these tissues.

There was no significant difference in biopsy weights among the 3 groups of subjects ($P = .43$ by ANOVA) or between control and allergen-stimulated explants within any group (all $P > .23$). The statistical differences reported above for allergen-induced responses and the effects of anti-IL-16, anti-B7 and CTLA4-Ig were also found when data were corrected for wet weight of tissue.

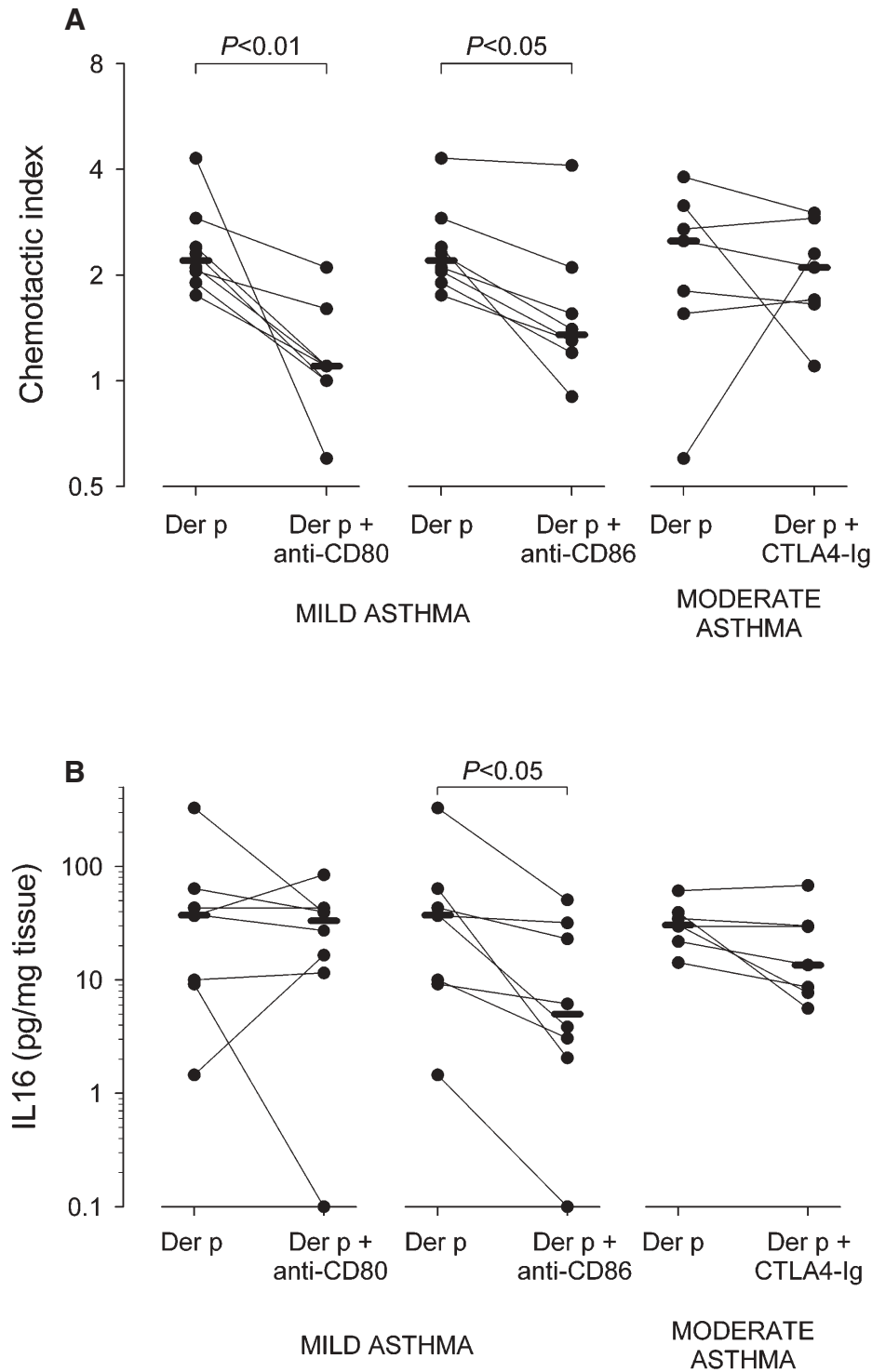


FIG 3. A, Dependence of allergen-induced release of T-cell chemotactic activity from asthmatic bronchial explants on CD28/B7 costimulation. Bronchial biopsy specimens were cultured as described for Fig 1 in the absence or presence of 10 μ g/mL neutralizing mAb to CD80 ($n = 8$, left) or CD86 ($n = 8$, center), or 25 μ g/mL CTLA4-Ig fusion protein ($n = 7$, right). Supernatants were assayed for chemotactic activity as in Fig 1, A. Data are individual points from the indicated numbers of samples per group. **B,** Dependence of allergen-induced release of IL-16 from asthmatic bronchial explants upon CD28/B7 costimulation. Bronchial biopsy specimens were cultured as described in Fig 3, A. Supernatants were assayed for IL-16 by ELISA. Data are individual values from 8 mildly asthmatic and 7 moderately asthmatic subjects. Horizontal bars represent median values.

DISCUSSION

We have previously demonstrated that spontaneous T-cell chemotactic activity generated *ex vivo* by bronchial mucosal tissue is not raised constitutively in mild atopic asthma but can be induced both *in vivo*⁷ and *in vitro*¹² by allergen to which the patient is sensitized. Extending our observation that CD28-dependent activation of T cells is important in mild disease,¹² we now show that this activation involves both CD86 and CD80 as ligands of CD28, without any preferential usage of either ligand. In accord with the notion that allergen exposure plays an important role in moderate-severe disease, we show that culture of mucosal tissue in the presence of allergen further stimulates the generation of T-cell chemoattractants but that in contrast to mild disease, IL-16 does not play an important role in this phenomenon. Importantly, the release of chemotactic activity from moderate asthmatic airways is not dependent on CD28-mediated mechanisms of resident T-cell activation, suggesting that either resident T cells are not the source of the activity or other costimulatory pathways are involved. Furthermore, in the