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Sézary syndrome with elevated serum IgE and hypereosinophilia: Role of dysregulated cytokine production

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A 62-year-old man presented with a 4-year history of a pruritic erythematous rash. Initial workup was not diagnostic, and the rash was refractory to standard treatment. A complete blood cell count demonstrated a white cell count of 9100 cells/ μ l with 61% polymorphonuclear neutrophils, 16% eosinophils, and 17% lymphocytes. A serum protein electrophoresis revealed an M spike identified as IgG κ . His IgE level was 11,900 IU/ml. A bone marrow biopsy specimen demonstrated "atypical plasma cells" but was not diagnostic for myeloma. Peripheral blood smear was remarkable for highly convoluted lymphoid cells diagnostic for Sézary T cells. Consistent with this diagnosis, 89% of his peripheral blood CD2+ cells expressed CD4. The eosinophilia, elevated IgE level, and monoclonal gammopathy led to further investigations. Circulating adherent monocytes were 96% positive for presumed low-affinity IgE receptor expression as shown by surface IgE binding. In a 9-day lymphocyte coculture, the patient's T lymphocytes induced IgE production in vitro (1.25 ng/ml) by a normal donor's cultured B cells. A healthy donor's T cells failed to induce IgE production (0 ng/ml) in a similar culture system. In situ hybridization with an Sulfur-35-labeled cDNA probe revealed interleukin-4 mRNA expression by 84% and interleukin-2 mRNA expression by 62% of nonadherent peripheral blood mononuclear cells. Interleukin-5 mRNA was shown by reverse transcription and the polymerase chain reaction. These studies demonstrate a subject with Sézary T cell leukemia with hypereosinophilia and elevated IgE due to presumed enhanced interleukin-4 and interleukin-5 production by the Sézary T cells. (J ALLERGY CLIN IMMUNOL 1993;92:123-31.)

Key words: Allergies, Sézary syndrome, IgE, eosinophilia, interleukin-4, interleukin-5, low-affinity IgE receptors (Fc ϵ RII)

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Sézary syndrome belongs to a group of relatively uncommon cutaneous T-cell lymphomas, which includes mycosis fungoides and the adult T-cell leukemias.^{1, 2} The presence of generalized erythroderma and circulating hyperchromatic convoluted lymphoid cells, termed Sézary cells, is characteristic of this disease. These leukemic cells represent metabolically active mature T lymphocytes.^{3, 4} We report the case of a patient with Sézary syndrome who presented with the distinct-

Abbreviations used

FcεRII:	Low-affinity IgE receptor
GM-CSF:	Granulocyte-macrophage colony-stimulating factor
ICAM:	Intercellular adhesion molecule
IL:	Interleukin
PBMCs:	Peripheral blood mononuclear cells
PCR:	Polymerase chain reaction
RT-PCR:	Reverse transcription-polymerase chain reaction
TNF:	Tumor necrosis factor

tive features of a markedly elevated serum IgE level (11,900 IU/ml) and significant hypereosinophilia (16%). We reasoned that these findings might represent the sequelae of metabolic products of the patient's leukemia cells. We therefore investigated the capacity of the patient's leukemic cells to induce IgE production and phenotyped these cells on the basis of their cytokine production. Our data demonstrate that the patient's leukemic T cells induce the production of IgE and produce cytokines known to participate in the induction of the IgE isotype switch (interleukin-4 [IL-4]) and hypereosinophilia (IL-5 and granulocyte-macrophage colony-stimulating factor [GM-CSF]). These data support the concept that these features of allergic diseases are regulated by T lymphocytes and that different subsets of T cells may produce different and distinct profiles of cytokines.

CASE REPORT

The patient is a 62-year-old man who initially presented in 1986 with a pruritic rash on his lower extremities. Initial therapy for "nonspecific dermatitis" provided no relief. A biopsy specimen obtained in 1988 was nondiagnostic. In 1989 axillary and inguinal lymphadenopathy developed. An axillary node biopsy specimen obtained in February 1989 was reported as showing benign reactivity. In 1989 the patient was found to have eosinophilia, with 16% eosinophils in his peripheral blood. Treatment for presumptive atopic dermatitis with topical corticosteroids and ultraviolet B phototherapy was only partially successful, and the rash continued to progress.

