

Brief Report

EOSINOPHILIA WITH ABERRANT T CELLS AND ELEVATED SERUM LEVELS OF INTERLEUKIN-2 AND INTERLEUKIN-15

MELISSA MEANS-MARKWELL, M.D.,
TIMOTHY BURGESS, M.D., DOMINIC DEKERATRY, M.D.,
KEVIN O'NEIL, M.D., JOHN MASCOLA, M.D.,
THOMAS FLEISHER, M.D., AND DANIEL LUCEY, M.D.

THE hypereosinophilic syndrome comprises various idiopathic myeloproliferative disorders with sustained eosinophilia and damage to the heart, lungs, skin, and other organs by infiltrating eosinophils.¹ A single mechanism for this syndrome has not been identified, and multiple factors are likely to be involved. The serum level of interleukin-5, an eosinophilopoietic cytokine, is increased in some patients with the hypereosinophilic syndrome,² and a few have been found to have unusual T-cell abnormalities, including CD3⁺CD4⁺CD8⁻ T cells^{3,4} and so-called double-negative CD3⁺CD4⁻CD8⁻ T cells.⁵ CD3, a complex of five proteins associated with the T-cell receptor, is a distinctive feature of all normal T cells. CD4 and CD8 identify helper and cytotoxic T cells, respectively. Recently, Simon et al. described 16 patients with eosinophilia and unusual T cells, some of which secreted abnormal amounts of interleukin-5 when cultured *in vitro*.⁶ The serum levels of interleukin-5 were not reported.

We describe a patient with the hypereosinophilic syndrome who presented with a pericardial effusion, endobronchial lesions, and a population of activated T cells that expressed markers of natural killer cells (CD16 and CD56). Serum levels of interleukin-2 and interleukin-15, which are potent stimulators of the proliferation of T cells and natural killer cells, were elevated. After the initiation of therapy with hydroxyurea, the patient's condition improved markedly and the immunologic abnormalities resolved.

CASE REPORT

A 34-year-old man was referred to the pulmonary clinic at the National Naval Medical Center in April 1997 after nine months

From the Department of Medicine, National Naval Medical Center, Bethesda, Md. (M.M.-M., T.B., D.D., K.O.); the Division of Retrovirology, Walter Reed Army Institute of Research, Rockville, Md. (J.M.); the Warren G. Magnuson Clinical Center, National Institutes of Health, Bethesda, Md. (T.E.); and the Infectious Disease Service, Washington Hospital Center, Washington, D.C. (D.L.). Address reprint requests to Dr. Lucey at the Infectious Disease Service, Rm. 2A-56, Washington Hospital Center, 110 Irving St. NW, Washington, DC 20010.

©2000, Massachusetts Medical Society.

of worsening cough with dyspnea. He had no personal or family history of asthma, atopy, or serious illnesses. He had received antibiotics intermittently and short courses of oral glucocorticoids for presumed postinfectious bronchospasm, but he otherwise took no medications routinely and had never taken tryptophan or any type of antileukotriene therapy. He had no known drug allergies and did not smoke. A complete blood count obtained during a routine examination in 1989 showed an elevated absolute eosinophil count of 770 per cubic millimeter (normal value, <450). This measurement was not repeated until his presentation in 1997.

The physical examination revealed a pulse of 115 beats per minute and diffuse wheezing in both lungs. There were no skin lesions or organomegaly, and the neurologic examination was normal. Initial laboratory studies were remarkable only for the presence of eosinophilia (23 percent eosinophils; absolute eosinophil count, 2074 per cubic millimeter). The serum IgE level was normal. A chest radiograph revealed an enlarged cardiac silhouette but no parenchymal abnormalities. Echocardiography demonstrated a large pericardial effusion.

