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Eosinophilia Associated With Adult T-Cell Leukemia: Role of Interleukin 5 and Granulocyte-Macrophage Colony-Stimulating Factor

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To clarify the mechanism of eosinophilia in adult T-cell leukemia (ATL), we studied three ATL patients having marked eosinophilia. Eosinophil-predominant colony-stimulating activity was detected in the serum of one patient and in the conditioned media (CM) from cultured ATL cells from two patients. Soluble interleukin 5 (IL-5), but no interleukin 3 (IL-3) and granulocyte-macrophage colony-stimulating factor (GM-CSF), was detected in sera from all patients. On the other hand, GM-CSF was produced in vitro by ATL cells from all cases, whereas detectable IL-3 and IL-5 was produced by cells from only one, suggesting that in the other two cases, the serum IL-5 was produced by the normal reacting lymphocytes. The fact that no patient showed marked neutrophilia supports the possibility that IL-5 may have a leading role in the development of eosinophilia, with GM-CSF produced by ATL cells playing a complementary role. Am. J. Hematol. 59:242–245, 1998. © 1998 Wiley-Liss, Inc.

Key words: adult T-cell leukemia; eosinophilia; interleukin 5; granulocyte-macrophage colony stimulating factor

INTRODUCTION

Adult T-cell leukemia (ATL) is an aggressive T-lymphoproliferative disorder that is endemic to southwestern Japan and the Caribbean basin [1,2]. The human T-cell leukemia virus type 1 (HTLV-1) plays a causative role in its development [3–5].

Eosinophilia is one of the major complications of ATL. It occurs in about 20% of ATL patients [6] and is sometimes severe. Interleukin 3 (IL-3) [7], interleukin 5 (IL-5) [8], and granulocyte-macrophage colony-stimulating factor (GM-CSF) [9] influence eosinophil production in normal subjects [10]. However, the role of these cytokines in the development of eosinophilia in ATL patients has not been investigated so far. A few studies [11,12] suggest that GM-CSF produced by ATL cells might associate with the development of eosinophilia, but that does not seem likely because most ATL patients with eosinophilia do not show marked neutrophilia. Because IL-5 specifically acts on eosinophil production [8,10], it seems to be a more likely candidate.

In this study, we examined the eosinophil-stimulating activity in serum and conditioned medium (CM) from three ATL patients with marked eosinophilia, and we identified the cytokines responsible for the activity, and presumably for the development of eosinophilia.

PATIENTS AND METHODS

Patient 1

In September 1988, a 77-year-old Japanese man was admitted to our hospital with fever and general fatigue. On admission, enlargement of bilateral cervical lymph nodes, pleural effusion, and ascites were noted. The leukocyte count was 40,200/μl, with 9% abnormal lymphocytes, 51% eosinophils, and 26% neutrophils. No parasite infection or allergic disease was found. Antibody for HTLV-1 was positive, and monoclonal integration of proviral DNA in peripheral blood mononuclear cells

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(PBMNC) was demonstrated by Southern blot analysis. Treatment with combination chemotherapy was initially effective and the ATL cell and eosinophil counts decreased; the leukemia became refractory to chemotherapy, however, and the patient died of pneumonia in July 1989.

Patient 2

In April 1997, a 65-year-old Japanese man was referred to our hospital for hypereosinophilic syndrome and thrombocytopenia. The leukocyte count was 17,910/μl, with 12% abnormal lymphocytes, 80% eosinophils, and 7% neutrophils. The platelet count was 25,000/μl. No parasite infection or allergic disease was found. Examination of bone marrow biopsy and cerebrospinal fluid revealed abnormal lymphocytes. Surface marker analysis of mononuclear cells (MNCs) from cerebrospinal fluid and peripheral blood showed CD2, CD3, and CD25 positive cells, but CD4 negative cells. Based on positivity of antibody against HTLV-1 and monoclonal integration of proviral DNA in PBMNC, the disease was diagnosed as acute-type ATL. After the initial treatment with combination chemotherapy, the eosinophil count rapidly decreased. Later, the leukemia and eosinophilia became refractory although various combination chemotherapies were used, and he died of tumor involvement in November 1997.

