

Expression of IL-15 in inflammatory pulmonary diseases

Shigeo Muro, MD, PhD,^a Rame Taha, MD,^a Anne Tsicopoulos, MD, PhD,^b
Ron Olivenstein, MD,^a Andre-Bernard Tonnel, MD,^b Pota Christodouloupoulos, MSc,^a
Benoit Wallaert, MD,^b and Qutayba Hamid, MD, PhD^a *Montreal, Quebec, Canada, and
Lille, France*

Background: IL-15 is a T_H1-related cytokine that shares many biologic activities with IL-2. Both cytokines bind a specific α subunit, and they share the same β and γ common receptor subunits for signal transduction. IL-15 has recently been shown to be upregulated in T cell-mediated inflammatory disorders, such as rheumatoid arthritis and inflammatory bowel diseases. However, the role and expression of IL-15 in inflammatory lung disease has not been investigated.

Objective: In the present study we have evaluated the expression of IL-15 mRNA and protein in bronchial biopsy specimens obtained from patients with sarcoidosis (n = 8), tuberculosis (n = 7), chronic bronchitis (n = 10), and bronchial asthma (n = 8) and compared its expression with that seen in normal control subjects (n = 11).

Methods: In situ hybridization and immunocytochemistry were used to detect the number of cells expressing IL-15 mRNA and protein, respectively, within sections of bronchial tissues from all subject groups. In addition, double immunocytochemistry was used to characterize the cellular source of IL-15.

Results: The number of IL-15⁺ cells was significantly higher within tissue from patients with sarcoidosis, tuberculosis, and chronic bronchitis compared with that in asthmatic patients and normal control subjects. Similar results were obtained for IL-15 immunoreactivity by using immunohistochemistry. Furthermore, double immunostaining revealed that neutrophils and macrophages are the major source of IL-15.

Conclusion: These results suggest that the expression of IL-15 may be associated with T_H1-mediated chronic inflammatory diseases of the lung. (*J Allergy Clin Immunol* 2001;108:970-5.)

Key words: IL-15, IL-2, sarcoidosis, tuberculosis, chronic bronchitis, neutrophils, natural killer cells

Cytokines play a pivotal role in the recruitment, proliferation, and survival of inflammatory cells in lung disorders. IL-15 is a novel multipotent cytokine with similar biologic activity to IL-2, the receptor of which is also composed of 3 subunits: α , β , and γ . The β and γ subunits of the IL-15 receptor are shared with IL-2. IL-15 has been shown to be produced by macrophages and struc-

Abbreviations used

ATS: American Thoracic Society
BM: Basement membrane
ISH: In situ hybridization
MBP: Major basic protein

tural cells, such as intestinal epithelial cells, in response to environmental stimuli and infectious agents, but not by T lymphocytes, which are the major source of IL-2.

The coexistence of IL-2 and IL-4 induces the differentiation of naive T cells into a T_H2-type phenotype,^{1,2} whereas IL-12 promotes their differentiation toward a T_H1 phenotype.^{3,4} Seder⁵ has demonstrated that even in the presence of IL-4, IL-15 could not substitute for IL-2 in the differentiation of CD4⁺ T cells into a T_H2-type phenotype, whereas the coexistence of IL-15 and IL-12 increased the IFN- γ production from T cells. IL-15 has also been shown to stimulate the proliferation of T lymphocytes and to induce the activation of natural killer cells and the production of IFN- γ from natural killer cells. These findings suggest that IL-15 has the potential to skew the immunologic response in favor of a T_H1, rather than a T_H2, response. In contrast, the findings of Mori et al⁶ that IL-15 promotes the production of IL-5 in human T_H cells and the fact that IL-15 has been shown to be a growth factor of mast cells⁷ suggests a role of this cytokine in T_H2 immune responses. IL-15 has also been shown to induce the production of IL-8 in human neutrophils,⁸ which is an important chemoattractant of neutrophils, as well as eosinophils. All these observations suggest a potential role for this cytokine in a variety of pathologic conditions, including T_H1- and T_H2-mediated inflammatory diseases.

We have previously reported a high expression of T_H1-type cytokines, such as IFN- γ and IL-12, in active sarcoidosis⁹ and tuberculosis,¹⁰ in contrast to bronchial asthma, which is predominantly mediated by T_H2 cytokines. In this study we examined the expression of IL-15 in patients with chronic inflammatory lung diseases, such as asthma, sarcoidosis, tuberculosis, and chronic bronchitis, as well as in normal control subjects. Because the expression of IL-15 is regulated at multiple steps, including transcriptional control and translational control,¹¹ we examined both the mRNA expression and immunoreactivity by using in situ hybridization (ISH) and immunohistochemistry, respectively.

