

II. Organ-specific effects of systemic inflammatory activation

T lymphocytes in asthma: Bronchial versus peripheral responses

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Recent evidence points to the recruitment of T_H2 cells, phenotype T lymphocytes, their activation, and the generation of T_H2 cytokines, particularly IL-4 and IL-5, in both peripheral blood and bronchial mucosa of asthmatic patients, leading to local tissue eosinophilia and IgE-dependent mast-cell activation. Activation of T_H2 T lymphocytes appears to be specific for asthma (as opposed to airway obstructive disease) and was shown to correlate with asthma severity as evidenced by the inverse correlation between CD25⁺/CD4⁺ cells and peak expiratory flow rates. These findings support the fundamental importance of T-lymphocyte responses in bronchial asthma and delineate potential therapeutic strategies, such as broad-based immunosuppression versus a more selective approach targeted against CD4⁺ T lymphocytes. The high efficacy of topical treatments (ie, inhalation) supports the notion that changes that are detectable in peripheral blood merely reflect a "spill-over" of local T-lymphocyte responses in the target organ. Conversely, the multiple systemic manifestations of allergy (such as allergic rhinitis and atopic dermatitis in atopic patients) support systemic therapeutic approaches. (*J Allergy Clin Immunol* 2000;106:S221-6.)

Key words: Bronchial asthma, cytokines, *in situ* hybridization

T lymphocytes (T cells) are the only cells capable of responding to the antigenic peptides that are expressed on the surface of antigen-presenting cells with the relevant major histocompatibility complex class II molecule and other costimulatory signals. On the basis of murine studies, T-cell responses are classified into 2 subtypes, depending on their cytokine profile in response to antigenic stimulation.¹ T_H1 cells predominantly express IFN- γ and IL-2, whereas T_H2 cells preferentially express IL-4 and IL-5. Although less clearly delineated, this classification was shown to be relevant to human T-cell responses.^{2,3}

Abbreviations used

BAL: Bronchoalveolar lavage
IL-2R: IL-2 receptor

Both allergic and parasitic infections in humans are characterized by T_H2-cell responses. IL-4 was shown to induce B-cell production of a sterile germline gene transcript,⁴ which is a necessary first step toward immunoglobulin heavy-chain gene rearrangement and subsequent IgE messenger RNA and protein production.⁵ IL-5 promotes the terminal differentiation of eosinophils from precursor cells in the bone marrow⁶ and selectively activates eosinophils and prolongs their survival at sites of allergic inflammation.⁷ Typically, exposure to aeroallergens on mucosal surfaces occurs at very low allergen doses over prolonged periods of time. In general, low allergen concentrations, IL-4,⁸ and preferential antigen presentation by B cells^{9,10} all favor a T_H2-cell phenotype. In contrast, high antigen concentration, antigen presentation by macrophages, and the absence of IgE receptors on the surface of antigen-presenting cells favor the development of a T_H1-cell phenotype. Preferential T_H2-cell development leads to increased local IgE production and tissue eosinophilia, which characterize late-phase responses and ongoing chronic allergic inflammation, comparable to day-to-day allergic asthma.^{11,12}

We present evidence that points to the recruitment of T_H2 cells, their activation, and the generation of T_H2-cell cytokines, particularly IL-4 and IL-5, in both the peripheral blood and the bronchial mucosa of asthmatic patients.

PERIPHERAL T-CELL RESPONSES

The activation of T cells *in vivo* was accompanied by increased surface expression of certain markers including CD25 (ie, IL-2 receptors).¹³ With the use of flow cytometry, a marked increase in IL-2 receptor (IL-2R) expression was observed on the surface of circulating CD4⁺ T cells in patients with severe acute asthma when compared with the T cells in patients with asymptomatic mild asthma. These changes appeared unrelated to atopy *per se*, because they were not detected in the peripheral blood of atopic patients (ie, those with positive skin tests to common allergens but without bronchial asthma).

