

Evidence for expression of eosinophil-associated IL-12 messenger RNA and immunoreactivity in bronchial asthma

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Background: Eosinophils are a source of cytokines within the airways of asthmatic individuals that may exert an important immunoregulatory influence.

Objectives: We examined IL-12 messenger (m)RNA and protein expression in eosinophils from peripheral blood and bronchoalveolar lavage (BAL) fluid obtained from subjects with atopic asthma (n = 7), patients with chronic bronchitis (n = 5), and nonatopic healthy control subjects (n = 7). To further define this IL-12⁺ population of eosinophils for the expression of other cytokines, we colocalized IL-12 and IL-5 within the peripheral blood eosinophils.

Methods: To detect IL-12 mRNA and protein expression, we used in situ hybridization and immunocytochemistry techniques. The double-immunocytochemistry technique was used to localize IL-12 protein to BAL eosinophils and to colocalize IL-5 and IL-12 in peripheral blood eosinophils.

Results: IL-12 mRNA and immunoreactive protein were localized to peripheral blood eosinophils. BAL fluid-derived eosinophils from asthmatic subjects were also reactive to IL-12. The percentage of peripheral blood eosinophils expressing mRNA for IL-12 was significantly lower in asthmatic subjects compared with that found in eosinophils obtained from patients with chronic bronchitis ($P < .001$) and control patients ($P < .05$). Colocalization studies demonstrated that the percentages of IL-12⁺ eosinophils that are also IL-5⁺ were 72% in asthmatic subjects and only 11% in control subjects ($P < .001$). **Conclusion:** These results suggest that eosinophils are a potential source of IL-12. Eosinophil-derived IL-12 may contribute and modulate the local allergic inflammatory responses. (*J Allergy Clin Immunol* 2000;106:288-92.)

Key words: Eosinophils, IL-12, cytokines, asthma, magnetic cell sorting technique, immunofluorescence staining

Eosinophilic inflammation is a hallmark of allergic disorders and is orchestrated by the expression of T_{H2}-type cytokines (IL-4, IL-5, and IL-13) from CD4⁺ T lymphocytes.^{1,2} Eosinophils are believed to contribute to the pathophysiology of allergic inflammation, both directly through the release of lipid and granular mediators and indirectly through the release of cytokines.³ Recently, it has been shown that like CD4⁺ T lymphocytes, eosinophils can be polarized in their capacity to synthesize and release T_{H1}- and T_{H2}-type cytokines, including IL-2, IL-4, IL-5, IL-6, and GM-CSF.^{4,5} These findings may suggest that as cytokine-producing cells, eosinophils may play a role in the regulation of the host immune response.

IL-12 is a heterodimeric 70-kd glycoprotein composed of two covalently linked chains, p35 and p40, both of which are required for functional activity.⁶ IL-12 has been established as a T_{H1}-type cytokine by virtue of its ability to induce T_{H1}-type lymphocyte differentiation and proliferation and to inhibit the T_{H2}-type responses.^{7,8} Administration of IL-12 in vivo abrogates antigen-induced airway hyperresponsiveness, IgE synthesis, and the associated pulmonary eosinophilia in animal models of allergic inflammation.⁹⁻¹² Consistent with this activity of IL-12 in promoting T_{H1}-type responses, the level of IL-12 messenger (m)RNA expression has been reported as being decreased within the airways of asthmatic subjects compared with those of nonasthmatic control subjects.² These data reflect the preferential switching of CD4⁺ T lymphocytes to a T_{H2}-like phenotype within the airways of asthmatic individuals¹ and suggest a potential modulatory role for IL-12 as a suppressor of allergic inflammation.

To extend our investigations into the role of eosinophils as a cellular source of cytokines, we examined IL-12 transcription and translation in eosinophils derived from the peripheral blood and bronchoalveolar lavage (BAL) fluid of asthmatic patients and compared that with eosinophils obtained from patients with chronic bronchitis and control subjects.