From ^aMeakins-Christie Laboratories and Montreal Chest Research Institute, McGill University, Montreal; and ^bPathologie Immuno-allergique Respiratoire et Cellules Inflammatoires, Institut Pasteur de Lille.

Supported by the Medical Research Council Canada.

Received for publication February 7, 2001; revised August 10, 2001; accepted for publication August 13, 2001.

Reprint requests: Qutayba Hamid, MD, PhD, Meakins-Christie Laboratories, 3626 St Urbain, Montreal, Quebec, Canada, H2X 2P2.

Copyright © 2001 by Mosby, Inc.

0091-6749/2001 \$35.00 + 0 1/83/119556

doi:10.1067/mai.2001.119556

METHODS

Subjects

Patients with active pulmonary tuberculosis ($n = 7$), active pulmonary sarcoidosis ($n = 8$), chronic bronchitis ($n = 10$), and atopic asthma ($n = 8$) and normal control subjects ($n = 11$) were entered into the study. Ethical approval for this study was obtained from the Montreal Chest Institute, Quebec, Canada, and Hôpital Calmette, Lille, France. Written informed consent was obtained from all the subjects enrolled in this study.

Patients with abnormal chest radiographs suggesting a diagnosis of pulmonary tuberculosis were recruited from the Montreal Chest Institute after referral for bronchoscopy. All chest radiographs were reviewed by a respiratory burst respiratory physician who deemed that the probability of pulmonary tuberculosis was likely enough to warrant further investigation. Subjects who were already receiving antituberculosis chemotherapy, HIV-positive subjects, and pregnant women were excluded from this study. Subjects were nonatopic and had positive skin test results for purified protein derivative (>10 mm). Active pulmonary tuberculosis was defined as microbiologically confirmed tuberculosis on the basis of positive cultures for *Mycobacterium tuberculosis* (sputum or bronchial washing fluid). Patients were followed for 6 months, and all microbiologic and clinical data were used to establish a final diagnosis of active tuberculosis.

Patients with newly diagnosed active pulmonary sarcoidosis were recruited from Hôpital Calmette, Lille, France. The diagnosis of pulmonary sarcoidosis was based on previously described criteria,¹² including a lung or mediastinal lymph node biopsy specimen exhibiting histologic evidence of noncaseating epithelial-cell granuloma. The disease was classified as active according to conventional clinical and radiographic criteria.¹² The criteria used to determine disease activity were as follows: (1) recently developed or increasing cough or dyspnea; and/or (2) systemic symptoms, such as cutaneous lesions, weakness, fever, or arthralgia; and/or (3) increasing opacities on chest radiography. As determined with chest roentgenographic staging, 4 patients had stage I and 4 had stage II disease. None of the patients were smokers. None had any evidence of atopy, as defined by the absence of clinical history and negative skin prick test results to common aeroallergens. None had taken corticosteroids during the previous year.

Subjects fulfilling the American Thoracic Society (ATS) criteria for asthma¹³ and having demonstrated documented airway reversibility to inhaled β_2 -agonists and increased airway responsiveness to methacholine ($PC_{20} < 8$ mg/mL) were recruited from the Montreal Chest Hospital. Asthma symptoms were controlled only by on-demand use of inhaled selective β_2 -agonists. Subjects had taken inhaled corticosteroids during the preceding 12 weeks. None were smokers or had any other serious lung disease.

Chronic bronchitis was also defined according to the criteria of the ATS.¹³ Briefly, subjects were given a diagnosis of chronic bronchitis if they had a history of cough and sputum production on most days of the month for at least 3 months a year during the previous 2 years. In addition, they had a documented lack of β_2 -induced airflow reversibility recorded as a less than 10% change of FEV_1 . All subjects had a history of cigarette smoking, had no history of asthma, were nonatopic, and were not using oral or inhaled steroids. Subjects with lung diseases other than chronic obstructive lung disease or systemic diseases affecting the lung were excluded from the study. Eleven subjects, recruited through the Asthma Clinic at the Montreal Chest Hospital, volunteered to participate in the study as control subjects. None were smokers, and none had any evidence of atopy or had taken corticosteroids during the year preceding the study.