In May 1990, the patient came to the National Jewish Center for Immunology and Respiratory Medicine for further evaluation. He gave a history of seasonal allergic rhinitis and childhood asthma. He had no history of travel to areas endemic for parasite exposure. Results of physical examination were significant for a diffuse erythematous rash involving much of his body surface area with relative sparing of his face, scalp, and

TABLE I. Phenotypic analysis of the patient's mononuclear cells

Marker	Percent positive
T lymphocytes*	
CD2	92 (>90)
CD3	94 (>90)
CD4	89 (~50)
CD8	5 (~22)
CD7	4 (~65)
CD25	2 (0)
Monocytes†	
CD23	96 (0)

*Data represent percent of PBMCs presenting the phenotypic marker on a T-cell-enriched population derived via adherent cell depletion and sheep erythrocyte rosetting. Numbers in parenthesis represent range in normal mononuclear cell populations.

†Monocytes were isolated from PBMCs via plastic adherence and were 87% pure as determined by staining with CD14. Monocytes were assessed for surface-bound IgE via sequential exposure to mouse monoclonal anti-human IgE and phycoerythrin-conjugated goat anti-mouse antibody. Data represent cells expressing surface-bound IgE.

groin. He had 2 × 3 cm axillary and 2 × 2 cm posterior cervical lymph nodes. Laboratory data included a lactate dehydrogenase level of 249 IU/L and an alkaline phosphatase level of 253 IU/L. His white blood cell count was 9100 cells/μl with 61% neutrophils, 16% eosinophils, 6% monocytes, and 17% lymphocytes. Immunophenotyping of circulating lymphocytes demonstrated: CD2, 92%; CD3, 94%, CD4, 89%; CD8, 5%; CD7, 4%; and CD25, 2% (Table I). Sedimentation rate was 15 mm/hr. Antinuclear antibody and rheumatoid factor were negative. Serum IgE was 11,900 IU/ml. He had an elevated total IgG level of 2280 mg/ml. Serum protein electrophoresis was remarkable for a monoclonal band, which was identified as an IgGκ on immunofixation electrophoresis. Results of bone marrow biopsy, however, were not consistent with myeloma. Flow cytometric analysis was performed on purified monocytes (>87% pure by CD14 expression; Becton-Dickinson, Mountain View, Calif.) for the presence of surface-bound IgE. These studies were accomplished via sequential staining with a monoclonal mouse anti-human IgE antibody (Sigma Chemical Co., St. Louis, Mo.) followed by a phycoerythrin-conjugated goat anti-mouse immunoglobulin antibody (Becton-Dickinson) and demonstrated that 96% expressed surface-bound IgE. Examination of peripheral blood revealed that the majority of lymphocytes had convoluted nuclei, and with the patient's medical history and skin appearance, these were considered diagnostic for Sézary syndrome.

The presence of hypereosinophilia and a markedly elevated serum IgE level in this patient with Sézary syndrome prompted us to investigate whether these

features may have developed secondary to the production of cytokines by the patient's lymphoma cells.

METHODS

Mononuclear cell purification

Mononuclear cells were obtained from the patient and two healthy nonatopic laboratory workers from heparinized, peripheral venous blood via Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) centrifugation. Isolated populations of T lymphocytes and B lymphocytes were obtained for lymphocyte coculture via nylon wool column adherence of B cells and E rosetting assay with neuraminidase-treated sheep erythrocytes to separate T lymphocytes from B lymphocytes. Mononuclear cells were removed by plastic adherence to obtain an enriched population of lymphocytes for *in situ* hybridization.

Induction of IgE synthesis in B lymphocytes by the patient's leukemia cells

Cocultures for IgE synthesis were performed in 96-well round-bottom microtiter plates in complete media (RPMI 1640 containing penicillin [200 U/ml], streptomycin [200 µg/ml], L-glutamine [4 mmol/L], and N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid buffer [25 mmol/L; pH 7.20]) supplemented with 10% fetal bovine serum.⁵ In parallel replicate wells, either the patient's T lymphocytes with B lymphocytes obtained from a healthy, nonatopic donor (2×10^4) were combined with B lymphocytes (1×10^5) purified from the second healthy, nonatopic donor. After 9 days of culture in a humidified 37° C/5% CO₂ incubator, the supernatants were collected and assayed for IgE content by a microparticle enzyme immunoassay having a sensitivity of 0.048 IU IgE/ml (Abbott Laboratories, Abbott Park, Ill.) according to the manufacturer's instructions. Data represent the mean of experiments performed in quadruplicate in the patient and the control subject.