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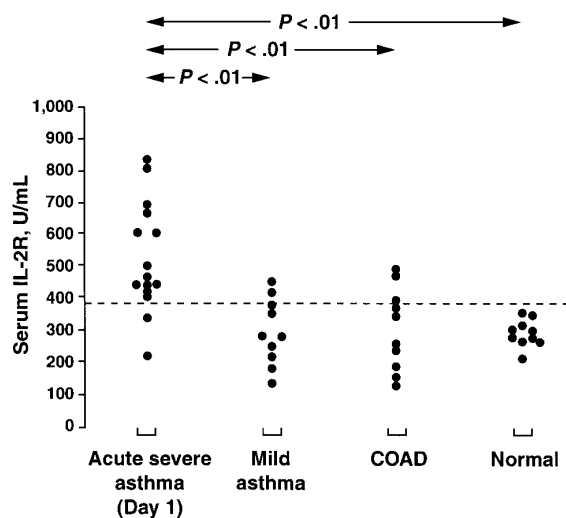


FIG 1. Serum IL-2R concentration in patients with acute severe asthma versus control groups. The dotted line represents the 95% confidence interval for the range of values in normal control subjects. COAD, Chronic obstructive airway disease. (From Corrigan CJ, Kay AB. CD4 T-lymphocyte activation in acute severe asthma: relationship to disease severity and atopic status. *Am Rev Respir Dis* 1990;141:970-7. With permission. Official Journal of the American Thoracic Society. © American Lung Association.)

These changes did not reflect airway obstruction because the numbers of CD4⁺/CD25⁺ cells detected in asthmatic patients were markedly higher than those observed in patients with chronic obstructive pulmonary disease (ie, airflow obstruction with no detectable airflow reversibility). Furthermore, the inverse correlation between CD25⁺/CD4⁺ cells and peak expiratory flow rates suggested a relationship between IL-2R expression on circulating cells and asthma severity.¹⁴ There were also increased peripheral blood concentrations of the soluble form of the IL-2R, an alternative indirect serum marker of T-cell activation (Fig 1).¹⁴

We also performed *in situ* hybridization studies that used gene probes to detect production of T_H2 cytokines by CD4⁺ and CD8⁺ T cells isolated from peripheral blood. These measurements were performed before and after oral corticosteroid therapy in patients with severe asthma. There was an increase in IL-4, IL-5 mRNA⁺ cells, and GM-CSF mRNA⁺ cells, but not in the number of IL-3, IL-2, or INF- γ mRNA⁺ T cells.¹⁵ There were marked reductions in the expression of these T_H2 cells after daily treatment with prednisolone for 7 days. Serum IL-5 was also detectable in a proportion of these patients with acute severe asthma. Serum IL-5 concentrations correlated with blood eosinophilia and with the percentage of CD25⁺/CD4⁺ T cells in peripheral blood and were also inhibited by oral corticosteroid treatment.¹⁶

More recently, the functional properties of circulating T cells were assessed by *in vitro* culture of peripheral blood mononuclear cells from subjects with mite-sensitive allergy.¹⁷ Short-term cultures were incubated with

concentrations of house dust mite allergen that were optimal for proliferation and for cytokine production. IL-5 was measured in supernatants that were harvested from 6-day cultures, and proliferation was assessed after a further 16 hours by a measurement of the radioactive thymidine incorporation (Fig 2).¹⁷ In general, the highest level of mite-induced T-cell proliferation and IL-5 production occurred in atopic asthmatic patients. Intermediate levels were detected in patients with allergic rhinitis, and very low levels were detected in atopic, asymptomatic, and nonatopic normal individuals. The principle sources of IL-5 in these experiments were CD4⁺ T cells. This was demonstrated by the complete inhibition of IL-5 production after depletion of peripheral blood mononuclear cells with the use of magnetic separation techniques with negative selection for CD4⁺, but not CD8⁺ T cells. In asthmatic subjects, IL-5 expression correlated closely with the level of bronchial hyperreactivity measured by histamine challenge.¹⁷

BRONCHIAL T-CELL RESPONSES

Extensive evidence now supports a primary, proinflammatory role for T_H2 T cells in the bronchial mucosa of asthmatic patients.