Study design

Fiberoptic bronchoscopy was performed according to ATS guidelines.¹³ Three to 5 biopsy specimens per patient were taken

from the segmental divisions of the right middle- and right lower-lobe bronchi with an alligator forceps. For immunocytochemistry, endobronchial biopsy specimens were immediately snap-frozen in isopentane cooled in liquid nitrogen. For ISH, biopsy specimens were fixed immediately in a 4% paraformaldehyde-PBS solution for 2 hours, washed in 15% PBS-sucrose 3 times, embedded in ornithine carbamyltransferase compound (Tissue-Tek, Miles Inc), and then snap-frozen in isopentane cooled in liquid nitrogen. Endobronchial biopsy specimens for immunocytochemistry were cut with a cryostat in 5- μ m sections and fixed in acetone-methanol and stored at -80°C . Cryostat sections 10- μ m thick for ISH were cut on 0.1% poly-L-lysine-coated slides, baked in an oven at 37°C , and stored at -80°C until further use.

Probe preparation

Radiolabeled cRNA probes coding for IL-15 were prepared from cDNA (gift from Dr Michael Widmer, Immunex Corp), as previously described.¹⁴ In brief, cDNA was inserted into vectors, linearized, and transcribed in vitro in the presence of ^{35}S -labeled uridine triphosphate and either T3 or T7 polymerases. Antisense (complementary to mRNA) and sense probes (identical to mRNA) were prepared. The percentage of the incorporation of the radioisotopes was approximately 40% to 50%.

In situ hybridization

ISH was performed as previously described.¹⁵ Briefly, cryostat sections from biopsy specimens were permeabilized with Triton X-100 and proteinase K solution (1 $\mu\text{g/mL}$) in 0.1 TRIS containing 50 mmol EDTA for 20 minutes at 37°C . The samples were subsequently incubated with 0.1 mol/L triethanolamine and 0.5% acetic anhydride for 20 minutes at 37°C to prevent the nonspecific binding of the ^{35}S -labeled cRNA probes. Prehybridization of the samples was carried out in 50% formamide in 2 $\times$ standard saline citrate for 15 minutes at 37°C . Hybridization was carried out with the use of a hybridization mixture containing the appropriate sense or antisense probes (0.75×10^6 counts/min per slide). Posthybridization involved high-stringency washing of the samples in decreasing concentrations of standard saline citrate at 42°C . To remove any unbound RNA probes, the samples were washed with ribonuclease solution for 20 minutes at 42°C . The samples were then dehydrated with increasing concentrations of ethanol and air-dried. After this, the samples were dipped in Amersham LM-2 emulsion and then exposed for a period of 14 days. The autoradiographs were then developed in Kodak D-19 developer, fixed, and counterstained with hematoxylin. The samples were then mounted with a coverslip and examined under a graduated microscope for positive signals.

Immunohistochemistry and double immunohistochemistry

Immunostaining was performed with the use of the alkaline phosphatase-antialkaline phosphatase technique to detect IL-15 immunoreactivity, as previously described.¹⁶ Briefly, sections were incubated with the primary antibody, rabbit anti-human IL-15 polyclonal antibody (Biosource), at 4°C overnight. Sections were then incubated with the biotinylated swine anti-rabbit immunoglobulin as a secondary antibody, followed by the streptavidin-alkaline phosphatase conjugate. The reaction was visualized with Fast Red. For negative control preparations, the primary antibody was replaced by either nonspecific immunoglobulin or TRIS-buffered saline solution.

Cryostat sections of bronchial biopsy specimens were studied by using the technique of double immunostaining to examine the phenotype of IL-15-immunoreactive cells. Briefly, endogenous peroxidase was blocked with 1% H_2O_2 in TRIS-buffered saline for 30 minutes. A mixture of primary antibodies was applied to the sec-