Reverse transcription-polymerase chain reaction for detection of cytokines

Specific 20-oligomer primers that specifically amplify IL-2, IL-4, IL-5, GM-CSF, and interferon-γ transcripts were obtained from a commercial source (Clontech, Palo Alto, Calif.). These primers span at least 2 introns and amplify cDNA of predicted sizes as follows: IL-2, 458 bp; IL-4, 456 bp; IL-5, 293 bp; GM-CSF, 424 bp; and interferon-γ, 494 bp. Total RNA was extracted from the patient's peripheral blood mononuclear cells in RNeasy (Qiagen Biotech, Friendswood, Texas) according to the manufacturer's instructions. The RNA was washed and 1 µg aliquots were subsequently denatured (at 65° C for 5 minutes) in the additional presence of oligo d(T) 12-18 mer (Promega, Madison, Wis.). RNA was rapidly cooled to 4° C and subsequently exposed to Moloney murine leukemia virus reverse transcriptase (600 U; Bethesda Research Laboratories, Bethesda, Md.) in the additional presence of

RNAasin (40 U; Promega), dithiothreitol (100 mmol/L; Bethesda Research Laboratories), deoxynucleotide triphosphate mix (10 mmol/L each; Promega), and the supplied reverse transcriptase buffer. The reverse transcriptase reaction was allowed to continue for 1 hour at 42° C. Aliquots of the newly synthesized cDNA at dilutions of 1:10 and 1:40 were then subjected to the polymerase chain reaction (PCR) in the presence of cytokine primers, Taq polymerase (1 U; Promega), deoxynucleotide triphosphate mix (1.25 mmol/L each), and the supplied reaction buffer. The DNA thermal cycler (Perkin-Elmer Corp., Norwalk, Conn.) was programmed to perform 30 cycles with the following conditions: 94° C for 1 minute, 55° C for 2 minutes, and 72° C for 3 minutes. At the completion of the PCR, 9 µl of PCR product was mixed with 1 µl of gel loading buffer (0.25% bromophenol blue, 0.25% xylene cyanole, 30% glycerol) and electrophoresed in 2% agarose in the presence of 0.5 µg/ml ethidium bromide. The amplified DNA bands were visualized with an ultraviolet transilluminator. The presence of cytokine-specific mRNA transcripts was confirmed by visual inspection as the presence of bands having the predicted number of base pairs, with the use of a DNA ladder (Sigma) electrophoresed in parallel.^{6, 7}

Nested probe hybridization

The data obtained via reverse transcription-polymerase chain reaction (RT-PCR) were confirmed by hybridization with a nested oligonucleotide probe. Briefly, the agarose gels that contained the PCR product were denatured in 0.5 mmol/L NaOH and neutralized, and the DNA was electrophoretically transferred to nylon paper. A nested, antisense oligonucleotide single stranded probe of IL-5 cDNA was commercially obtained (Clontech). The primer was 5' end-labeled with α-phosphorous 33-adenosine triphosphate (DuPont, Boston, Mass.) with T4 polynucleotide kinase (Promega) and purified by molecular sieve chromatography. The nylon filters were prehybridized for 2 hours and were then hybridized to the ³³P-labeled nested probe overnight at 45° C. After washing, the nylon paper was applied to X-ray films to obtain an autoradiographic image.