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METHODS

Subjects and study design

A total of 25 subjects were included in this study. Patients were recruited from the outpatient clinic at the Montreal Chest Institute, McGill University. Asthma and chronic bronchitis were defined

Abbreviations used

BAL: Bronchoalveolar lavage

ISH: In situ hybridization

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Supported by the Medical Research Council of Canada.

Received for publication Jan 12, 2000; revised Apr 13, 2000; accepted for publication Apr 13, 2000.

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0091-6749/2000 \$12.00 + 0 1/107932

doi:10.1067/mai.2000.107932

according to the American Thoracic Society criteria.¹³ All of the asthmatic subjects were atopic to one or more of the tested panel of allergens. The asthmatic patients ($n = 7$; 4 male subjects) exhibited mild-to-moderate asthma; the mean FEV₁ percent predicted was 77% (range, 62%–87%). The asthmatic subjects had a greater number of eosinophils in their blood when expressed as the mean percentage of total granulocytes ($\pm$ SEM) compared with the nonasthmatic control subjects and the patients with chronic bronchitis (asthmatic subjects, $13.2\% \pm 3.5\%$; nonasthmatic control subjects, $3.5\% \pm 0.5\%$; patients with chronic bronchitis, $3.9\% \pm 1.2\%$). The nonasthmatic control subjects ($n = 7$; 3 male subjects) and the patients with chronic bronchitis ($n = 7$; 7 male patients) had negative skin prick test results. Mean FEV₁ percent predicted for the patients with chronic bronchitis was 64% (range, 60%–65%). All subjects except patients with chronic bronchitis were nonsmokers.

Informed consent, approved by the Ethics Committee of the Montreal Chest Hospital, was obtained from all subjects before entry into this study. Fifty milliliters of blood was retrieved from each patient by means of venous section. To confirm the expression of IL-12-immunoreactive protein in eosinophils recovered by BAL, fiberoptic bronchoscopy was performed on the asthmatic patients, as previously described.¹⁴ The expression of IL-12 immunoreactivity was not colocalized in the BAL fluid of the nonasthmatic subjects because these individuals have few eosinophils within their airways.

Peripheral blood eosinophil purification

Human granulocytes were purified from peripheral blood by using Ficoll-Paque (Pharmacia) density centrifugation and dextran sedimentation. Eosinophils were further purified by means of negative selection with anti-CD16- and anti-CD3-coated immunomagnetic microbeads by using a magnetic cell-sorting system (MACS system; Miltenyl Biotech, Bergish-Gladbach, Germany) at 4°C. The degree of purity of the eosinophil population estimated after staining with Giemsa was greater than 95%. Major contaminating cells were neutrophils.

In situ hybridization

Riboprobes (³⁵S-labeled RNA) for IL-12 mRNA (p35 and p40) were prepared from complementary DNA, and in situ hybridization (ISH) was performed as previously described.¹⁵ To ensure ISH specificity, negative controls were performed, including the use of sense probes and pretreatment of the cytospin preparations with RNAase A solution before hybridization with the antisense probe. The specificity of these probes has already been established in previous experiments with Northern blot analysis and ISH.

Immunocytochemistry

We used the alkaline phosphatase–antialkaline phosphatase technique and immunofluorescence staining to detect IL-12 protein in eosinophils. Briefly, mouse anti-human IL-12 mAb (anti-IL-12 p70, 25 μ g/mL; R&D Systems, Minneapolis, Minn) recognizing both subunits of IL-12 was used as the primary antibody. After an overnight incubation at 4°C, the secondary antibody, alkaline phosphatase–antialkaline phosphatase–conjugated rabbit anti-mouse antibody, was added (5 μ g/mL; DAKO A/S, Glostrup, Denmark). The reaction was visualized by using alkaline phosphatase substrate added to fast red dye (TR Salt; Sigma Chemical Co, Poole, UK). For the immunofluorescence staining, a secondary biotinylated rabbit anti-mouse antibody (110 μ g/mL, DAKO A/S) and FITC-labeled extravidin (30 μ g/mL, Sigma Chemical Co) were used. Slides were then washed, mounted in PBS-glycerol, and stored in the dark at 4°C until viewed by means of immunofluorescence microscopy (Nixon Diaphot-TMD, 500 nm excitation wavelength with an objective magnification 100 $\times$).