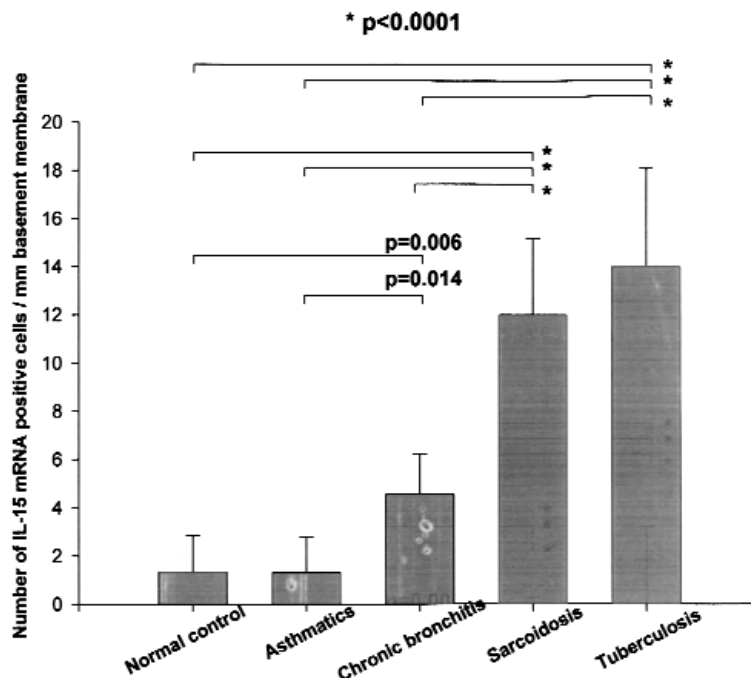


FIG 1. IL-15 mRNA expression in normal control subjects and in subjects with asthma, chronic bronchitis, sarcoidosis, and tuberculosis. The levels of IL-15 mRNA expression were significantly higher in subjects with sarcoidosis, tuberculosis, and chronic bronchitis compared with those in normal control subjects. The number of positive cells in the bronchial biopsy specimens from patients with sarcoidosis and tuberculosis were significantly higher compared with those from patients with chronic bronchitis. Results are expressed as the number of positive cells per millimeter of BM.

tions consisting of rabbit anti-human IL-15 polyclonal antibody (Biosource) and mAb to determine cellular phenotype: anti-CD4 (Dako); anti-eosinophil major basic protein (MBP; a generous gift from Dr Moqbel); anti-CD68 (Dako); and anti-neutrophil elastase (Dako). After incubating with the appropriate secondary antibodies, a tertiary layer of peroxidase and alkaline-phosphatase conjugate was applied. Sections were developed sequentially in Fast Red (the alkaline-phosphatase substrate) and diaminobenzidine (a peroxidase substrate). IL-15-immunoreactive cells were stained brown, and cells immunoreactive for CD4, CD68, MBP, or elastase were stained red. Double-immunoreactive cells were stained reddish-brown. In all immunocytochemical studies, the negative controls included omission of the primary antibody or the use of irrelevant isotype- and species-matched primary antibodies.

Quantification and data analysis

Slides were coded and counted blindly by using 200 $\times$ magnification with an eyepiece graticule. Cells expressing IL-15 mRNA or IL-15-immunoreactive cells were counted to a depth of 0.45 mm below the basement membrane (BM), and the results were expressed as the mean number of positive cells per millimeter of BM.

The number of cells that were positive for both IL-15 and cell markers (CD4, CD68, MBP, and elastase) were counted together with the number of cells individually staining for IL-15. Results are presented as the percentage of IL-15-immunoreactive cells that coexpressed immunoreactivity for CD4, CD68, MBP, or elastase.

Statistical analysis

The within-observer coefficient of variation for repeat measures was less than 5%. The numbers of cells expressing IL-15 mRNA or IL-15-immunoreactive cells in patients with asthma, chronic bronchitis, tuberculosis, and sarcoidosis and in healthy control subjects

were compared with the use of a 1-way ANOVA, followed by a post hoc Fisher LSD test for multiple comparisons. Significance was accepted at the 5% level of confidence. Statistical analyses were performed with use of a standard computer package (Systat version 8.0).

RESULTS

Hybridization between the labeled cRNA probes and mRNA encoding IL-15 was demonstrated by means of specific deposit of silver grains in the photographic emulsion overlaying the tissue sections. We detected the expression of IL-15 mRNA in all groups of patients with bronchial asthma, chronic bronchitis, sarcoidosis, and tuberculosis and in the normal control subjects. The levels of IL-15 expression were significantly higher in patients with sarcoidosis (mean mRNA⁺ cells/mm of BM $\pm$ SD, 12.0 ± 3.16 , $P < .0001$), tuberculosis (14.0 ± 4.08 , $P < .0001$), and chronic bronchitis (4.56 ± 1.67 , $P = .006$) compared with those in the normal control subjects (1.33 ± 1.5 ; Figs 1 and 2, A). Furthermore, there were significantly more IL-15 mRNA-expressing cells in the bronchial biopsy specimens from subjects with sarcoidosis ($P < .0001$) and tuberculosis ($P < .0001$) compared with those from patients with chronic bronchitis. However, the level of IL-15 expression in asthmatic subjects (1.3 ± 1.49) was not significantly different from that in control subjects.