In situ hybridization

In situ hybridization was performed to identify mRNA specific for IL-2, IL-4, and interferon-γ.^{8, 9} Monocyte-depleted peripheral blood mononuclear cells (PBMCs) (10^4) were obtained as above from the patient with Sézary syndrome and a healthy, nonatopic donor and were cytopun and fixed in 95% ethyl alcohol to glass microscope slides. These specimens via immunohistochemical analysis were estimated to represent ~85% Sézary cells. Sulfur 35-ATP-labeled (³⁵S-ATP from DuPont) cytokine cDNA was prepared with a random primers DNA labeling system (Bethesda Research Laboratories) according to the manufacturer's

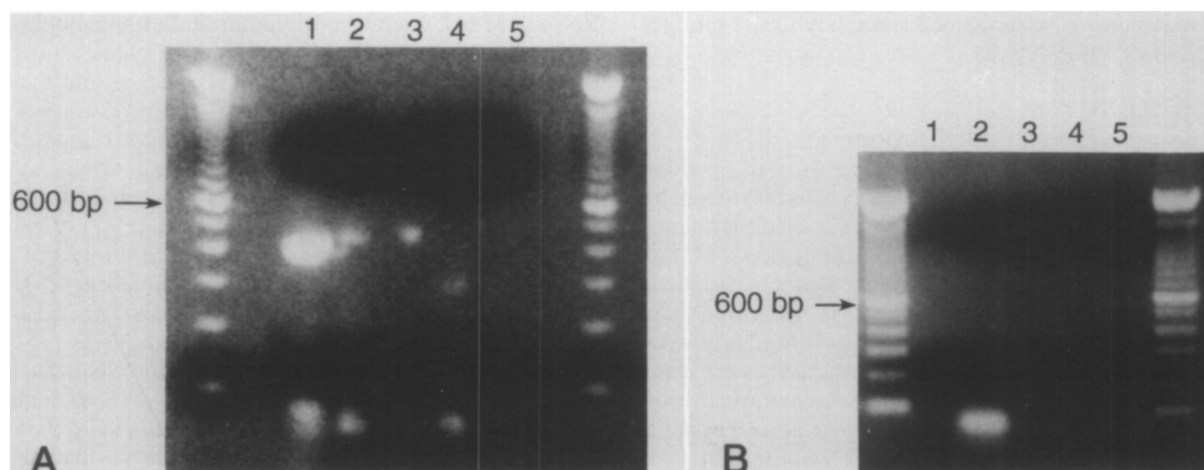


FIG. 1. RT-PCR of cytokine transcripts within Sézary cells. Total RNA was extracted from the patient's Sézary cells, and cDNA was generated in the presence of Moloney murine leukemia virus reverse transcriptase. The PCR was performed with cDNA diluted 1:10 (**A**) in the presence of oligonucleotide primers specific for: GM-CSF (lane 1), IL-4, (lane 2), IL-2 (lane 3), IL-5 (lane 4), and interferon- γ (lane 5). Results for cDNA diluted to 1:40 are shown in **B**. The positive band (lane 2) represents IL-5-specific primers. PCR products were electrophoresed on 2% agarose and visualized in the presence of ethidium bromide by ultraviolet transillumination. A 100 bp ladder is present, with the band representing 600 bp labeled. These primers span at least 2 introns and amplify cDNA of predicted sizes as follows: IL-2, 458 bp; IL-4, 456 bp; IL-5, 293 bp; GM-CSF, 424 bp; and interferon- γ , 494 bp.

TABLE II. Induction of IgE synthesis in B lymphocytes by the patient's leukemia cells

Source of T lymphocytes	IgE concentration (ng/ml)
Control subject	0.0
Patient	1.25

T cells (2×10^4) from the patient with Sézary syndrome and a control volunteer were cocultured with B lymphocytes (10^5) from a healthy, nonatopic donor for 14 days. Secreted IgE concentrations in the supernatants were determined via ELISA.