Activated CD25⁺ T cells are present in bronchial biopsy specimens,¹⁸ and bronchoalveolar lavage (BAL)¹² fluid obtained at fiberoptic bronchoscopy from patients with atopic asthma. In general, T-cell activation and the preferential production of IL-4 and IL-5 (as opposed to INF- γ and IL-2) correlated with local tissue eosinophilia, asthma severity (determined by symptom scores), FEV₁, diurnal variation in peak expiratory flow rates, and bronchial hyperreactivity.¹⁹

T-cell activation and tissue eosinophilia are also features of occupational asthma.²⁰ They are detectable in patients with so-called "intrinsic" late-onset asthma in whom skin prick tests to common inhaled allergens are negative and in whom an allergic cause could not be detected.²¹ Intrinsic asthma is additionally characterized by a more intense inflammation with an increase in CD68⁺ macrophages and an increase in the expression of GM-CSF receptors, particularly on the surface of CD68⁺ macrophages.²²

T-cell activation, T_H2 cytokine expression, and local eosinophilia (detected in BAL²³ and bronchial biopsy specimens²⁴), are upregulated after allergen inhalation challenge. In BAL fluid, a close association was observed between CD25 expression by CD4⁺ T cells, local IL-5 mRNA⁺ cells, and bronchoalveolar eosinophilia. Furthermore, in agreement with previous authors, we found a close correlation between bronchoalveolar eosinophilia and the magnitude of the late asthmatic response (Fig 3).²⁵ These changes can be inhibited by both topical²⁶ and oral corticosteroid treatment (Fig 4).²⁷ Moreover, local T-cell activation and expression of T_H2-type cytokines are specific for bronchial asthma as opposed to other inflammatory airway disorders, including tuberculosis and bronchiectasis.²⁸

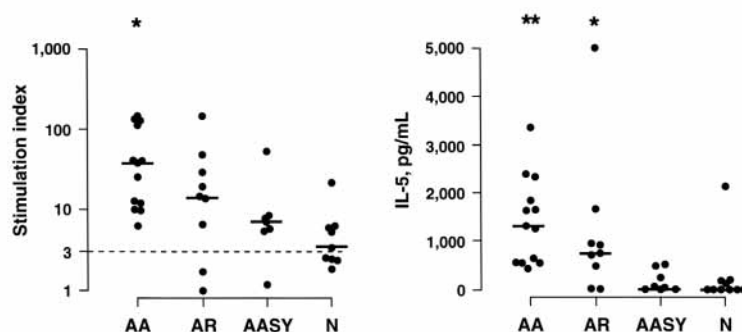


FIG 2. Proliferation and IL-5 production by peripheral blood T-cell cultures in patients with asthma and sensitive allergy to house dust mite (AA), in patients with allergic rhinitis (AR), in atopic asymptomatic patients (AASY), and in nonatopic normal control subjects (N). Peripheral blood monocytes were cultured for 6 days in the presence of optimal concentrations of house dust mite before the measurement of IL-5 production in culture supernatants by enzyme-linked immunosorbent assay and tritiated thymidine incorporation. (From Till S, Dickason R, Huston D, Humbert M, Robinson D, Larche M, et al. IL-5 secretion by allergen-stimulated CD4⁺ T cells in primary culture: relationship to expression of allergic disease. *J Allergy Clin Immunol* 1997;99:563-9. With permission.)

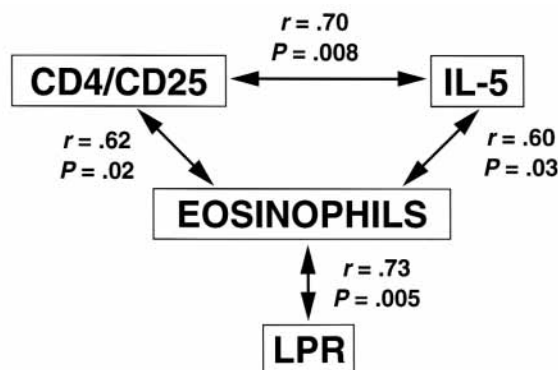


FIG 3. T-cell activation, IL-5 mRNA expression, bronchoalveolar eosinophilia, and the magnitude of the late-phase asthmatic response (LPR) in bronchoalveola of atopic asthmatic patients after allergen inhalation challenge. Patients were challenged with house dust mite or grass pollen extract. Twenty-four hours later, fiberoptic bronchoscopy and BAL were performed. CD25 expression on T cells was measured by flow cytometry. Eosinophils and IL-5 mRNA expression were detected with immunostaining and in situ hybridization of BAL cytospin preparations, respectively. The late-phase asthmatic response was recorded as maximal fall in FEV₁ at 3 to 24 hours after the challenge. Correlations among cell counts and the late-phase asthmatic response were determined by the Spearman's rank method. (Adapted with permission from Durham SR, Kay AB. Late allergic responses. In: Kaplan AP, ed. *Allergy*. 2nd ed. Philadelphia, PA: WB Saunders; 1997:292.)