Double immunocytochemistry

Double immunocytochemistry was performed, as previously described,¹⁶ to confirm the phenotype of the IL-12-immunoreactive cells within the BAL fluid of asthmatic subjects. A mixture of primary antibodies was applied to detect eosinophils (anti-major basic protein antibody, BMK-13, a kind gift from Dr R. Moqbel, University of Alberta, Edmonton, Canada) and IL-12 immunoreactivity (polyclonal IL-12 [p70] antibody, R&D Systems). Double immunocytochemistry was also used to colocalize IL-12 and IL-5 in purified peripheral blood eosinophils. In these experiments monoclonal IL-5 antibody (a kind gift from Dr J. Tavernier, University of Ghent, Belgium) was used in the primary antibody mix in combination with the same polyclonal IL-12 (p70) antibody mentioned above. In all immunochemical studies the appropriate negative controls were included as either omission of the primary antibodies or the use of an irrelevant isotype-specific antibody.

Quantification and statistical analysis

Slides were coded, and positive cells were counted blindly by using 200 $\times$ magnification with an eyepiece graticule. At least 500 cells per slide were counted, and the results were expressed as the percentage of positive cells per high-power field ($\pm$ SEM). The percentage of eosinophils expressing IL-12 immunoreactivity was compared between the groups by using a nonparametric Kruskal-Wallis ANOVA, and subsequent post hoc analyses were performed by using a Mann-Whitney *U* test. Results were considered statistically significant at a *P* value of less than .05. Statistical analyses were performed by using a standard computer package (Systat v7.0; SPSS Inc, Chicago, Ill).

RESULTS

IL-12 mRNA expression in peripheral blood eosinophils

Hybridization of the labeled complementary RNA probes and mRNA encoding IL-12 p35 and p40 was demonstrated by specific deposits of silver grains in the photographic emulsion overlying the tissue sections. No positive hybridization signals were observed when the sense probe was used nor when the cells were pretreated with RNase before antisense hybridization. Peripheral blood eosinophils obtained from asthmatic and normal healthy control subjects expressed mRNA for both p35 and p40 subunits of IL-12 (Fig 1, A). In the asthmatic subjects there were significantly less p35 mRNA⁺ eosinophils compared with the amounts found in the nonasthmatic control subjects (mean $\pm$ SEM; asthmatic subjects, $53.6\% \pm 6.2\%$ [$n = 7$]; control subjects, $71.4\% \pm 3.8\%$ [$n = 7$]; $P < .05$). Compared with the control subjects, there were also significantly lower numbers of IL-12 p40 mRNA⁺ eosinophils in the asthmatic patients (mean $\pm$ SEM; asthmatic subjects, $72.0\% \pm 7.1\%$ [$n = 6$]; control subjects, $89.7\% \pm 3.8\%$ [$n = 6$]; $P < .05$; Fig 2, A).