instructions. Full-length cytokine cDNA for labeling was prepared via the PCR. As an additional negative control, hybridizations were also performed with ^{35}S -labeled DNA derived from λ -DNA *Hind*III fragments (Bethesda Research Laboratories). ^{35}S -labeled cDNA was used at a specific activity of $>10^7$ cpm/ μg DNA as determined by trichloroacetic acid precipitation. Fixed cells were incubated for 2 hours at 42°C in prehybridization solution ($5\times$ saline-sodium citrate buffer [Sigma], $1\times$ Denhardt's solution [Sigma], 10 mmol/L dithiothreitol [Sigma], 10% deionized formamide [Sigma], 100 $\mu\text{g}/\text{ml}$ salmon sperm DNA [Sigma], and 10% dextran sulfate [Pharmacia Fine Chemicals, Uppsala, Sweden]). Hybridization occurred in prehybridization solution supplemented with 1×10^6 cpm of the cDNA probe. Hybridization was allowed to proceed overnight

at 42°C . After rinsing in room temperature saline-sodium citrate followed by 42°C saline-sodium citrate, detection was accomplished with application of Kodak NTB 2 autoradiography emulsion (Kodak, Rochester, N.Y.). The exposure occurred in a light-proof box at -4°C for 7 days. Slides were developed with Kodak D19 developer (Kodak), fixed, dried and, in certain experiments, counterstained with Giemsa stain. One hundred consecutive lymphocytes were examined, and grains corresponding to ^{35}S activity were counted. The distribution in the number of grains per cell observed when the control (cytokine) and experimental (plasmid) probes were applied was compared on PBMCs derived from the patient and the control subject. It was determined that more than 10 grains associated with a cell represented statistically greater activity than that observed when the ^{35}S -control DNA was applied to the slides ($p < 0.05$) and therefore was considered to demonstrate the presence of cytokine transcripts. The observer was blinded as to the contents of the slide at the time these observations were made. Data are presented as the percent of lymphocytes demonstrating cytokine-specific mRNA and represent the mean of three experiments for each cytokine.

RESULTS

Induction of IgE synthesis in B lymphocytes by the patient's leukemia cells

T lymphocytes obtained from the patient with Sézary syndrome and a healthy, nonatopic control

TABLE III. Cytokine transcripts detected in Sézary cells by RT-PCR

Cytokine	cDNA dilution	
	1:10	1:40
Interferon- γ	—	—
GM-CSF	+	—
IL-2	+	—
IL-4	+	—
IL-5	+	+

Total RNA was extracted from the Sézary cells, and a cDNA library was generated via exposure to reverse transcriptase. Complementary DNA at varying dilutions was subjected to the PCR in the presence of cytokine-specific primers. Transcript expression was determined via gel electrophoresis as the presence of expanded DNA having the predicted number of base pairs for the respective primers.

subject were cocultured with B lymphocytes purified from a second unrelated healthy, nonatopic control subject. After 9 days, supernatants were collected and assayed for IgE concentration by ELISA. The data are displayed in Table II. Supernatants derived from the coculture of T and B lymphocytes obtained from the two control subjects did not demonstrate IgE production. However, T lymphocytes obtained from the patient with Sézary syndrome induced increased IgE secretion by B lymphocytes from a nonallergic control subject as shown by the presence of 1.25 ng/ml of IgE in these supernatants.

RT-PCR for detection of cytokines

Total RNA was extracted from peripheral blood T lymphocytes and cDNA generated via exposure to Moloney murine leukemia virus reverse transcriptase. PCRs were performed with 1:40 (Fig. 1, *A*) and 1:10 (Fig. 1, *B*) dilutions of the cDNA in the presence of primers specific for IL-2, IL-4, IL-5, GM-CSF, and interferon- γ . The results are displayed in Table III. At a 1:40 dilution of the cDNA, only IL-5 transcripts were detected. At a 1:10 dilution IL-2, IL-4, IL-5, and GM-CSF—but not interferon- γ —were detected. Specificity of IL-5 was confirmed by hybridization of the PCR product to a ^{33}P -end-labeled single stranded nested oligonucleotide probe (Fig. 2).

In situ hybridization

PBMCs enriched with presumed Sézary cells were fixed to glass slides, and in situ hybridization was performed to detect IL-2, IL-4, and interferon- γ transcripts. Representative fields for the