Patient 3

In May 1997, a 49-year-old Japanese man was admitted to our hospital with abdominal pain and continuous diarrhea. Enlargement of cervical lymph nodes and pleural effusion were noted. Computed tomography revealed a thickened stomach wall. The leukocyte count was 20,540/μl, with 38% abnormal lymphocytes, 42% eosinophils, and 11% neutrophils. Antibody for HTLV-1 was positive, and monoclonal integration of proviral DNA was demonstrated in PBMNC. Treatment with combination chemotherapy resulted in a good response with relief of the symptoms and a decrease of the eosinophil count in the peripheral blood. The patient's condition deteriorated, however, and he died due to lung infiltration of ATL cells five months after admission.

Cell Preparation and Culture

MNC samples from the three patients and two normal healthy subjects were separated from pleural effusion (patient 1) or peripheral blood (patients 2 and 3, and normal subjects) by Ficoll-Conray density gradient centrifugation. In patient 1, the isolated MNCs were suspended in RPMI-1640 medium containing 30% patient serum and 1% silica (KAC-2; Nihon Kohtai Kenkyusho, Gunma, Japan). After incubation for one hr, the monocyte-free MNC were collected by Ficoll-Conray centrifugation. Samples of MNCs from the other two patients and the normal subjects were depleted of adherent cells by plastic adherence. More than 90% of those separated cells were morphologically identified as ATL cells. The surface markers of those cells are shown in Table I. The cells (1×10^6/ml) were cultured in RPMI-1640 medium containing 10% fetal calf serum (FCS) (GIBCO, Grand Island, NY) at 37°C with 5% CO_2. CM from three-day cultures was collected and stored at −30°C until assayed.

TABLE I. Surface Markers of Mononuclear Cells From Patients*

		Immunophenotype (%)			
Patient	Source	CD3	CD4	CD8	CD25
1	PE	82.6	82.4	6.4	ND
2	PB	89.2	17.1	22.3	79.1
3	PB	92.2	87.3	6.9	43.6

*PE, pleural effusion; PB, peripheral blood; ND, not done.

Semi-Solid Culture Procedures

Serum and CM from patients or normal subjects were added to semi-solid culture medium formed by α-MEM (Flow Laboratories, McLean, VA), 20% FCS, and 0.3% Bacto-agar (Difco Laboratories, Detroit, MI). Mobilized peripheral blood precursor cells collected from a subject with nonhematological disorder were used as target cell, and the CD34-positive cells were seeded in the culture at a concentration of 2,000/1 ml/dish. Stimulating factors used as positive controls were GM-CSF (Kirin Corp., Tokyo, Japan) and IL-3 (GIBCO). Culture without stimulating factors was used as the negative control. After incubation for 14 days at 37°C with 5% CO_2, the aggregates of 30 or more cells were counted as colonies with the aid of an inverted microscope. The preparations were air dried and stained with May-Giemsa, and the type of colony was analyzed. If more than 50% of colony-forming cells were eosinophils, the colony was described as an ''eosinophil-predominant colony.''

Quantification of IL-3, IL-5, and GM-CSF in Serum and CM

IL-3, IL-5, and GM-CSF levels in sera and CM from patients as well as from two normal subjects were measured with a commercially available enzyme-linked immunosorbent assay kit (Amersham Corp., Arlington Heights, IL, for the IL-3 and GM-CSF; Immunotec S.A., Marseille, France for the IL-5) according to the manufacturer's instructions. The detection limits for the IL-3, IL-5, and GM-CSF assays were 31.0 pg/ml, 5.0 pg/ml, and 8.0 pg/ml, respectively.

RESULTS

CM from all patients showed colony-stimulating activity and CM from two patients showed eosinophil-

TABLE II. Colony Stimulating Activities of Sera and Conditioned Media From Patients and Normal Subjects*

Stimulus	Number of colonies	
	All colonies	Eo. colonies (%[a])
None	0	0
IL-3 (25 ng/ml)	76.0 ± 5.2	16.5 ± 2.1 (21.7)
GM-CSF (20 ng/ml)	93.3 ± 7.5	32.0 ± 7.1 (34.3)
Serum (20%)		
Normal (n = 2)	0.2 ± 0.4	0
Patient 2	13.7 ± 7.8	12.0 ± 6.6 (87.6)
Patient 3	0.3 ± 0.6	0
CM (20%)		
Normal (n = 2)	0.2 ± 0.4	0
Patient 1	32.7 ± 8.3	10.7 ± 3.8 (32.7)
Patient 2	53.7 ± 2.9	21.0 ± 1.7 (39.1)
Patient 3	11.7 ± 5.7	0

*Data represent mean ± SD of the colony counts for triplicate cultures. Eo. colonies, eosinophil-predominant colony; IL, interleukin; GM-CSF, granulocyte-macrophage colony-stimulating factor; CM, conditioned medium.
[a]Ratio of Eo. colony to total colonies.