IL-12 immunoreactivity in peripheral blood eosinophils

Cells exhibiting positive immunoreactivity for IL-12 were detected by the presence of discrete red cytoplasmic staining and positive immunofluorescence (Fig 1, B). Eosinophils obtained from all subjects were immunoreactive for IL-12 (Fig 2, B). However, the percentages of

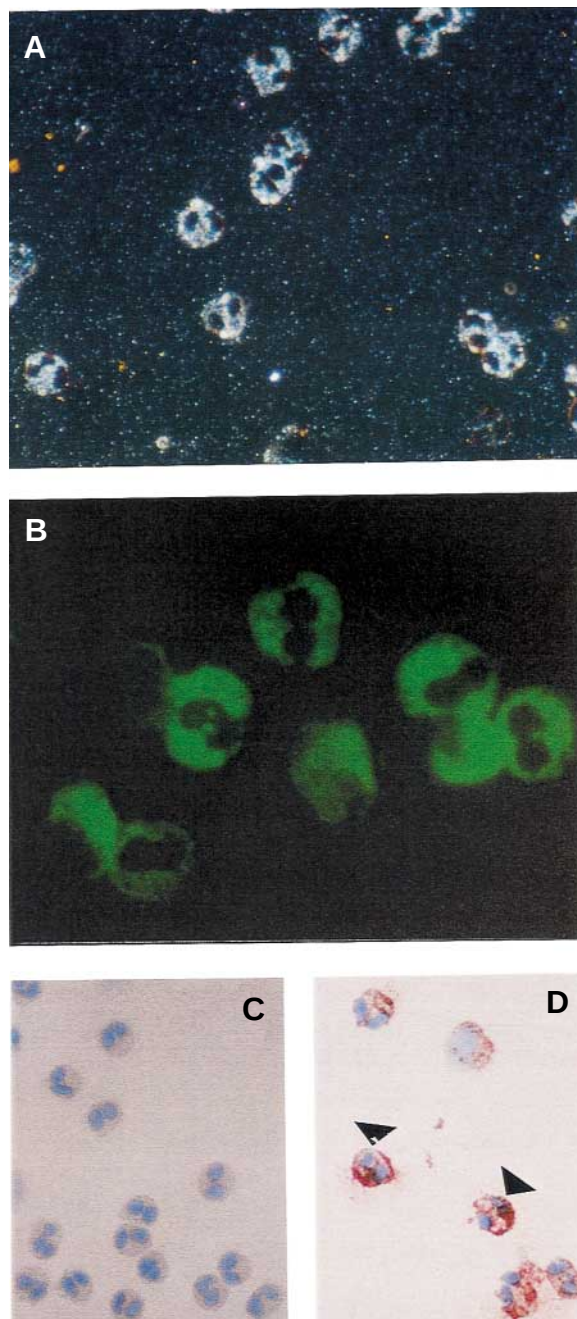


FIG 1. **A**, A representative example of IL-12 (p40) mRNA in peripheral blood eosinophils from an asthmatic individual. Positive hybridization signals are localized as silver grains (magnification 200 $\times$). **B**, Immunofluorescent staining with an FITC-conjugated antibody against both subunits of IL-12 is shown in peripheral blood eosinophils from asthmatic subjects (magnification 1000 $\times$). **C**, Control experiment (no staining). **D**, Double-staining experiments to colocalize IL-5 and IL-12 in peripheral blood eosinophils. Arrows indicate the positive double staining.

IL-12-immunoreactive eosinophils were significantly lower in asthmatic patients (mean $\pm$ SEM; asthmatic subjects, $5.5\% \pm 0.7\%$ [$n = 6$]) compared with patients with chronic bronchitis ($25.7\% \pm 1.9\%$ [$n = 7$], $P < .001$) and nonatopic control subjects ($12\% \pm 1.5\%$ [$n = 7$], $P < .05$).

IL-12 immunoreactivity in BAL eosinophils

Double immunostaining was performed on BAL fluid obtained from asthmatic subjects to ensure the local production of IL-12 by eosinophils within the airways. In phenotyping the cells eosinophils were detected by the presence of red staining indicating major basic protein immunoreactivity, and IL-12 immunoreactivity was detected by a brown staining. Those eosinophils expressing the IL-12-immunoreactive protein stained reddish brown. Of the total cells recovered in BAL fluid, $7.0\% \pm 1.9\%$ were eosinophils. Approximately $77\% \pm 10.5\%$ of the eosinophil population within the BAL fluid stained positive for IL-12.