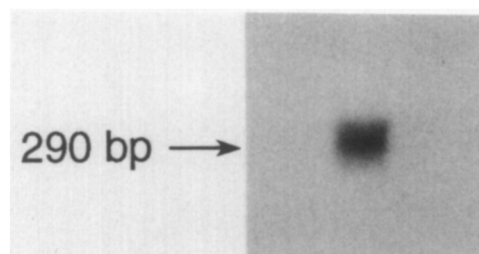


FIG. 2. Hybridization of RT-PCR product to IL-5-specific nested oligonucleotide probe. Reverse transcriptase and IL-5-specific PCR were performed as described. The PCR products at a 1:40 dilution of cDNA were electrophoresed on 2% agarose, denatured, and electrophoretically transferred to nylon paper. Hybridization was performed in the presence of a 5' $\gamma^{33}\text{P}$ -ATP end-labeled nested IL-5-specific probe, and autoradiography was performed.

negative control probe and for IL-4 are displayed in Fig. 3, *A* and *B*, respectively and the results for all three cytokines are summarized in Table IV. Transcripts for IL-4 were detected in 84% of cells, whereas 62% of cells contained IL-2 mRNA and 18% contained interferon- γ mRNA. No cytokine-specific transcripts were observed in unstimulated lymphocytes obtained from the control subject (data not shown).

DISCUSSION

We report the case of a 62-year-old man with Sézary syndrome who presented with an elevated serum IgE level (11,900 IU/ml) and hypereosinophilia (16% of 9100 white blood cells). Sézary syndrome is a lymphoma associated with the diffuse cutaneous infiltration of malignant T lymphocytes, producing an erythroderma.^{1, 2} This disease is diagnosed on the basis of the characteristic appearance of the malignant cells, with hyperchromatic lymphoid cells containing a convoluted nucleus. The malignant cells usually represent mature T lymphocytes of the T_H phenotype. Thus these cells demonstrate surface expression of CD3 and CD4. In addition, these cells are characterized by the absence of CD7.¹⁰ In addition to displaying the phenotype of T_H lymphocytes, Sézary cells may behave as metabolically active helper cells, the functional profile of which includes induction of immunoglobulin synthesis and the production of cytokines.^{1, 2, 11-14} Thus Sézary cells of the HUT 78 human T-cell line have been demonstrated to produce IL-2, interferon- γ , tumor necrosis factor- α [TNF- α], and transforming growth factor- β , cytokines characteristic of T_H cells.⁶

Our patient's Sézary syndrome presented with

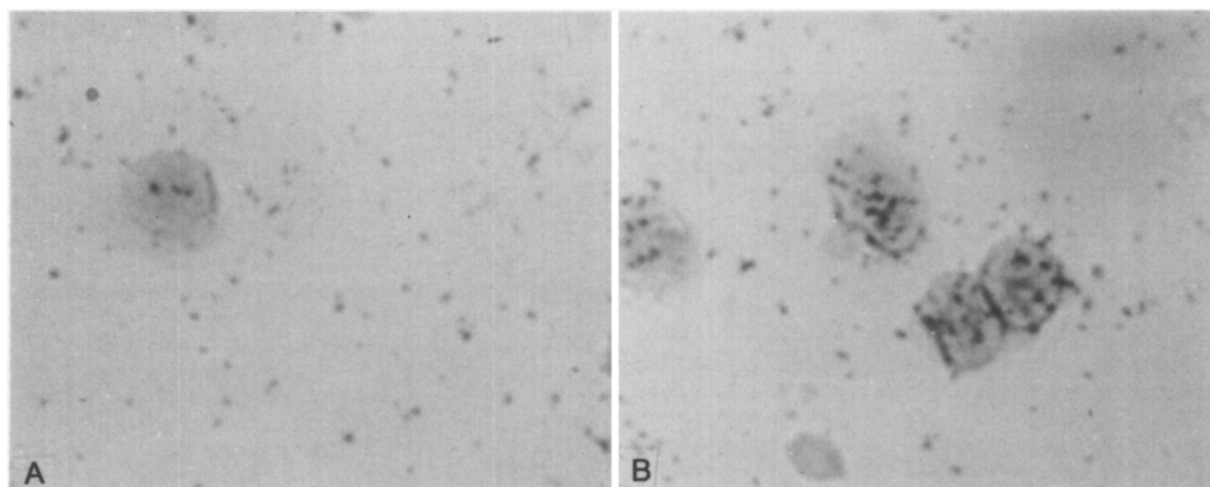


FIG. 3. In situ hybridization of Sézary cells. Sézary cells were fixed on glass slide and allowed to hybridize in the presence of ^{35}S -labeled cDNA probes. After extensive washing, the hybridized label was visualized by immersion in silver emulsion. **A** represents the negative control probe derived from λ DNA *Hind*III fragments. **B** was performed with an IL-4-specific cDNA probe.