The number of IL-15-immunoreactive cells was also greater in the airways of patients with tuberculosis (mean IL-15 immunoreactive cells/mm of BM $\pm$ SD, $9.04 \pm$

3.95, $P < .0001$), sarcoidosis (7.12 ± 2.36 , $P < .0001$), and chronic bronchitis (2.83 ± 2.42 , $P = .018$) compared with that from normal control subjects (0.27 ± 0.47 ; Figs 2, B, and 3). There were higher numbers of IL-15-immunoreactive cells in patients with sarcoidosis ($P < .0001$) and tuberculosis ($P < .0001$) than in patients with chronic bronchitis. There was no increase in the number of IL-15-immunoreactive cells within bronchial tissue from asthmatic subjects compared with that in normal control subjects.

When the IL-15-immunoreactive cells were phenotyped, the majority of IL-15-immunoreactive cells were CD68⁺ macrophages (36.6%) and elastase-positive neutrophils (47.9%) in patients with tuberculosis. The numbers were similar in patients with sarcoidosis (CD68⁺ cells, 54.5%; elastase-positive neutrophils, 43.6%). In patients with chronic bronchitis, 40.4% of the IL-15-immunoreactive cells were CD68⁺, and 57.6% were neutrophil-elastase positive. The percentage of CD4⁺ T cells or MBP⁺ eosinophils that coexpress IL-15 immunoreactivity was less than 2% in all diseases.

DISCUSSION

In this study we evaluated the *in vivo* expression of IL-15 in human bronchial tissue obtained from patients with sarcoidosis, tuberculosis, bronchial asthma, and chronic bronchitis, as well as that from normal subjects. Using ISH and immunohistochemistry, our results provide direct evidence that IL-15 is expressed both at the mRNA and protein level in human airways. IL-15 is highly expressed, particularly in patients with sarcoidosis and tuberculosis and, to a lesser extent, in patients with chronic bronchitis compared with in patients with bronchial asthma and normal subjects. The results of this study may suggest that IL-15 is associated primarily with T_H1-mediated chronic inflammatory diseases of the lung.

The findings in this study support previous work showing that alveolar macrophages isolated from bronchoalveolar lavage of patients with active sarcoidosis^{17,18} and tuberculosis¹⁸ produce increased levels of IL-15 compared with those of normal subjects. Our demonstration of increased IL-15 expression in bronchial biopsy specimens of patients with sarcoidosis and tuberculosis is consistent with these previous findings.

Bronchoalveolar lavage cells of individuals with granulomatous sarcoid lesions show a marked increase in the numbers of CD4⁺ lymphocytes,^{12,19} which have been shown to proliferate in response to IL-15.¹⁷ As such, IL-15 may contribute to the development of T-cell alveolitis in this disease.

Recently, Liu et al²⁰ reported that IL-15 is highly expressed in the intestinal mucosa of patients with inflammatory bowel diseases. They also showed that IL-15 enhances local T-cell activation and proliferation and the production of proinflammatory cytokines, such as IFN- γ and TNF, by T cells and IL-12 and TNF by monocytes in patients with inflammatory bowel disease. These responses, however, were not observed in cells from

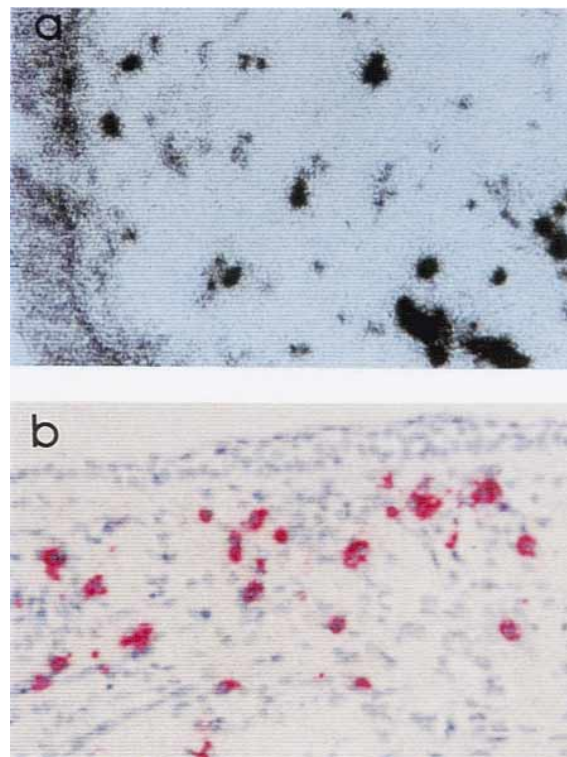


FIG 2. a, Representative example of IL-15 mRNA⁺ cells in a bronchial biopsy specimen from a subject with tuberculosis, as demonstrated by the dense collection of silver grains. b, Representative example of IL-15-immunoreactive cells in a bronchial biopsy specimen from a subject with sarcoidosis, as indicated by the red staining.