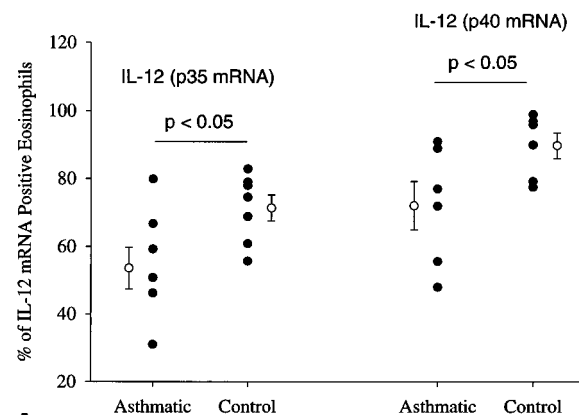
Colocalization of IL-12 and IL-5 in peripheral blood eosinophils

Eosinophil-associated IL-12 immunoreactivity was detected by the presence of red staining, and IL-5 immunoreactivity was detected by the presence of brown staining. Eosinophils immunoreactive for both IL-12 and IL-5 were stained reddish brown (Fig 1, *D*). Colocalization studies demonstrated that the percentage of IL-12⁺ eosinophils that were also IL-5⁺ was 72% in asthmatic subjects, whereas in control subjects it was only 11% ($P < .001$). A total of $4.03\% \pm 0.4\%$ of peripheral blood eosinophils were positive for IL-12 and IL-5 compared with $1.3\% \pm 0.31\%$ in control subjects (Fig 3, $P < .001$).

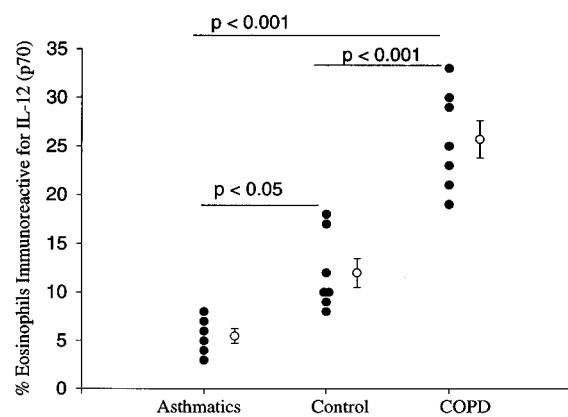
DISCUSSION

In this study we have demonstrated the expression of IL-12 (p35 and p40) mRNA in human peripheral blood eosinophils. Evidence for the translation of this cytokine mRNA into IL-12-immunoreactive protein was also confirmed in circulating eosinophils. In addition, IL-12 immunoreactivity localized to eosinophils in the BAL fluid of asthmatic subjects, establishing these cells as potential sources of this immunomodulatory cytokine within the airways.

To date, many studies have demonstrated the capacity of eosinophils to express cytokines both constitutively and after the appropriate stimulation.¹⁶⁻²² By using confocal microscopy, several of these cytokines have been localized to the cytoplasmic granules.^{16,21,22} The presence of IL-12 mRNA and immunoreactive protein in freshly isolated peripheral blood eosinophils from all the subjects studied suggests that IL-12 expression by eosinophils is constitutive. The presence of IL-12 mRNA within these cells indicates that this cytokine is synthesized *de novo* and that the eosinophil-associated immunoreactivity for IL-12 could not be attributed to the release of preformed protein, or the endocytosis of IL-12 by these cells. It has been previously shown that the expression of both p35 and p40 subunits is required for the biologic activity of IL-12.⁶ In this study we demonstrated that eosinophils express mRNA for both p35 and p40, suggesting they synthesize biologically active IL-12. Further evidence to support the coordinated expres-