present study we demonstrate the existence of increased spontaneously generated chemotactic activity in more severe asthma that might not require acute allergen stimulation. Together, these results suggest that T-cell chemotaxis is an important component of mucosal inflammation in both mild and more severe asthma but that the mechanisms that underlie its generation and the profile of chemotactic factors might vary depending on disease severity.

For safety reasons, allergen challenge has not been possible in asthmatic individuals treated with inhaled corticosteroids; our study is therefore the first to show allergen effects on mucosal tissue in more severe forms of asthma. Our finding that lymphocytes were not raised in sputum, together with previously published reports,^{20,21} would suggest that increased circulation of T cells from the blood into the tissue, rather than lymphocyte accumulation, is a feature of both mild and more severe asthma.

The relevance and mechanisms of increased spontaneous chemotactic activity release in explants from individuals with moderate asthma remain unclear. It is possible that *in vivo* stimulation by chronic exposure to allergen is simply carried over *ex vivo*. It could be argued that topical corticosteroids—which the subjects with moderate asthma had received until 5 days before biopsy—might have altered allergen responses. However, any suppressive effect of corticosteroids was far from complete, given that subjects with moderate asthma had the greatest degree of histamine airway hyperresponsiveness. They also had sputum eosinophilia and were symptomatic—all despite treatment with topical corticosteroids.

We have shown that both patients with mild asthma and patients with moderate asthma produce sputum possessing T-cell chemotactic activity. Neutralizing experiments showed that IL-16 accounted for a substantial component of the activity in mild asthmatic sputum; the same was not true for moderate asthma. Thus, our findings in bronchial explants reflect the situation in airway secretions sampled

in vivo as induced sputum. It is important to note, however, that the immunoreactivity of exogenous IL-16 “spikes” in sputum supernatants was low and highly variable, and this variability might extend to the biological activity of the cytokine in sputum. In this case, the contribution of IL-16 to the T-cell chemotactic activity of sputum supernatants might have been underestimated.