healthy control subjects. Moreover, the expression of IFN- γ and IL-12 was upregulated in patients with tuberculosis¹⁰ and sarcoidosis.⁹ It is thus logical to suggest that IL-15 may play a role in the pathogenesis of chronic pulmonary inflammation, such as tuberculosis and sarcoidosis, through its ability to induce proinflammatory cytokines, such as IL-12 and IFN- γ , and its involvement in the proliferation of inflammatory cells.

In sputum of patients with chronic bronchitis, the concentration of IL-8,²¹ an important chemoattractant of neutrophils, and the number of IL-8-immunoreactive cells,²² were shown to be increased and to correlate with myeloperoxidase and elastase activity.²³ Because IL-15 has been shown to induce the expression of IL-8 in T cells²⁴ and in monocytes,²⁵ its upregulation might contribute to airway inflammation, in which the presence of neutrophils is very common through the induction of IL-8. IL-15 has also been shown to induce the expression of monocyte chemotactic protein 1 and macrophage inflammatory protein 1 β ,^{24,25} both of which are chemokines highly expressed in the bronchoalveolar lavage fluid of patients with chronic bronchitis.²⁶ Taken together, these findings suggest that IL-15 may play an important role in the inflammatory process in chronic bronchitis by means of chemokine induction.

In our study we demonstrated that the number of IL-15⁺ cells in bronchial biopsy specimens from asthmatic

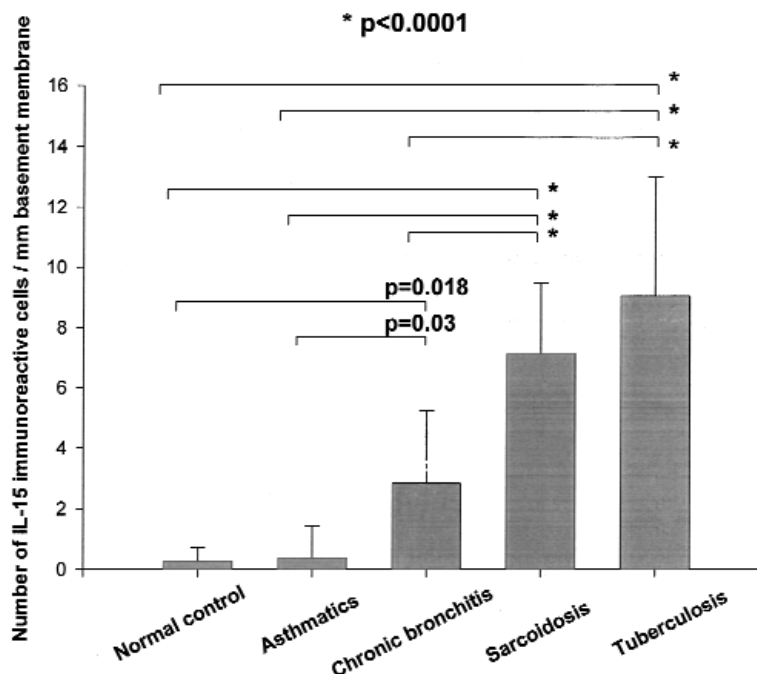


FIG 3. Number of IL-15-immunoreactive cells in normal control subjects and in subjects with asthma, chronic bronchitis, sarcoidosis, and tuberculosis. Results are expressed as the number of positive cells per millimeter of BM.

subjects is not different from that of normal subjects, suggesting that the role of IL-15 in T_H2 immune responses may be limited. On the contrary, it has previously been reported that IL-15 can induce the production of IL-5 from T_H2 -type cells⁶ and the proliferation of mast cells.⁷

Neutrophils, which have been shown to express the IL-15R α subunit,²⁷ respond to IL-15, which induces their morphologic cell shape typical of activated neutrophils, increases their ability to phagocytose opsonized sheep red blood cells, and affects their gene and protein expression.²⁸ To date, this is the first report showing IL-15 immunoreactivity in neutrophils, an important cell type in the defense of bacterial infection. Our findings suggest that IL-15 may act in an autocrine fashion to enhance the neutrophil response at the inflammatory site. The abundant release of IL-15 from the alveolar macrophages in patients with pneumonia, as reported by Zissel et al,¹⁸ and our finding that this cytokine is upregulated in chronic bronchitis both suggest a protective role for this cytokine in bacterial infections.