A



B

FIG 2. A, Mean percentages of p35 and p40 mRNA⁺ eosinophils in peripheral blood taken from asthmatic subjects ($n = 7$) and nonasthmatic control subjects ($n = 7$). There were statistically significantly greater percentages of IL-12 mRNA⁺ (both p35 and p40) cells in nonasthmatic subjects compared with asthmatic subjects. **B,** IL-12 immunoreactivity in peripheral blood eosinophils obtained from asthmatic subjects ($n = 6$), nonasthmatic control subjects ($n = 7$), and patients with chronic bronchitis ($n = 7$). The percentage of eosinophils expressing IL-12-immunoreactive protein was lower in asthmatic subjects compared with that found in patients with chronic bronchitis ($P < .001$) and healthy control subjects ($P < .05$).

sion of both subunits came from immunocytochemistry studies with an anti-p70-specific antibody. Our findings suggest that eosinophils produce a biologically active heterodimeric form of IL-12. These data are consistent with a previous publication demonstrating that the IL-4-induced release of IL-12 by these cells is sufficient to promote IFN- γ expression in T_{H1} -type cells.²³ Taken together, these findings demonstrate that eosinophils are a potential cellular source of IL-12. Eosinophil-derived IL-12 may contribute and modulate the local allergic inflammatory responses.

Our findings demonstrate that there was a difference in the percentages of IL-12-positive eosinophils determined by ISH and immunoreactivity. The number of eosinophils immunoreactive for IL-12 was lower compared with that of eosinophils positive for IL-12 mRNA.

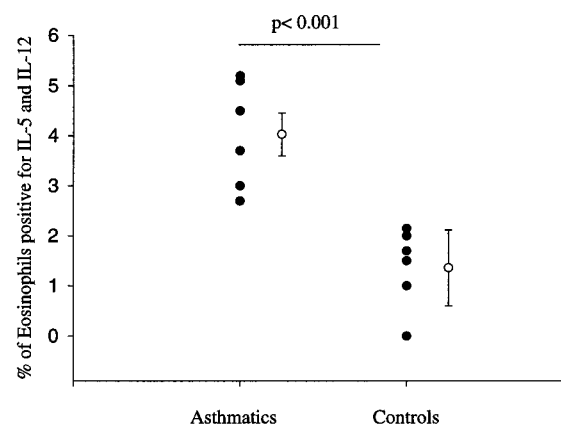


FIG 3. A representative graph demonstrating the percentages of eosinophils positive for IL-12 and IL-5 in asthmatic and control subjects ($P < .001$).

This discrepancy in cytokine mRNA and protein expression suggests that protein expression might be regulated at the posttranscriptional, translational, or posttranslational level. This may also suggest that IL-12 is released rather than stored inside the cell. Moreover, in our immunocytochemistry experiments we used an mAb that recognizes the functional IL-12 protein (both subunits, IL-12p70), which may prevent the detection of the protein expression for each individual subunit of IL-12 (p40 and p35). Additionally, previous studies have demonstrated that production of IL-12 both by cell lines and by normal monocytes results in the secretion of 5- to 500-fold excess of p40 relative to the IL-12 heterodimer,^{24,25} which may also explain our findings presented here.

In our study IL-12 mRNA and protein were expressed in peripheral blood eosinophils obtained from all the subjects, including asthmatic subjects, patients with chronic bronchitis, and control subjects. The percentage of cells expressing this cytokine in asthmatic subjects was less compared with patients with nonallergic airway diseases (chronic bronchitis) and nonatopic nonasthmatic control subjects. These findings are consistent with the previous studies demonstrating the relative paucity of IL-12⁺ cells in allergic inflammation.^{2,26}