INFLUENCE OF IMMUNOTHERAPY

The value of allergen injection immunotherapy in patients with allergy and IgE-mediated disease was highlighted in a recent World Health Organization report.²⁹ In pollen-sensitive patients, immunotherapy induces changes in circulating antibody levels³⁰ (blunting of seasonal increases in IgE and profound increases in allergen-specific IgG concentrations), decreased mast cell migration³¹ and mediator release,³² and decreased tissue recruitment and activation of eosinophils after allergen provocation.³³ These changes are considered secondary to the effect of immunotherapy on T-cell responses that are detectable in both the peripheral blood and the target organ. Immunotherapy induces an alteration in the

T_H2/T_H1 T-cell balance in favor of T_H1 responses. This may occur as a consequence of immune deviation of T_H2/T_H0 responses in favor of T_H1 responses or the downregulation of T_H0/T_H2 responses (eg, through T-cell anergy; Fig 5).^{34,35}

Suppression of late responses in the skin by immunotherapy was accompanied by an increase in IL-12 mRNA expression, predominantly by macrophages.³⁶ This raised the question whether local T cells at allergic mucosal sites remain susceptible to the influence of inhibitory cytokines (including IL-12, IFN- γ , IL-10, or transforming growth factor- β). To determine whether the proliferation and production of IL-5 by T cells from the target organ could be inhibited by pretreatment with IL-12, we recently examined cultures of enriched T cells