In our study we demonstrated that neutrophils are a considerable source of IL-15, suggesting a role of this cell type in chronic inflammation. As in chronic bronchitis, IL-8, a potent chemoattractant for neutrophils, has also been shown to be upregulated in patients with sarcoidosis²⁹ and tuberculosis.³⁰ It is possible that once the neutrophil is attracted to the inflammatory site, it subsequently produces IL-15, inducing T cells²⁴ and monocytes²⁵ to produce IL-8, thus enhancing or lengthening the time of airway inflammation.

In our study we were able to detect the upregulation of IL-15 in bronchial biopsy specimens from patients with sarcoidosis and tuberculosis, whose main inflammatory lesion is not located in the airways and instead in the parenchyma. The bronchial biopsy specimens from patients with sarcoidosis and tuberculosis did not have any apparent granulomatous or infectious lesions. Furthermore, the expression of IL-15 in the airways of patients with these diseases was significantly greater than that in patients with chronic bronchitis, whose prominent feature is airway inflammation. This suggests that the cytokine milieu of the tracheobronchial tree is similar to the parenchyma in these diseases.

In summary, our results show an increased expression of IL-15 in T_H1 -mediated pulmonary diseases at both the mRNA and protein levels and demonstrate that monocytes-macrophages and neutrophils are the major cell types that produce IL-15. The current findings support the idea that IL-15 might be an important mediator for the development of T_H1 -mediated diseases. Further research is needed, however, to determine the role of IL-15 in the pathophysiology of these diseases. Moreover, future studies are required to determine the relationship between the expression of IL-15 and the activity and progression of T_H1 -mediated diseases. These studies could provide a rational basis to determine whether IL-15 could be a good candidate for anticytokine therapy in these chronic inflammatory lung diseases.

We thank E. Schotman for her technical assistance.