This finding may also support the preferential deviation of eosinophils from asthmatic individuals toward a T_{H2} -type pattern of cytokine expression. IL-12 is the latest cytokine to be recognized as being associated with eosinophils, and its importance lies in the observations of eosinophil heterogeneity and the skewing of these cells toward T_{H1} -type (IFN- γ and IL-2) and T_{H2} -type (IL-4, IL-5, and IL-13) cytokine production.^{4,5} Extending these findings further, our colocalization studies defined the IL-12⁺ eosinophil population for the expression of other types of cytokines, particularly IL-5. The difference in the expression of eosinophil-associated IL-12 and IL-5 in asthmatic and control subjects suggests that the production of such cytokines by eosinophils is exclusive to the patient groups studied. Our findings of higher expression of IL-12 in eosinophils from patients with chronic bronchitis fur-

ther demonstrate that decreased IL-12 expression in eosinophils is specific for asthma.

These results demonstrate that eosinophils may perpetuate the immune reaction within the airways of asthmatic subjects and amplify the local ongoing allergic inflammation. However, the significance of a reduced IL-12 expression at the cellular level in eosinophils and the contribution made by eosinophils to the total expression of IL-12 within the airways still remains to be determined. Moreover, this differential regulation of eosinophil IL-12 expression in allergic inflammation may be due to stimuli in the local milieu, particularly increased levels of cytokines, such as IL-4 and IL-13, which have been shown to inhibit IL-12 expression.²⁷

In summary, the data presented here support the hypothesis that peripheral blood-derived, as well as BAL-derived, eosinophils contribute to the cytokine milieu through the production of IL-12. Eosinophils from asthmatic individuals are less likely to express IL-12 than cells from patients with chronic bronchitis and nonatopic control subjects. Studies to address the regulatory mechanisms of cytokine production by eosinophils and to characterize the physiologic significance of cytokines produced by eosinophils will be necessary to understand the role of eosinophil-derived cytokines in various human diseases.

We thank Ms Elsa Schotman for her excellent technical assistance.