TABLE III. Cytokine Concentration in Patient Serum and Conditioned Medium From Patient ATL Cells*

Patient	Serum (pg/ml)[a]			Conditioned medium (pg/ml)[a]		
	IL-3	IL-5	GM-CSF	IL-3	IL-5	GM-CSF
1	<31.0	537.0	<8.0	<31.0	<5.0	92.0
2	<31.0	681.0	<8.0	67.0	10800.0	2740.0
3	<31.0	16.5	<8.0	<31.0	<5.0	418.0

*ATL, adult T-cell leukemia; IL, interleukin; GM-CSF, granulocyte-macrophage colony-stimulating factor.
[a]IL-3, IL-5, and GM-CSF were not detected in samples from normal subjects (n = 2).

predominant colony-stimulating activity, but no colony stimulating activity was seen in CM from the normal subjects (Table II). Colony-stimulating activity was also detected in the serum from patient 2, and most of the colonies induced were eosinophil colonies containing very few neutrophils or macrophages.

Detectable levels of soluble IL-5, but not of IL-3 and GM-CSF, were present in all serum samples (Table III). Although CM from unstimulated MNCs always contained detectable GM-CSF, IL-3 and IL-5 were only detected in the CM from the cells of one patient (patient 2).

DISCUSSION

Eosinophilia complicates about 20% of ATL cases [6], and it is sometimes marked. Several investigators have reported eosinophil-stimulating cytokine involvement in other lymphoid malignancies that are accompanied by eosinophilia. In Hodgkin's disease, for example [13,14], tumor cells [15] or normal MNCs [16] produce IL-5, and T-cell lymphoma cells from patients with eosinophilia secrete different combinations of IL-3, IL-5, and GM-CSF [17,18]. In spite of the high frequency of eosinophilia, the information about its cause in ATL is limited. To our knowledge, cells from only two ATL patients have been studied [11,12], and the data suggest that GM-CSF produced by ATL cells might associate with the development of eosinophilia.

In malignant lymphoma accompanied by eosinophilia, eosinophilopoietic factors are produced by either malignant cells themselves [15,17–19] or normal MNCs [16]. Our in vitro data indicated that GM-CSF was produced by MNCs from all patients, whereas IL-3 and IL-5 was produced by cells from only one (patient 2). As most of cultured MNCs were ATL cells, the cytokine level of CM probably reflects the production pattern of ATL cells. On the other hand, IL-5 was always detected in serum. IL-5 is ordinarily produced by activated T cells [20]. In patient 2, the serum IL-5 was probably produced by ATL cells. We think that IL-5 in patients 1 and 3, whose circulating ATL cells did not produce IL-5 in vitro, was probably produced by reacting normal lymphocytes in malignant lesions, rather than by noncirculating ATL cells. In those patients, normal lymphocytes may react to ATL cells or tumor necrosis through the anaphylactic reaction in the ATL lesions, such as lymph nodule, organ, or skin.

Previous reports [11,13] and our study suggest that ATL cells from patients with eosinophilia may always produce GM-CSF. However, the fact that not all patients showed severe neutrophilia and that detectable IL-5 were always present in their serum support that IL-5 may have a leading role in the development of eosinophilia, whereas GM-CSF produced by ATL cells may play a complementary role.

In patient 2, ATL cells produced three kinds of eosinophilopoietic cytokines, especially IL-5. To our knowledge, accelerated IL-5 production by fresh ATL cells has not been reported before. A previous report showed that mRNA expression and production of IL-5 were decreased in the ATL cells [21]. A more recent study showed, however, that HTLV-1-encoded tax could regulate the IL-5 gene [22]. These contradictory results suggest that the cytokine secretion pattern in ATL cells may not be uniform. In some patients, ATL cells may produce mainly IL-5, resulting in eosinophilia.

In conclusion, GM-CSF may be produced by leukemic cells in ATL patients with eosinophilia, but IL-5 produced by normal-reacting lymphocytes or ATL cells is probably the more important cytokine for the development of eosinophilia.

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