Pericardiocentesis yielded 600 ml of exudate, with 44 percent eosinophils, 6 percent neutrophils, and 50 percent monocytes. The eosinophils appeared to be morphologically mature. Drainage of the pericardial fluid transiently relieved the orthopnea, but the effusion reaccumulated and a pericardial window was placed for continued drainage. Histologically, the pericardial tissue was characterized by chronic inflammation with an infiltration of eosinophils.

Pulmonary function was not assessed, but flexible fiberoptic bronchoscopy was performed because of persistent asymmetric wheezing. Findings included diffuse erythema and edema of the endobronchial mucosa, with numerous small yellow papules throughout the airways and nearly complete occlusion of the bronchus of the left upper lobe. Biopsies of these lesions revealed an inflammatory infiltrate containing eosinophils, lymphocytes, and macrophages. Nasopharyngoscopy revealed similar yellow papular lesions on the nasal mucosa. Biopsy of pericardial tissue, endobronchial tissue, and nasal turbinates did not reveal any granulomas or vasculitis. Stains and cultures for fungus, acid-fast organisms, and bacteria were negative. Bone marrow biopsies revealed a predominance of eosinophils, without granulomas or evidence of cancer. The results of karyotyping were normal. Computed tomography of the chest and abdomen showed no evidence of adenopathy or masses.

A provisional diagnosis of the hypereosinophilic syndrome was made after the results of an extensive workup for infectious, vasculitic, rheumatologic, and malignant causes were found to be negative. This evaluation included tests for serum antibodies against strongyloides, toxocara, trichinella, coccidioides, human immunodeficiency virus type 1, human T-cell lymphotropic virus type 1, and human herpesvirus 6, as well as tests for antinuclear antibody and antineutrophil cytoplasmic antibodies. Three stool examinations for ova and parasites were also negative.

Given the diagnosis of the hypereosinophilic syndrome, worsening cough, and wheezing that was unresponsive to inhaled glucocorticoids and beta-agonists, the patient was treated with 1 mg of prednisone per kilogram of body weight per day. These symptoms and the eosinophilia persisted, however, and intermittent paresthesias, flushing and weakness of the right forearm, and paresthesias of the chest wall developed. Magnetic resonance imaging of the right arm revealed abnormal enhancement of the brachial plexus, and findings on electromyography were consistent with the presence of brachial plexopathy. After four weeks of glucocorticoid therapy, treatment with hydroxyurea (1 g per day) was initiated and the dose of prednisone was tapered. Within a month after the initiation of hydroxyurea therapy, the pulmonary and neurologic abnormalities decreased. The absolute eosinophil count remained between 250 and 550 per cubic millimeter for eight months while the patient was receiving 1 g of hydroxyurea per day.

RESULTS

Immunologic evaluation of the patient during the period of prednisone tapering and shortly after the

initiation of hydroxyurea therapy revealed several remarkable findings. Flow cytometry (Fig. 1) demonstrated that essentially all CD3+ T cells, including both CD3+CD4+ and CD3+CD8+ subgroups, were activated — that is, they expressed CD25, the α chain of the interleukin-2 receptor that appears on activated T cells. All these T cells also expressed the natural-killer-cell markers CD16 and CD56. We did not detect increased numbers of CD3–CD4+CD8– T cells or CD3+CD4–CD8– T cells (data not shown). These findings were confirmed when flow cytometry was repeated one week later.

These unusual cells, which displayed markers of both T cells (CD3) and natural killer cells (CD16 and CD56, the former being the low-affinity receptor for IgG and the latter an adhesion molecule), led us to measure serum levels of interleukin-2 and interleukin-15, because these cytokines are potent stimulators of T cells and natural killer cells. The serum interleukin-2 level was markedly elevated, at 1160 pg per milliliter, on enzyme-linked immunosorbent assay (ELISA, Dupont, Boston). Normally, serum levels of interleukin-2 are undetectable, even with the use of an assay with a limit of detection of 10 pg per milliliter.