REFERENCES

1. Le Gros G, Ben-Sasson SZ, Seder R, Finkelman FD, Paul WE. Generation of interleukin 4 (IL-4)-producing cells in vivo and in vitro: IL-2 and IL-4 are required for in vitro generation of IL-4-producing cells. *J Exp Med* 1990;172:921-9.
2. Ben-Sasson SZ, Le Gros G, Conrad DH, Finkelman FD, Paul WE. IL-4 production by T cells from naive donors. IL-2 is required for IL-4 production. *J Immunol* 1990;145:1127-36.
3. Hsieh CS, Macatonia SE, Tripp CS, Wolf SF, O'Garra A, Murphy KM. Development of TH1 CD4+ T cells through IL-12 produced by Listeria-induced macrophages. *Science* 1993;260:547-9.
4. Seder RA, Gazzinelli R, Sher A, Paul WE. Interleukin 12 acts directly on CD4+ T cells to enhance priming for interferon gamma production and diminishes interleukin 4 inhibition of such priming. *Proc Natl Acad Sci U S A* 1993;90:10188-92.
5. Seder RA. High-dose IL-2 and IL-15 enhance the in vitro priming of naive CD4+ T cells for IFN-gamma but have differential effects on priming for IL-4. *J Immunol* 1996;156:2413-22.
6. Mori A, Suko M, Kaminuma O, Inoue S, Ohmura T, Nishizaki Y, et al. IL-15 promotes cytokine production of human T helper cells. *J Immunol* 1996;156:2400-5.
7. Tagaya Y, Burton JD, Miyamoto Y, Waldmann TA. Identification of a novel receptor/signal transduction pathway for IL-15/T in mast cells. *EMBO J* 1996;15:4928-39.
8. McDonald PP, Russo MP, Ferrini S, Cassatella MA. Interleukin-15 (IL-15) induces NF-kappaB activation and IL-8 production in human neutrophils. *Blood* 1998;92:4828-35.
9. Minshall EM, Tsicopoulos A, Yasruei Z, Wallaert B, Akoum H, Vorng H, et al. Cytokine mRNA gene expression in active and nonactive pulmonary sarcoidosis. *Eur Respir J* 1997;10:2034-9.
10. Taha RA, Kotsimbos TC, Song YL, Menzies D, Hamid Q. IFN-gamma and IL-12 are increased in active compared with inactive tuberculosis. *Am J Respir Crit Care Med* 1997;155:1135-9.
11. Waldmann TA, Tagaya Y. The multifaceted regulation of interleukin-15 expression and the role of this cytokine in NK cell differentiation and host response to intracellular pathogens. *Annu Rev Immunol* 1999;17:19-49.
12. NIH conference. Pulmonary sarcoidosis: a disease characterized and perpetuated by activated lung T-lymphocytes. *Ann Intern Med* 1981;94:73-94.
13. Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, November 1986. *Am Rev Respir Dis* 1987;136:225-44.
14. Hamid Q, Wharton J, Terenghi G, Hassall CJ, Aimi J, Taylor KM, et al. Localization of atrial natriuretic peptide mRNA and immunoreactivity in the rat heart and human atrial appendage. *Proc Natl Acad Sci U S A* 1987;84:6760-4.
15. Hamid Q, Azzawi M, Ying S, Moqbel R, Wardlaw AJ, Corrigan CJ, et al. Expression of mRNA for interleukin-5 in mucosa bronchial biopsies from asthma. *J Clin Invest* 1991;87:1541-6.
16. Hamid Q, Springall DR, Riveros-Moreno V, Chanez P, Howarth P, Redington A, et al. Induction of nitric oxide synthase in asthma. *Lancet* 1993;342:1510-3.
17. Agostini C, Trentin L, Faccio M, Sancetta R, Cerutti A, Tassinari C, et al. Role of IL-15, IL-2, and their receptors in the development of T cell alveolitis in pulmonary sarcoidosis. *J Immunol* 1996;157:910-8.
18. Zissel G, Baumer I, Schlaak M, Muller-Quernheim J. In vitro release of interleukin-15 by broncho-alveolar lavage cells and peripheral blood mononuclear cells from patients with different lung diseases. *Eur Cytokine Netw* 2000;11:105-12.
19. Hunninghake GW, Crystal RG. Pulmonary sarcoidosis: a disorder mediated by excess helper T- lymphocyte activity at sites of disease activity. *N Engl J Med* 1981;305:429-34.
20. Liu Z, Geboes K, Colpaert S, D'Haens GR, Rutgeerts P, Ceuppens JL. IL-15 is highly expressed in inflammatory bowel disease and regulates local T cell-dependent cytokine production. *J Immunol* 2000;164:3608-15.
21. Richman-Eisenstat JB, Jorens PG, Hebert CA, Ueki I, Nadel JA. Interleukin-8: an important chemoattractant in sputum of patients with chronic inflammatory airway diseases. *Am J Physiol* 1993;264:L413-8.
22. Hoshi H, Ohno I, Honma M, Tanno Y, Yamauchi K, Tamura G, et al. IL-5, IL-8 and GM-CSF immunostaining of sputum cells in bronchial asthma and chronic bronchitis. *Clin Exp Allergy* 1995;25:720-8.
23. Hill AT, Bayley D, Stockley RA. The interrelationship of sputum inflammatory markers in patients with chronic bronchitis. *Am J Respir Crit Care Med* 1999;160:893-8.
24. Perera LP, Goldman CK, Waldmann TA. IL-15 induces the expression of chemokines and their receptors in T lymphocytes. *J Immunol* 1999;162:2606-12.
25. Badolato R, Ponzi AN, Millesimo M, Notarangelo LD, Musso T. Interleukin-15 (IL-15) induces IL-8 and monocyte chemotactic protein 1 production in human monocytes. *Blood* 1997;90:2804-9.
26. Capelli A, Di Stefano A, Gnemmi I, Balbo P, Cerutti CG, Balbi B, et al. Increased MCP-1 and MIP-1beta in bronchoalveolar lavage fluid of chronic bronchitis. *Eur Respir J* 1999;14:160-5.
27. Girard D, Boiani N, Beaulieu AD. Human neutrophils express the interleukin-15 receptor alpha chain (IL-15Ralpha) but not the IL-9Ralpha component. *Clin Immunol Immunopathol* 1998;88:232-40.
28. Girard D, Paquet ME, Paquin R, Beaulieu AD. Differential effects of interleukin-15 (IL-15) and IL-2 on human neutrophils: modulation of phagocytosis, cytoskeleton rearrangement, gene expression, and apoptosis by IL-15. *Blood* 1996;88:3176-84.
29. Girgis RE, Basha MA, Maliarik M, Popovich J Jr, Iannuzzi MC. Cytokines in the bronchoalveolar lavage fluid of patients with active pulmonary sarcoidosis. *Am J Respir Crit Care Med* 1995;152:71-5.
30. Zhang Y, Broser M, Cohen H, Bodkin M, Law K, Reibman J, et al. Enhanced interleukin-8 release and gene expression in macrophages after exposure to *Mycobacterium tuberculosis* and its components. *J Clin Invest* 1995;95:586-92.