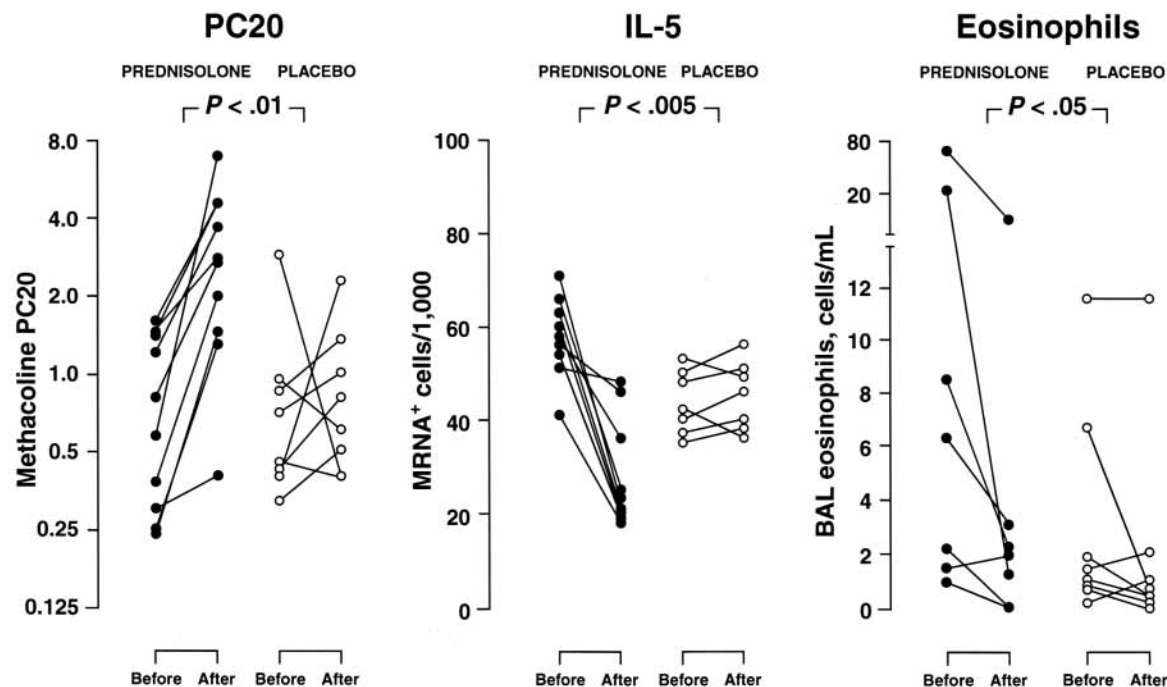


FIG 4. The influence of prednisolone tablets (0.6 mg/kg daily) versus placebo on airway methacholine responsiveness, the numbers of IL-5 mRNA⁺ cells, and the number of eosinophils in the BAL fluid that was harvested before and 2 weeks after treatment. Between-group differences (after/before treatment) were significant for methacholine PC₂₀, for IL-5 mRNA⁺ cells, and for eosinophils. Two-tailed Mann-Whitney *U* test probability values are shown. (Adapted with permission from Robinson D, Hamid Q, Ying S, Bentley A, Assoufi B, Durham S, et al. Prednisolone treatment in asthma is associated with modulation of bronchoalveolar lavage cell interleukin-4, interleukin-5, and interferon- γ cytokine gene expression. *Am Rev Respir Dis* 1993;148:401-6. Official Journal of the American Thoracic Society. © American Lung Association.)

that were purified from BAL fluid that was obtained 24 hours after segmental allergen challenge in atopic asthmatic patients. We found that allergen-stimulated T cells from BAL produced approximately 5- to 10-fold more IL-5 than equivalent numbers of peripheral blood T cells from the same patients.³⁷ This enhanced IL-5 production by T cells obtained directly from the lower airways was inhibited in a dose-dependent fashion by IL-12.³⁸ This effect of IL-12 was independent of IFN- γ , because it was not inhibited by the addition of a neutralizing concentration of anti-IFN- γ antibody to the cultures.⁵ Furthermore, IL-12 β 2 receptor mRNA⁺ transcripts were detectable by in situ hybridization in cytospin preparations of enriched BAL T cells that were obtained after allergen provocation, consistent with the persistence of susceptibility to IL-12.³⁸ The most likely explanation for these findings was that, unlike certain committed T_H2 clones, T cells harvested from the site of allergic inflammation in the bronchi remain susceptible to the effects of inhibitory cytokines.

CONCLUSIONS

Cardinal features of bronchial asthma include local tissue eosinophilia and IgE-dependent mast-cell activation. These mechanisms are under the regulation of

cytokines that are derived from T_H2 T cells. Activation of T_H2 T cells in the bronchi in atopic subjects appears specific for asthma, as opposed to other diseases that are associated with airway obstruction. The production of T_H2 cytokines correlated with asthma severity, was inducible by allergen, and was inhibited by corticosteroids. Allergen-specific immunotherapy induces a shift in the T_H2/T_H1 T-cell balance in favor of T_H1 responses. This may occur as a consequence of immune deviation of T_H2/T_H1 responses and/or induction of allergen-specific unresponsiveness of T_H2/T_H0 T cells (eg, through anergy).³⁴ These findings support the fundamental importance of T-cell responses in bronchial asthma and highlight potential strategies for therapy. Broad-based strategies include immunosuppression with alternatives to corticosteroids (eg, cyclosporin A)³⁹ and the use of more selective strategies directed against CD4⁺ T cells,⁴⁰ IL-5,^{41,42} and/or IL-4.⁴³

An important question is whether therapy should be directed locally to the affected mucosal surface or by the systemic route. The systemic nature of allergic disease is reflected by the parallel changes of T-cell activation and cytokine production that are detectable in peripheral blood and in the bronchial mucosa and BAL fluid. It is not clear whether changes that are detectable in the peripheral blood reflect a "spill-over" of T-cell responses