It is surprising that IL-16 levels in the supernatants of unstimulated healthy control explants are similar to those in supernatants of allergen-stimulated mild asthmatic explants (Fig 1, *B*), inasmuch as (a) the former possess no T-cell chemotactic activity and (b) the chemotactic activity of the latter is significantly attenuated by anti-IL-16. It is possible that healthy bronchial mucosal tissue also secretes 1 or more factors that neutralize chemotactic activity or otherwise suppress chemotactic response and that these factors are upregulated by a nonimmunologic action of Der p, as suggested by the decreased chemotactic index of healthy explant supernatants after allergen exposure (Fig 1, *A*). In contrast, the release of small additional quantities of IL-16 from mild asthmatic explants after allergen stimulation is associated with a substantial increase in IL-16-dependent chemotactic activity. Understanding of the mechanisms by which this occurs will require characterization of the full profile of substances secreted by the bronchial mucosa in health and disease to determine whether the presence of priming factors or the absence of endogenous inhibitors is responsible. The lack of inhibition of chemotactic activity in moderate asthmatic explant supernatants by anti-IL-16 is also puzzling in view of their significant baseline levels of IL-16. Again, a characterization of the other mediators present in the supernatants will be required to understand the reason for this discrepancy.

A further difference between the reactions of mild and moderate asthmatic airways was revealed in this study—namely, that blocking CD28 costimulation with CTLA4-Ig had no effect on the allergen-induced release of chemotactic activity from moderate asthmatic bronchial tissue. It is notable that the allergen-induced release of IL-5 from bronchial explants of individuals with moderate/severe asthma also occurs independently of CD28, indicating that an alternative costimulatory mechanism for T-cell activation by allergen might exist in these patients.²² Again, this finding might reflect a disease-state difference, a corticosteroid-treatment effect, or both. However, it is noteworthy that CTLA4-Ig has been demonstrated to suppress all inflammatory indices in a murine model of mild asthma while having only marginal effects in a model of more severe disease in which corticosteroids were not applied.²³ The dependence of allergen-induced release of IL-16 and T-cell chemotactic activity on CD28 costimulation in mild asthmatic airway tissues suggests involvement of allergen-specific T cells in the bronchial mucosa.¹² The lack of dependence on CD28 costimulation in moderate asthmatic explants might point to alternative costimulatory pathways, such as the OX40 (CD134)/OX40L interaction.²⁴ In the absence of T-cell activation, it would be possible for other cellular sources of IL-16 to contribute to release of

the cytokine. Expression of IL-16 by bronchial epithelial cell lines has been demonstrated.²⁵ Although we have observed primary epithelial cultures obtained by bronchial brushing to exhibit cytokine release in response to direct stimulation with Der p,²⁶ we have yet to show that allergen can induce IL-16 release. In common with T-helper cytokine release, IL-16 expression by bronchial epithelial cells is sensitive to suppression by corticosteroids,²⁵ precluding any compensatory production by the epithelium in response to the downregulation of T-cell responses by corticosteroid therapy.

The chemotactic activity release from mild asthmatic explant biopsy specimens is shown here to involve both CD80 (B7.1) and CD86 (B7.2), in common with the release of the T_H2 cytokines IL-5 and IL-13 in the same model.¹⁹ In contrast, the allergen-induced release of IL-16 was dependent on CD86 but not CD80, suggesting a possible differential dependence on B7 isoforms of the cells releasing different T-cell chemoattractants. Inasmuch as there was no significant allergen-induced release of IL-16 from moderate asthmatic biopsy specimens, we did not compare the relative contributions of CD80 and CD86 in steroid-dependent, moderate-severe disease. Studies using the same bronchial explant model have shown that whereas CD86 is preferentially used in allergic responses (generation of IL-5) of PBMCs, both CD80 and CD86 are used in mucosal tissue responses.²⁷ Taken together, the series of studies, including our own, indicate that CD86 is not a costimulatory molecule whose role is to preferentially drive allergic responses and that it cannot be seen as a selective therapeutic target.