TABLE IV. Cytokine production by Sézary cells as detected by in situ hybridization

Cytokine	Percent positive
IL-2	62
IL-4	84
Interferon- γ	18

Sézary cells were fixed on glass slides and hybridized with ^{35}S -labeled cDNA for the respective cytokines. Slides were washed, and the hybridized label was visualized after immersion in developing solution. Three slides were examined for each cytokine, and data represent the mean percent of 100 consecutively examined cells displaying cytokine-specific mRNA.

the unusual features of an elevated serum IgE level and hypereosinophilia. Immunologic factors that regulate the production of IgE and eosinophilia have been the subjects of intense investigations. Both of these features are typical of allergic diseases, and it has long been recognized that they are both regulated by secreted products of T lymphocytes.^{5, 15-18} We therefore investigated the capacity of the patient's lymphoma cells to induce IgE production by B lymphocytes. As shown in Table II, T cells derived from the patient but not T cells purified from a nonallergic donor induced IgE production. These data support the concept that the patient's marked elevation in serum IgE occurred as a result of the production of factors by his lymphoma cells.

The cytokines produced by T lymphocytes that are involved in the regulation of IgE and eosinophilia have recently become better understood. Participation in the induction of an isotype switch to IgE production is an activity of IL-4.¹⁹⁻²⁶ Thus IL-4 induces IgE production by B lymphocytes in vitro cultures, and the capacity of human T cell clones to support IgE production is directly proportional to their IL-4 production. On the contrary, interferon- γ is a counterbalance to this activity of IL-4 on B-cell IgE synthesis, and the capacity of a T-cell clone to support IgE production is inversely proportional to interferon- γ production and clones, which produce both IL-4 and interferon- γ will only induce IgE production in the additional presence of anti-interferon- γ .^{20, 23, 26-31} A second biologic activity of IL-4 is its capacity to induce expression of the low-affinity IgE receptor (Fc ϵ RII or CD23) on monocytes.^{32, 33} These receptors are normally not expressed on the monocytes of nonatopic subjects but are displayed at increased concentrations on those of allergic individuals.³³⁻³⁸ Support for the involvement of IL-4 in this patient's clinical syndrome was the presence of Fc ϵ RII on ~96% of his monocytes (Table I), as suggested by the presence of surface-bound IgE. Alternatively, however, CD23 may also be induced by other cytokines such as GM-CSF³⁹ or virus-derived transactivating factors.⁴⁰

Eosinophilia represents the sequela of the activity of three other cytokines. IL-5 represents the

most potent eosinophilopoietin, and incubation of bone marrow cells with IL-5 results in the generation of pure colonies of eosinophils.^{41, 42} IL-5 also activates and promotes the survival of mature eosinophils.^{43, 44} IL-3 and GM-CSF share with IL-5 this capacity to prolong the survival of mature eosinophils.⁴⁵⁻⁴⁷ With our observation that this patient's lymphoma cells induced IgE production, we therefore investigated their capacity to produce IL-4 and interferon- γ , and similarly, the patient's hypereosinophilia prompted us to investigate the production of IL-5 and GM-CSF.

An extremely sensitive technique for detection of RNA transcripts (~ 10 or less per cell) is the sequential application of reverse transcription and the polymerase chain reaction (RT-PCR).⁶⁻⁷ RT-PCR was performed for IL-2, IL-4, IL-5, interferon- γ , and GM-CSF; the results are displayed in Table III. To provide semiquantitative data on the relative concentrations of each cytokine transcript observed, the PCR was performed at a 1:10 and 1:40 dilution of the cDNA mix. At the higher dilution, only IL-5 transcripts were observed. At a 1:10 dilution, transcripts for all of the cytokines with the exception of interferon- γ were detected. Although less sensitive and less specific than RT-PCR, *in situ* hybridization was performed to confirm the data obtained by RT-PCR and to permit us to quantify the percent of the lymphoma cells involved in production of each of the cytokines. These data are displayed in Table IV. These data demonstrate that production of IL-2 and IL-4 was a characteristic feature of the patient's Sézary cells, whereas very few demonstrated interferon- γ .