REFERENCES

- Robinson DS, Hamid Q, Ying S, et al. Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma. *N Engl J Med* 1992;326:298-304.
- Naseer T, Minshall EM, Leung DY, et al. Expression of IL-12 and IL-13 mRNA in asthma and their modulation in response to steroid therapy. *Am J Respir Crit Care Med* 1997;155:845-51.
- Lacy P, Moqbel R. Eokines: synthesis, storage and release from human eosinophils. *Mem Inst Oswaldo Cruz* 1997;92(Suppl 2):125-33.
- Lamkhioed B, Gounni AS, Aldebert D, et al. Synthesis of type 1 (IFN gamma) and type 2 (IL-4, IL-5, and IL-10) cytokines by human eosinophils. *Ann NY Acad Sci* 1996;796:203-8.
- Woerly G, Roger N, Loiseau S, Capron M. Expression of Th1 and Th2 immunoregulatory cytokines by human eosinophils. *Int Arch Allergy Immunol* 1999;118:95-7.
- Wolf SF, Temple PA, Kobayashi M, et al. Cloning of cDNA for natural killer cell stimulatory factor, a heterodimeric cytokine with multiple biologic effects on T and natural killer cells. *J Immunol* 1991;146:3074-81.
- Kiniwa M, Gately M, Gubler U, Chizzonite R, Fargeas C, Delespesse G. Recombinant interleukin-12 suppresses the synthesis of immunoglobulin E by interleukin-4 stimulated human lymphocytes. *J Clin Invest* 1992;90:262-6.
- Manetti R, Parronchi P, Giudizi MG, et al. Natural killer cell stimulatory factor (interleukin 12 [IL-12]) induces T helper type 1 (Th1)-specific immune responses and inhibits the development of IL-4-producing Th cells. *J Exp Med* 1993;177:1199-204.
- Gavett SH, O'Hearn DJ, Li X, Huang SK, Finkelman FD, Wills-Karp M. Interleukin 12 inhibits antigen-induced airway hyperresponsiveness, inflammation, and Th2 cytokine expression in mice. *J Exp Med* 1995;182:1527-36.
- Hofstra CL, Van Ark I, Hofman G, Kool M, Nijkamp FP, Van Oosterhout AJ. Prevention of Th2-like cell responses by coadministration of IL-12 and IL-18 is associated with inhibition of antigen-induced airway hyperresponsiveness, eosinophilia, and serum IgE levels. *J Immunol* 1998;161:5054-60.
- Iwamoto I, Kumano K, Kasai M, Kurasawa K, Nakao A. Interleukin-12 prevents antigen-induced eosinophil recruitment into mouse airways. *Am J Respir Crit Care Med* 1996;154:1257-60.
- Schwarze J, Hamelmann E, Cieslewicz G, et al. Local treatment with IL-12 is an effective inhibitor of airway hyperresponsiveness and lung eosinophilia after airway challenge in sensitized mice. *J Allergy Clin Immunol* 1998;102:86-93.
- Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. *Am Rev Respir Dis* 1987;136:225-44.
- Azzawi M, Bradley B, Jeffery PK, et al. Identification of activated T lymphocytes and eosinophils in bronchial biopsies in stable atopic asthma. *Am Rev Respir Dis* 1990;142:1407-13.
- Hamid Q, Wharton J, Terenghi G, et al. Localization of atrial natriuretic peptide mRNA and immunoreactivity in the rat heart and human atrial appendage. *Proc Natl Acad Sci USA* 1987;84:6760-4.
- Moqbel R, Ying S, Barkans J, et al. Identification of messenger RNA for IL-4 in human eosinophils with granule localization and release of the translated product. *J Immunol* 1995;155:4939-47.
- Grewe M, Czech W, Morita A, et al. Human eosinophils produce biologically active IL-12: implications for control of T cell responses. *J Immunol* 1998;161:415-20.
- Hamid Q, Barkans J, Meng Q, et al. Human eosinophils synthesize and secrete interleukin-6, in vitro. *Blood* 1992;80:1496-501.
- Nakajima H, Gleich GJ, Kita H. Constitutive production of IL-4 and IL-10 and stimulated production of IL-8 by normal peripheral blood eosinophils. *J Immunol* 1996;156:4859-66.
- Moqbel R, Hamid Q, Ying S, et al. Expression of mRNA and immunoreactivity for the granulocyte/macrophage colony-stimulating factor in activated human eosinophils. *J Exp Med* 1991;174:749-52.
- Levi-Schaffer F, Barkans J, Newman TM, et al. Identification of interleukin-2 in human peripheral blood eosinophils. *Immunology* 1996;87:155-61.
- Moller GM, de Jong TA, van der Kwast TH, et al. Immunolocalization of interleukin-4 in eosinophils in the bronchial mucosa of atopic asthmatics. *Am J Respir Cell Mol Biol* 1996;14:439-43.
- Grewe M, Czech W, Morita A, et al. Human eosinophils produce biologically active IL-12: implications for control for T cell responses. *J Immunol* 1998;161:415-20.
- Podlaski FJ, Nanduri VB, Hulmes JD, et al. Molecular characterization of interleukin-12. *Arch Biochem Biophys* 1992;294:230-7.
- D'Andrea A, Rengaraju M, Valiante NM, et al. Production of natural killer cell stimulatory factor (interleukin-12) by peripheral blood mononuclear cells. *J Exp Med* 1992;176:1387-98.
- Van der Pouw Kraan TC, Boeije LC, de Groot ER, et al. Reduced production of IL-12 and IL-12-dependent IFN- γ release in patients with allergic asthma. *J Immunol* 1997;158:5560-5.
- de Waal Malefyt R, Figdor CG, Huijbens R, et al. Effects of IL-13 on phenotype, cytokine production, and cytotoxic function of human monocytes. Comparison with IL-4 and modulation by IFN- γ or IL-10. *J Immunol* 1993;151:6370-81.