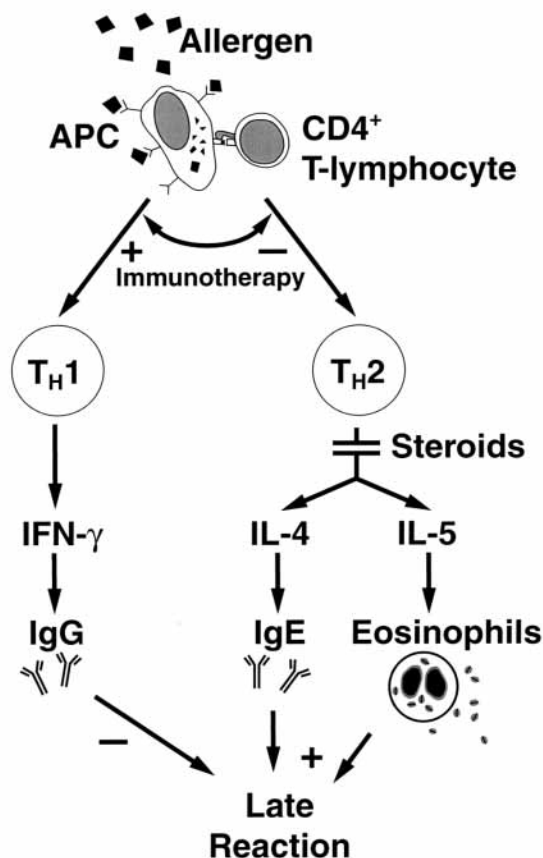


FIG 5. Hypothesis of the mechanism of allergen-induced late asthmatic response as a consequence of T_H2 -type T-lymphocyte activation. Whereas corticosteroids inhibit T_H2 -cytokine production, allergen injection immunotherapy may act by inducing T-cell unresponsiveness (anergy) and/or immune deviation in favor of T_H1 responses. APC, Antigen-presenting cell.

in the target organ. The high efficacy of topical treatments (ie, through inhalation) supports this concept. On the other hand, the presence of multiple systemic manifestations of allergy, including allergic rhinitis, bronchial asthma, atopic eczema, and food allergy in atopic subjects would support systemic therapeutic modalities.

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REFERENCES

- Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL. Two types of murine helper T cell clone: I. Definition according to profiles of lymphokine activities and secreted proteins. *J Immunol* 1986;136:2348-57.
- Wierenga EA, Snoek M, de Groot C, Chretien I, Bos JD, Jansen HM, et al. Evidence for compartmentalization of functional subsets of CD2⁺ T lymphocytes in atopic patients. *J Immunol* 1990;144:4651-6.
- Romagnani S. The role of lymphocytes in allergic disease. *J Allergy Clin Immunol* 2000;105:399-408.
- Del Prete G, Maggi E, Parronchi P, Chretien I, Tiri A, Macchia D, et al. IL-4 is an essential factor for the IgE synthesis induced in vitro by human T cell clones and their supernatants. *J Immunol* 1988;140:4193-8.