In summary, the release of T-lymphocyte chemotactic activity in response to allergen within the airways is a prominent feature of both mild and more severe forms of asthma. The mechanisms underlying this activity vary between these disease forms, possibly reflecting differences in cellular source, mechanisms of activation (co-stimulation), or treatment. Further elucidation of the complex mechanisms that attract T cells into inflamed airways is needed, inasmuch as T cells are key regulators of allergic inflammation and might be targets for novel therapies.

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REFERENCES

- Djukanovic R, Feather I, Gratziau C, Walls A, Peroni D, Bradding P, et al. Effect of natural allergen exposure during the grass pollen season on airways inflammatory cells and asthma symptoms. *Thorax* 1996;51:575-81.
- Montefort S, Gratziau C, Goulding D, Polosa R, Haskard DO, Howarth PH, et al. Bronchial biopsy evidence for leukocyte infiltration and upregulation of leukocyte-endothelial cell adhesion molecules 6 hours after local allergen challenge of sensitized asthmatic airways. *J Immunol* 1994;93:1411-21.
- Bentley AM, Meng Q, Robinson DS, Hamid Q, Kay AB, Durham SR. Increases in activated T lymphocytes, eosinophils, and cytokine mRNA

expression for interleukin-5 and granulocyte/macrophage colony-stimulating factor in bronchial biopsies after allergen inhalation challenge in atopic asthmatics. *Am J Respir Cell Mol Biol* 1993;8:35-42.