The serum interleukin-15 level was also elevated, at 36 pg per milliliter (ELISA performed in the laboratory of T.A. Waldmann, National Institutes of Health). Normally, serum levels of interleukin-15 are also undetectable. When the same sample was again assayed two months later with a commercial ELISA with a limit of detection of less than 1 pg per milliliter (R&D Systems, Minneapolis), the level of interleukin-15 was 13.8 pg per milliliter.

In contrast, serum levels of the primary eosinophilopoietic cytokines either were within normal ranges (interleukin-3, 21.5 pg per milliliter; normal range, 0 to 30) or were undetectable (interleukin-5 and granulocyte–macrophage colony-stimulating factor) soon after the initiation of hydroxyurea therapy. The serum levels of IgE, IgG, IgA, and IgM were also normal. The unusual T cells in the blood were not detectable when flow cytometry was repeated two, four, and eight months after the initiation of hydroxyurea therapy. The serum levels of interleukin-2 and interleukin-15 also became undetectable after two months of hydroxyurea therapy.

DISCUSSION

In agreement with previous reports, we found that the hypereosinophilic syndrome was associated with abnormal T cells. Cogan et al.³ described a patient with the hypereosinophilic syndrome who had high serum levels of interleukin-5 and a monoclonal population of CD3–CD4+ T cells that produced interleukin-5 in vitro. Several other patients with the hypereosinophilic syndrome have also been reported to have similar populations of CD3–CD4+ T cells.⁴ A

second type of abnormal T-cell population identified in some patients with the hypereosinophilic syndrome is the double-negative CD3+CD4–CD8– T-cell phenotype.⁵ These cells are called double-negative T cells because they lack CD4 and CD8; normally, they are found among immature thymocytes and constitute a small subpopulation of cells in the circulation. The CD3–CD4+ phenotype, by contrast, has no normal counterpart. Recently, Simon et al. described 16 patients with eosinophilia and populations of abnormal circulating T cells.⁶ These populations constituted only a fraction of all the circulating T cells, whereas in our patient virtually all T cells in the blood were activated and had an abnormal phenotype.

In our patient, essentially all circulating T cells expressed the activation marker CD25 and aberrantly expressed two surface proteins, CD16 and CD56, which are typical of natural killer cells and are not found on most normal T cells. Pathologic expansion of large granular lymphocytes can involve either T cells or natural killer cells; however, our patient's blood smear did not show any large granular lymphocytes. Southern blot analysis of the gene for the T-cell receptor β chain, with use of the restriction enzymes *Hind*III and *Eco*RI, in blood obtained while the patient was taking hydroxyurea demonstrated no evidence of a clonal population of T cells (Waldmann TA, National Institutes of Health: personal communication).

Serum levels of the primary eosinophilopoietic cytokines interleukin-3, interleukin-5, and granulocyte–macrophage colony-stimulating factor were normal or were undetectable in our patient when first measured after four weeks of treatment with high doses of prednisone. At the same time, by contrast, we found elevated serum levels of interleukin-2 and interleukin-15. These cytokines were no longer detectable during hydroxyurea therapy, when the abnormal T-cell population had disappeared and the eosinophilia and clinical abnormalities had resolved.

Interleukin-2, a product of activated T cells, is not a direct eosinophilopoietic cytokine. When therapeutic amounts of exogenous interleukin-2 are given to patients with cancer or human immunodeficiency virus type 1 infection, however, eosinophilia is frequent.^{7,8} This phenomenon may be due to the production of interleukin-5 by interleukin-2–activated T cells.⁹ In our patient, who had high serum levels of interleukin-2, we were unable to detect interleukin-5 in the serum, possibly because of the glucocorticoid therapy, because of the insensitivity of the test for detecting circulating interleukin-5, or because interleukin-5 was increased in tissues but not in the circulation. Interleukin-15 has also been reported to induce the production of interleukin-5 by T cells in vitro, independently of interleukin-2. This phenomenon was found only in T-cell clones that were allergen-specific and therefore likely to produce