- de Vries JE. The role of IL-13 and its receptor in allergy and inflammatory responses. *J Allergy Clin Immunol* 1998;102:165-9.
- Clutterbuck EJ, Hirst EM, Sanderson CJ. Human interleukin-5 (IL-5) regulates the production of eosinophils in human bone marrow cultures: comparison and interaction with IL-1, IL-3, IL-6, and GM-CSF. *Blood* 1989;73:1504-12.
- Her E, Frazer J, Austen KF, Owen WF Jr. Eosinophil hematopoietins antagonize the programmed cell death of eosinophils: cytokine and glucocorticoid effects on eosinophils maintained by endothelial cell-conditioned medium. *J Clin Invest* 1991;88:1982-7.
- Maggi E, Parronchi P, Manetti R, Simonelli C, Piccinni MP, Rugiu FS, et al. Reciprocal regulatory effects of IFN-gamma and IL-4 on the in vitro development of human Th1 and Th2 clones. *J Immunol* 1992;148:2142-7.
- Secrist H, DeKruyff RH, Umetsu DT. Interleukin 4 production by CD4⁺ T cells from allergic individuals is modulated by antigen concentration and antigen-presenting cell type. *J Exp Med* 1995;181:1081-9.
- Kapsenberg ML, Hilkens CM, Wierenga EA, Kalinski P. The role of antigen-presenting cells in the regulation of allergen-specific T cell responses. *Curr Opin Immunol* 1998;10:607-13.
- Durham SR, Ying S, Varney VA, Jacobson MR, Sudderick RM, Mackay IS, et al. Cytokine messenger RNA expression for IL-3, IL-4, IL-5, and granulocyte/macrophage-colony-stimulating factor in the nasal mucosa after local allergen provocation: relationship to tissue eosinophilia. *J Immunol* 1992;148:2390-4.
- Robinson DS, Hamid Q, Ying S, Tsicopoulos A, Barkans J, Bentley AM, et al. Predominant T_H2 -like bronchoalveolar T-lymphocyte population in atopic asthma. *N Engl J Med* 1992;326:298-304.
- Corrigan CJ, Hartnell A, Kay AB. T lymphocyte activation in acute severe asthma. *Lancet* 1988;1:1129-32.
- Corrigan CJ, Kay AB. CD4 T-lymphocyte activation in acute severe asthma: relationship to disease severity and atopic status. *Am Rev Respir Dis* 1990;141:970-7.
- Corrigan CJ, Hamid Q, North J, Barkans J, Moqbel R, Durham S, et al. Peripheral blood CD4 but not CD8 T-lymphocytes in patients with exacerbation of asthma transcribe and translate messenger RNA encoding cytokines which prolong eosinophil survival in the context of a Th2-type pattern: effect of glucocorticoid therapy. *Am J Respir Cell Mol Biol* 1995;12:567-78.
- Corrigan CJ, Haczku A, Gemou-Engesaeth V, Doi S, Kikuchi Y, Takatsu K, et al. CD4 T-lymphocyte activation in asthma is accompanied by increased serum concentrations of interleukin-5: effect of glucocorticoid therapy. *Am Rev Respir Dis* 1993;147:540-7.
- Till S, Dickason R, Huston D, Humbert M, Robinson D, Larche M, et al. IL-5 secretion by allergen-stimulated CD4⁺ T cells in primary culture: relationship to expression of allergic disease. *J Allergy Clin Immunol* 1997;99:563-9.
- Bradley BL, Azzawi M, Jacobson M, Assoufi B, Collins JV, Irani AM, et al. Eosinophils, T-lymphocytes, mast cells, neutrophils, and macrophages in bronchial biopsy specimens from atopic subjects with asthma: comparison with biopsy specimens from atopic subjects without asthma and normal control subjects and relationship to bronchial hyperresponsiveness. *J Allergy Clin Immunol* 1991;88:661-74.
- Robinson DS, Bentley AM, Hartnell A, Kay AB, Durham SR. Activated memory T helper cells in bronchoalveolar lavage fluid from patients with atopic asthma: relation to asthma symptoms, lung function, and bronchial responsiveness. *Thorax* 1993;48:26-32.
- Bentley AM, Maestrelli P, Sassetta M, Fabbri LM, Robinson DS, Bradley BL, et al. Activated T-lymphocytes and eosinophils in the bronchial mucosa in isocyanate-induced asthma. *J Allergy Clin Immunol* 1992;89:821-9.
- Bentley AM, Menz G, Storz C, Robinson DS, Bradley B, Jeffery PK, et al. Identification of T lymphocytes, macrophages, and activated eosinophils in the bronchial mucosa in intrinsic asthma: relationship to symptoms and bronchial responsiveness. *Am Rev Respir Dis* 1992;146:500-6.
- Kotsimbos AT, Humbert M, Minshall E, Durham S, Pfister R, Menz G, et al. Upregulation of alpha GM-CSF-receptor in nonatopic asthma but not in atopic asthma. *J Allergy Clin Immunol* 1997;99:666-72.