- Ray A, Cohn L. Altering the Th1/Th2 balance as a therapeutic strategy in asthmatic diseases. *Curr Opin Investig Drugs* 2000;1:442-8.
- Cruikshank WW, Center DM. Modulation of lymphocyte migration by human lymphokines. I. Identification and characterization of chemoattractant activity for lymphocytes from mitogen-stimulated mononuclear cells. *J Immunol* 1982;128:2563-8.
- Cruikshank WW, Center DM. Modulation of lymphocyte migration by human lymphokines. II. Purification of lymphocyte chemotactic factor (LCF). *J Immunol* 1982;128:2569-71.
- Cruikshank WW, Long A, Tarpy RE, Kornfeld H, Carroll MP, Teran L, et al. Early identification of interleukin-16 (lymphocyte chemoattractant factor) and macrophage inflammatory protein 1 alpha (MIP1 alpha) in bronchoalveolar lavage fluid of antigen-challenged asthmatics. *Am J Respir Cell Mol Biol* 1995;13:738-47.
- Mashikian MV, Tarpy RE, Saukkonen JJ, Lim KG, Fine GD, Cruikshank WW, et al. Identification of IL-16 as the lymphocyte chemotactic activity in the bronchoalveolar lavage fluid of histamine-challenged asthmatic patients. *J Allergy Clin Immunol* 1998;101:786-92.
- Laberge S, Ernst P, Ghaffar O, Cruikshank WW, Kornfeld H, Center DM, et al. Increased expression of interleukin-16 in bronchial mucosa of subjects with atopic asthma. *Am J Respir Cell Mol Biol* 1997;17:193-202.
- Laberge S, Pinsonneault S, Varga EM, Till SJ, Nouri-Aria K, Jacobson M, et al. Increased expression of IL-16 immunoreactivity in bronchial mucosa after segmental allergen challenge in patients with asthma. *J Allergy Clin Immunol* 2000;106:293-301.
- Krug N, Cruikshank WW, Tschernig T, Erpenbeck VJ, Balke K, Hohlfeld JM, et al. Interleukin 16 and T-cell chemoattractant activity in bronchoalveolar lavage 24 hours after allergen challenge in asthma. *Am J Respir Crit Care Med* 2000;162:105-11.
- Hidi R, Riches V, Al-Ali M, Cruikshank WW, Center DM, Holgate ST, et al. Role of B7-CD28/CTLA-4 co-stimulation and NFκB in allergen-induced T cell chemotaxis by IL-16 and RANTES. *J Immunol* 2000;164:412-8.
- Hidi R, Louis R, Holgate S, Djukanovic R. Chemotactic activity for T-cells detected in sputa from atopic asthmatics. *Am J Respir Crit Care Med* 1997;155:A542.
- Pizzichini E, Pizzichini MM, Kidney JC, Efthimiadis A, Hussack P, Popov T, et al. Induced sputum, bronchoalveolar lavage and blood from mild asthmatics: inflammatory cells, lymphocyte subsets and soluble markers compared. *Eur Respir J* 1998;11:828-34.
- Ellis JH, Burden MN, Vinogradov DV, Linge C, Crowe JS. Interactions of CD80 and CD86 with CD28 and CTLA4. *J Immunol* 1996;156:2700-9.
- American Thoracic Society. Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. *Am Rev Respir Dis* 1987;136:225-44.
- Louis R, Shute J, Biagi S, Stanciu L, Marrelli F, Tenor H, et al. Cell infiltration, ICAM-1 expression, and eosinophil chemotactic activity in asthmatic sputum. *Am J Respir Crit Care Med* 1997;155:466-72.
- Djukanovic R, Wilson JW, Lai CK, Holgate ST, Howarth PH. The safety aspects of fiberoptic bronchoscopy, bronchoalveolar lavage, and endobronchial biopsy in asthma. *Am Rev Respir Dis* 1991;143:772-7.
- Jaffar Z, Roberts K, Pandit A, Linsley P, Djukanovic R, Holgate S. B7 co-stimulation is required for IL-5 and IL-13 secretion by bronchial biopsy tissue of atopic asthmatic subjects in response to allergen stimulation. *Am J Respir Cell Mol Biol* 1999;20:153-62.
- Vrugt B, Wilson S, Underwood J, Bron A, de Bruyn R, Bradding P, et al. Mucosal inflammation in severe glucocorticoid-dependent asthma. *Eur Respir J* 1999;13:1245-52.
- Wenzel SE, Szefer SJ, Leung DY, Sloan SI, Rex MD, Martin RJ. Bronchoscopic evaluation of severe asthma: persistent inflammation associated with high dose glucocorticoids. *Am J Respir Crit Care Med* 1997;156:737-43.
- Lordan JL, Davies DE, Wilson SJ, Dent G, Corkhill A, Jaffar Z, et al. The role of CD28-B7 costimulation in allergen-induced cytokine release by bronchial mucosa from patients with moderately severe asthma. *J Allergy Clin Immunol* 2001;108:976-81.
- Deurloo DT, van Esch BCAM, Hofstra CL, Nijkamp FP, Van Oosterhout AJM. CTLA4-IgG reverses asthma manifestations in a mild but not in a more "severe" ongoing murine model. *Am J Respir Cell Mol Biol* 2001;25:751-60.
- Ohshima Y, Yang LP, Uchiyama T, Tanaka Y, Baum P, Sergerie M, et al.

- OX40 co-stimulation enhances interleukin-4 (IL-4) expression at priming and promotes the differentiation of naive human CD4+ T cells into high IL-4-producing effectors. *Blood* 1998;92:3338-45.
25. Arima M, Plitt J, Stellato C, Bickel C, Motojima S, Makino S, et al. Expression of interleukin-16 by human epithelial cells: inhibition by dexamethasone. *Am J Respir Cell Mol Biol* 1999;21:684-92.
 26. Lordan JL, Bucchieri F, Richter A, Buchanan D, Croudass A, Lau L, et al. Dermatophagoides pteronyssinus allergen stimulates GM-CSF release by bronchial epithelial cells of asthmatic subjects. *Am J Respir Crit Care Med* 2000;161:A207.
 27. Jaffar ZH, Stanciu L, Pandit A, Lordan J, Holgate ST, Roberts K. Essential role for both CD80 and CD86 costimulation, but not CD40 interactions, in allergen-induced Th2 cytokine production from asthmatic bronchial tissue: role for $\alpha\beta$, but not $\gamma\delta$, T cells. *J Immunol* 1999;163:6283-91.

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