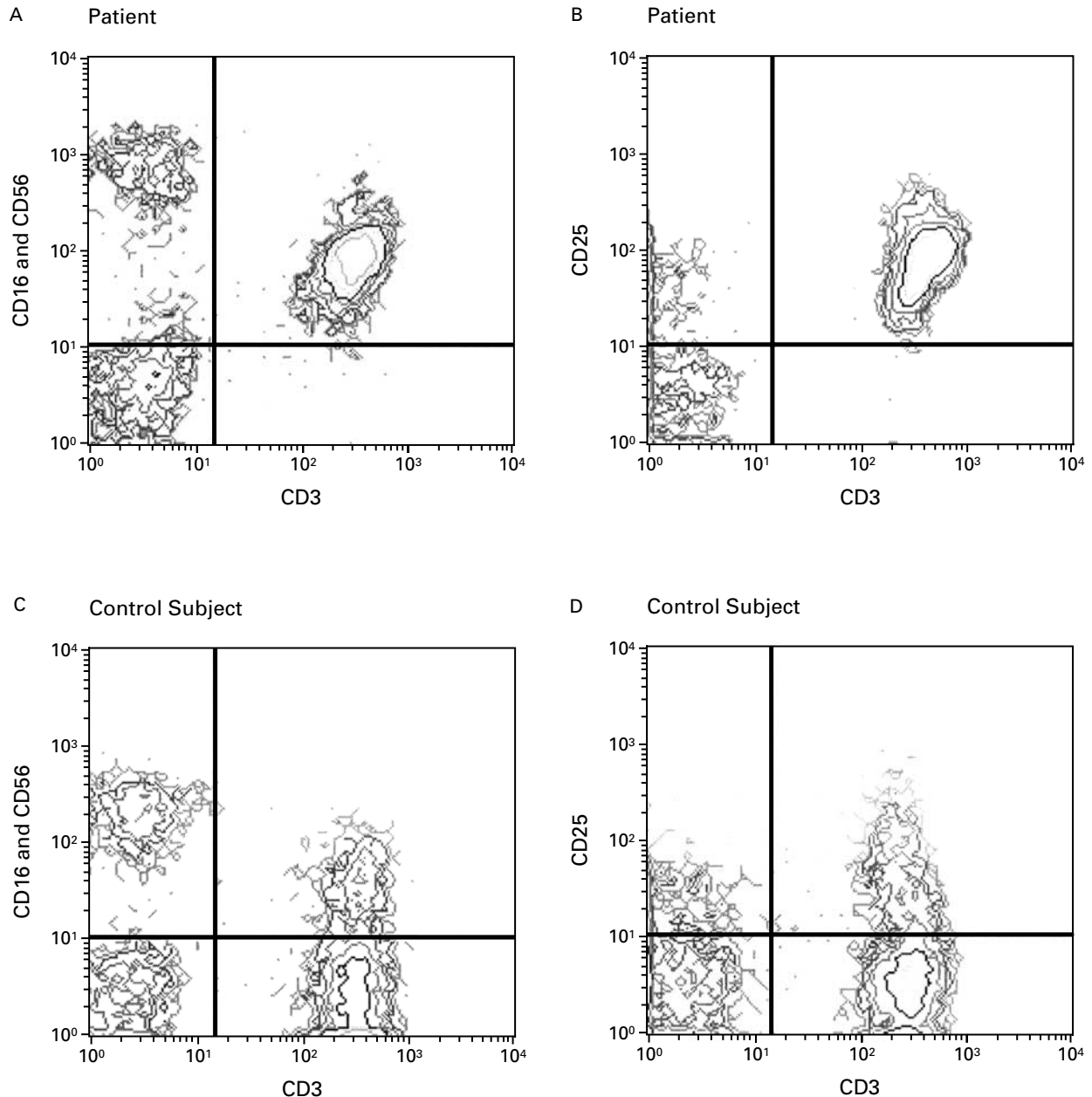


Figure 1. Identification of a Uniform Population of Peripheral-Blood T Cells Expressing Markers of Both Natural Killer Cells and Activation.