- Robinson D, Hamid Q, Bentley A, Ying S, Kay AB, Durham SR. Activation of CD4⁺ T cells, increased T_H2 -type cytokine mRNA expression and eosinophil recruitment in bronchoalveolar lavage after allergen inhalation challenge in patients with atopic asthma. *J Allergy Clin Immunol* 1993;92:313-24.
- 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.
25. Durham SR, Kay AB. Late allergic responses. In: Kaplan AP, ed. *Allergy*. 2nd ed. Philadelphia, PA: WB Saunders; 1997:287-298.
26. Trigg CJ, Manolitsas ND, Wang J, Calderon MA, McAulay A, Jordan SE, et al. Placebo-controlled immunopathologic study of four months of inhaled corticosteroids in asthma. *Am J Respir Crit Care Med* 1994;150:17-22.
27. Robinson D, Hamid Q, Ying S, Bentley A, Assoufi B, Durham S, et al. Prednisolone treatment in asthma is associated with modulation of bronchoalveolar lavage cell interleukin-4, interleukin-5, and interferon-gamma cytokine gene expression. *Am Rev Respir Dis* 1993;148:401-6.
28. Gaga M, Bentley AM, Humbert M, Barkans J, O'Brien F, Wathen CG, et al. Increases in CD4⁺ T lymphocytes, macrophages, neutrophils and interleukin 8 positive cells in the airways of patients with bronchiectasis. *Thorax* 1998;53:685-91.
29. Allergen immunotherapy: therapeutic vaccines for allergic diseases: Geneva: January 27-29, 1997. *Allergy* 1998;53 (suppl):1-42.
30. Djurup R, Malling HJ. High IgG4 antibody level is associated with failure of immunotherapy with inhalant allergens. *Clin Allergy* 1987;17:459-68.
31. Otsuka H, Mezawa A, Ohnishi M, Okubo K, Seki H, Okuda M. Changes in nasal metachromatic cells during allergen immunotherapy. *Clin Exp Allergy* 1991;21:115-9.
32. Creticos PS, Adkinson NF Jr, Kagey-Sobotka A, Proud D, Meier HL, Naclerio RM, et al. Nasal challenge with ragweed pollen in hay fever patients. Effect of immunotherapy. *J Clin Invest* 1985;76:2247-53.
33. Furin MJ, Norman PS, Creticos PS, Proud D, Kagey-Sobotka A, Lichtenstein LM, et al. Immunotherapy decreases antigen-induced eosinophil cell migration into the nasal cavity. *J Allergy Clin Immunol* 1991;88:27-32.
34. Durham SR, Till SJ. Immunologic changes associated with allergen immunotherapy. *J Allergy Clin Immunol* 1998;102:157-64.
35. Akdis CA, Blaser K. IL-10-induced anergy in peripheral T cell and reactivation by microenvironmental cytokines: two key steps in specific immunotherapy. *FASEB J* 1999;13:603-9.
36. Hamid QA, Schotman E, Jacobson MR, Walker SM, Durham SR. Increases in IL-12 messenger RNA⁺ cells accompany inhibition of allergen-induced late skin responses following successful grass pollen immunotherapy. *J Allergy Clin Immunol* 1997;99:254-60.
37. Till SJ, Durham SR, Rajakulasingam K, Humbert M, Huston D, Dickason R, et al. Allergen-induced proliferation and interleukin-5 production by bronchoalveolar lavage and blood T cells after segmental allergen challenge. *Am J Respir Crit Care Med* 1998;158:404-11.
38. Varga EM, Wachholz P, Nouri-Aria KT, Verhoef A, Corrigan CJ, Till SJ, Durham SR. T cells from human allergen-induced late asthmatic responses express IL-12 receptor beta 2 subunit mRNA and respond to IL-12 in vitro. *J Immunol* 2000;165:2877-85.
39. Lock SH, Kay AB, Barnes NC. Double-blind, placebo-controlled study of cyclosporin A as a corticosteroid-sparing agent in corticosteroid-dependent asthma. *Am J Respir Crit Care Med* 1996;153:509-14.
40. Kon OM, Sihra BS, Compton CH, Leonard TB, Kay AB, Barnes NC. Randomised, dose-ranging, placebo-controlled study of chimeric antibody to CD4 (keliximab) in chronic severe asthma. *Lancet* 1998;352:1109-13.
41. Mauser PJ, Pitman AM, Fernandez X, Foran SK, Adams GK III, Kreutner W, et al. Effects of an antibody to interleukin-5 in a monkey model of asthma. *Am J Respir Crit Care Med* 1995;152:467-72.
42. Egan RW, Wang P, Garlisi C, Mauser P, Chapman RW, Kreutner W, et al. Mechanism of extended duration of anti-IL-5 antibodies against pulmonary eosinophilia [abstract]. *J Allergy Clin Immunol* 1997;99:S122.
43. Borish LC, Nelson HS, Lanz MJ, Claussen L, Whitmore JB, Agosti JM, et al. Interleukin-4 receptor in moderate atopic asthma: a phase I/II randomized, placebo-controlled trial. *Am J Respir Crit Care Med* 1999;160:1816-23.