Neither our RT-PCR experiments nor the *in situ* hybridizations are able to distinguish cytokine production by Sézary cells from that of the patient's nonmalignant T lymphocytes. However, the nonmalignant T lymphocytes were not activated, as demonstrated by their failure to express high-affinity IL-2 receptors (CD25) (Table I). Because resting T lymphocytes do not produce cytokines, these nonmalignant cells are not likely to be contributing to our results. Furthermore, insofar as $\sim 85\%$ of the patients' peripheral blood T lymphocytes represented Sézary cells and 62% and 84% were synthesizing IL-2 and IL-4, respectively, then most if not all of the cytokine production must be ascribed to the leukemic cells.

In addition to its role in regulating IgE synthesis, the diminished synthesis of interferon- γ has been proposed to have a role in evolution of cutaneous T-cell leukemias from a localized (my-

cosis fungoides) to a leukemic phase (Sézary syndrome). Thus interferon- γ is an important inducer of intercellular adhesion molecule-1 (ICAM-1) on many cell types including epidermal keratinocytes.^{48, 49} Sézary cells express lymphocyte function associated antigen -1 (or CD11a) and an ICAM-1-lymphocyte function associated antigen-1 interaction has been proposed to support the localized binding of Sézary cells to the skin in mycosis fungoides.⁵⁰ Decreased synthesis of interferon- γ is associated with loss of ICAM-1 expression and possibly with dissemination of the Sézary cells.⁵¹

T lymphocytes do not necessarily produce all of their cytokines on activation. In the mouse subclasses of T helper lymphocytes can be identified among antigen-specific T-cell clones on the basis of their repertoire of cytokines.^{52, 53} Type 1 helper cells produce interferon- γ , IL-2, and TNF- β but not IL-4 and IL-5. Type 2 helper cells produce IL-4, IL-5, IL-6, and IL-10 but not interferon- γ , IL-2, or TNF- β . Both classes produce GM-CSF and IL-3. Type 1 helper lymphocytes, through their production of IL-2 and interferon- γ , will induce T-cell and monocyte activation and therefore promote cell-mediated immune responses. IL-4, IL-5, and IL-6 production by type 2 helper cells will induce B-cell maturation and are therefore important in antibody-dependent immunity. Type 2 helper lymphocytes, which either produce excessive IL-4 and IL-5 or function in the absence of interferon- γ , are likely candidates for inducing an allergic immune response.

This T_{H1} and T_{H2} dichotomy is less apparent in human T-cell clones. The primary difference is that virtually all human T-cell clones produce IL-2 and may produce both interferon- γ and IL-4. However, there is an inverse relationship between the tendency of a T-cell clone to produce interferon- γ as opposed to producing IL-4 or IL-5.^{23, 29} Similarly, T lymphocytes derived from the lungs of subjects with allergic asthma are more likely to express mRNA transcripts for IL-2, IL-4, and IL-5 and not for interferon- γ .^{54, 55} The presumed malignant T-lymphocyte clone present in our patient had these typical features of human type 2 helper lymphocytes, lending support for the concept that this subclass of lymphocytes exists in human beings and may be critically important in generating atopic disease.

In summary, this patient presented with Sézary syndrome, with leukemia cells characterized by the presumed production of IL-2, IL-4, and IL-5 and GM-CSF and the relative absence of inter-

feron- γ . This is in contrast to previously characterized Sézary syndrome T lymphocytes, which produced IL-2, interferon- γ , TNF- α , and transforming growth factor- β . Consistent with our current understanding of the regulation of IgE, our patient's production of IL-4 in the relative absence of interferon- γ was associated with a marked elevation of his serum IgE. Similarly, his production of IL-5 and GM-CSF was associated with hypereosinophilia. Together these data support the concept that these cytokines perform functions *in vivo* in a manner similar to that described for them *in vitro* and, by extension, that these cytokines may be involved in the dysregulation of IgE production and eosinophilia that characterize allergic diseases.

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