Peripheral-blood T cells from the patient (Panels A and B) and a control subject (Panels C and D) were incubated with monoclonal antibodies against CD16, CD56, and CD25 conjugated to phycoerythrin and with monoclonal antibody against CD3 conjugated to fluorescein isothiocyanate. As Panel A shows, nearly all the patient's CD3+ T cells expressed the natural-killer-cell marker CD16, CD56, or both (upper right quadrant). Of his circulating lymphocytes, 83.3 percent were CD3+ T cells and 83.0 percent expressed CD3 as well as CD16, CD56, or both. Panel B demonstrates that nearly all the patient's CD3+ T cells expressed the activation marker CD25 (upper right quadrant). Of his circulating lymphocytes, 83.3 percent were CD3+ T cells and 82.7 percent expressed both CD3 and CD25. Panel C and Panel D show the results of the same analyses in peripheral-blood T cells from a control subject. In our laboratory, the normal ranges for peripheral-blood lymphocytes are as follows: CD3+ T cells, 60.9 to 84.0 percent; CD3+CD16+, CD3+CD56+, or CD3+CD16+CD56+ T cells, 1.3 to 15.3 percent; and CD3+CD25+ T cells, less than 37.5 percent.

cytokines like interleukin-5. The production of interleukin-5 could be blocked by monoclonal antibodies against the interleukin-15 receptor.¹⁰

The receptors for interleukin-15 on T cells share the same β and γ chains but have distinctive α chains. The functions of interleukin-15 and interleukin-2 also overlap. Both cytokines cause the proliferation of T cells and the activation of natural killer cells.^{11,12} We propose that another shared function of interleukin-15 and interleukin-2 is the stimulation of eosinophilia, but only in the presence of T cells that are primed to produce interleukin-5, granulocyte-macrophage colony-stimulating factor, or interleukin-3.

Elevated serum levels of interleukin-15 have been reported in five of eight patients with moderate-to-severe ulcerative colitis, but not in control subjects or in patients with Crohn's disease.¹³ No other disease states with increased levels of circulating interleukin-15 have been reported. However, secretion of interleukin-15 in vitro and the overexpression of interleukin-15 in tissue have been linked to various conditions (e.g., infection with human T-cell lymphotropic virus type I and human herpesvirus 6, rheumatoid arthritis, and large granular lymphocyte leukemia).^{11,14-16} Interleukin-15 appears to be produced primarily by monocytes and macrophages^{11,17} or other antigen-presenting cells, such as dendritic cells. Unlike interleukin-2, interleukin-15 is not produced by T cells.

We hypothesize that our patient had T cells that were primed to produce eosinophilopoietic cytokines and that an antigenic challenge, probably from an infectious agent, triggered the production of interleukin-15 by antigen-presenting cells such as macrophages or dendritic cells. The presentation to the T cells of the foreign antigen by the antigen-presenting cells in the presence of interleukin-15 resulted in the production of interleukin-2 by the T cells. This caused T cells to proliferate and express the CD25 activation marker and the natural-killer-cell markers CD16 and CD56. These activated T cells produced one or more eosinophilopoietic cytokines. The resulting eosinophilia damaged the heart, lungs, and nerves.

This aberrant reactive T-cell population was polyclonal and not monoclonal, which is evidence against the presence of a malignant clone of T cells. The expression of such a seemingly aberrant phenotype by our patient's T cells may not have been recognized previously in patients with eosinophilia because it was not looked for or because it was transient. The novel T-cell phenotype that we found on flow cytometry

led us to look for elevated serum levels of interleukin-15 and interleukin-2, since these are two of the major cytokines that stimulate the proliferation and activation of T cells and natural killer cells. Future study of serum levels of interleukin-15 and interleukin-2 might provide insight into the role of these cytokines in idiopathic eosinophilic diseases and might suggest new therapeutic options, such as the use of monoclonal antibody against the α chain of the interleukin-2 receptor (i.e., monoclonal antibody against CD25) or antibodies against the shared β chain of the interleukin-15 and interleukin-2 receptors.

REFERENCES

1. Weller PF, Bubley GJ. The idiopathic hypereosinophilic syndrome. *Blood* 1994;83:2759-79.
2. Owen WF, Rothenberg ME, Petersen J, et al. Interleukin 5 and phenotypically altered eosinophils in the blood of patients with the idiopathic hypereosinophilic syndrome. *J Exp Med* 1989;170:343-8.
3. Cogan E, Schandené L, Crusiaux A, Cochaux P, Velu T, Goldman M. Clonal proliferation of type 2 helper T cells in a man with the hypereosinophilic syndrome. *N Engl J Med* 1994;330:535-8.
4. Brugnani D, Airo P, Rossi G, et al. A case of hypereosinophilic syndrome is associated with expansion of a CD3-CD4+ T-cell population able to secrete large amounts of interleukin-5. *Blood* 1996;87:1416-22.
5. Simon H-U, Yousefi S, Dommann-Scherrer CC, et al. Expansion of CD4-CD8- T cells associated with abnormal Fas expression and hypereosinophilia. *J Exp Med* 1996;183:1071-82.
6. Simon H-U, Plotz SG, Dummer R, Blaser K. Abnormal clones of T cells producing interleukin-5 in idiopathic eosinophilia. *N Engl J Med* 1999;341:1112-20.
7. Silberstein DS, Schoof DD, Rodrick ML, et al. Activation of eosinophils in cancer patients treated with IL-2 and IL-2-generated lymphokine-activated killer cells. *J Immunol* 1989;142:2162-7.
8. Schwartz DH, Skowron G, Merigan TC. Safety and effects of interleukin-2 plus zidovudine in asymptomatic individuals infected with human immunodeficiency virus. *J Acquir Immune Defic Syndr* 1991;4:11-23.
9. Macdonald D, Gordon AA, Kajitani H, Enokihara H, Barrett AJ. Interleukin-2 treatment-associated eosinophilia is mediated by interleukin-5 production. *Br J Haematol* 1990;76:168-73.
10. Mori A, Suko M, Kaminuma O, et al. IL-15 promotes cytokine production of human T helper cells. *J Immunol* 1996;156:2400-5.
11. Tagaya Y, Bamford RN, DeFilippis AP, Waldman TA. IL-15: a pleiotropic cytokine with diverse receptor/signaling pathways whose expression is controlled at multiple levels. *Immunity* 1996;4:329-36.
12. Lucey DR, Pinto LA, Bethke FR, et al. In vitro immunologic and virologic effects of interleukin 15 on peripheral blood mononuclear cells from normal donors and human immunodeficiency virus type 1-infected patients. *Clin Diagn Lab Immunol* 1997;4:43-8.
13. Kirman I, Nielsen OH. Increased numbers of interleukin-15-expressing cells in active ulcerative colitis. *Am J Gastroenterol* 1996;91:1789-94.
14. Zambello R, Facco M, Trentin L, et al. Interleukin-15 triggers the proliferation and cytotoxicity of granular lymphocytes in patients with lymphoproliferative disease of granular lymphocytes. *Blood* 1997;89:2011-11.
15. Flamand L, Stefanescu I, Menezes J. Human herpesvirus-6 enhances natural killer cell cytotoxicity via IL-15. *J Clin Invest* 1996;97:1373-81.
16. Carson DA. Unconventional T-cell activation by IL-15 in rheumatoid arthritis. *Nat Med* 1997;3:148-9.
17. Carson WE, Ross ME, Baiocchi RA, et al. Endogenous production of interleukin 15 by activated human monocytes is critical for optimal production of interferon-gamma by natural killer cells in vitro. *J Clin Invest* 1995;96:2578-82.