Abnormal Cytokine Expression in Sézary and Adult T-Cell Leukemia Cells Correlates with the Functional Diversity between These T-Cell Malignancies


Abstract

The Sézary syndrome (Séz) and adult T-cell leukemia (ATL) are malignant proliferations of mature T-lymphocytes that possess distinct functions. Sézary cells function as helper cells, whereas ATL cells are usually suppressor effectors. Although phenotypically similar (CD4+/CD7−/CD8−), these functional differences between the T-cell lymphoproliferative disorders suggest different patterns of cytokine expression. We wished to delineate the cytokine mechanisms potentially underlying the diverse functional characteristics of Séz and ATL. Therefore, we analyzed the expression of interleukins (IL) 2, 4, and 5, γ-interferon, and transforming growth factor β1 in the highly purified leukemic T-cells from 5 Séz and 5 ATL patients. Decreased mRNA and protein levels of IL-2, γ-interferon, and IL-5 were detected in mitogen-stimulated ATL and Séz cells when compared to similarly cultured normal CD4+ cells. In contrast, IL-4 production was markedly up-regulated in the leukemic cells of 4/5 Séz patients as compared to ATL and normal controls. Finally, fresh ATL cells secreted higher levels of transforming growth factor β1 into the culture medium than the malignant T-cells from Séz patients. Collectively these results suggest that, similar to the murine CD4-expressing T-cell subsets Th1 and Th2, distinct cytokine profiles exist in a human population of CD4+ T-cells. Moreover, the distinct patterns of IL-4 and transforming growth factor β1 expression by Séz and ATL cells, respectively, are most consistent with the functional differences (i.e., helper versus suppressor activity) between these T-cell malignancies and thus may play important roles in the pathogenesis of the paraneoplastic features associated with these two leukemias.

Introduction

The malignant expansion of mature CD4+ T-cells is represented clinically by two distinct human leukemias known as the Sézary syndrome (Séz) and ATL. Séz is a cutaneous T-cell lymphoma associated with diffuse skin involvement known as erythroderma, generalized lymphadenopathy, and circulating malignant cells in the blood (1, 2). ATL occurs in patients infected with the retrovirus HTLV-I and is also characterized by infiltration of the skin with malignant T-cells (3). However, it tends to have a more aggressive course than Séz and is often complicated by hepatosplenomegaly, hypercalcemia, and an extremely high incidence of opportunistic infections (4, 5).

The circulating leukemic cells in Séz and ATL patients share the identical mature T-cell phenotype, CD4+, but lack CD7 and CD8 antigen expression (6, 7). Although phenotypically similar, biological differences between these T-lymphoproliferative disorders suggest an altered pattern of cytokine expression. For example, in immunoglobulin biosynthesis assays the neoplastic T-cells from Séz patients function as helper cells when cocultured with stimulated normal B-cells, whereas in the same assay ATL cells effectively suppress normal immunoglobulin production (8). Moreover, peripheral blood lymphocytes from ATL patients have impaired proliferative responses to T-cell mitogens (5). This in vitro immunosuppressor activity of ATL cells is paralleled clinically by the uniform finding of cutaneous anergy, failure to make antibody to infused murine monoclonal antibodies, and increased incidence of opportunistic infections in ATL patients (5, 9–10). In contrast, lack of anergy, elevated levels of IgE, and the production of antibodies following antigenic stimulation have all been demonstrated in patients with Séz (1, 2). These functional and clinical immunological differences between the CD4+ leukemic T-cells from Séz and ATL patients suggest that, similar to the murine T-helper cell populations designated Th1 and Th2, distinctive cytokine profiles exist among human CD4-expressing T-cell subsets as well.

Recently, mitogen-activated peripheral blood mononuclear cells isolated from Séz patients were shown to produce significantly higher protein levels of IL-4 and lower levels of IL-2 and IFN-γ than did similarly cultured cells from normal controls (11). However, this study did not provide direct evidence that it is the malignant Sézary cell which is responsible for the abnormal immune response since enriched CD4+ leukemic populations from Séz patients were not analyzed. Moreover, there has been no investigation comparing the cytokine profile in Séz with respective levels of cytokine expression in ATL to better define their relevance to the pathogenesis of the immunological abnormalities associated with these T-cell malignancies. Therefore, we have examined the expression of IL-2, IFN-γ, IL-4, IL-5, and TGF-β1 in highly purified populations of leukemic T-cells isolated from Séz and ATL patients to determine which cytokines are potential mediators of the diverse immune abnormalities observed in these T-cell lymphoproliferative disorders and to gain insight into the regulatory network of cells that control the human immune response.

Materials and Methods

Patients. Five patients with Séz and five with ATL were studied. Healthy blood bank donors, all males with a mean age of 46 years (range, 25–70), served as normal controls. Informed consent was obtained from all patient and volunteer blood donors. The Séz group consisted of four males and one female from the United States with a mean age of 64 years (range, 50–71). This patient population was characterized by the presence of erythroderma, lymphadenopathy, and a circulating pool of abnormal lymphocytes with cerebrospinal fluid and a predominant CD4+/CD7−/CD8− T-cell phenotype. Except for one Séz patient with a total WBC of 7,100/mm3, leukocytosis was a consistent finding in this group ranging from 18,100 to 45,800 cells/mm3. All
of the SzS patients tested were seronegative for HTLV-I antibodies and had normal IgE levels and eosinophil counts. The ATL population was composed of 4 females and 1 male with a mean age of 42 years (range, 32-55). Two were of African-Caribbean descent and three were African-American. The diagnosis of ATL was established by the following criteria: presence of abnormal CD4+, CD7−, CD8−, and CD25+ lymphocytes in the blood; demonstration of a clonal integration of the HTLV-I genome in the circulating T-cells; and the presence of anti-HTLV-I antibodies in the serum by ELISA. All of the ATL patients had WBC<14,000/mm³ (range, 14,600-40,800/mm³), as well as impaired delayed-type hypersensitivity manifested by cutaneous anergy. Southern blot analysis of the T-cell receptor β-chain gene rearrangement from the peripheral blood lymphocytes of all patients studied revealed a clonal population of leukemic T-cells. At the time of study each patient had not received immunosuppressive therapy for at least 4 weeks and none had evidence of acute infection.

**Purification of Leukemic T-Cells from Study Patients.** PBMCs were isolated from patient venous blood samples by density gradient centrifugation over lymphocyte separation medium (Organon Teknika, Durham, NC). The PBMC interface cells were further purified by negative selection with immunomagnetic beads to obtain highly enriched CD4+ T-cell populations as described previously (8). Briefly, isolated PBMCs were centrifuged through 46.5% Percoll (Pharmacia, Uppsala, Sweden). Percoll-dense cells at 2×10⁷ cells/ml were used for DNA and RNA isolation.

Cell culture conditions were as follows: CD4+ cells from each (GIBCO Laboratories, Grand Island, NY), and 10% heat-inactivated FBS (Biofluids, Inc., Rockville, MD), penicillin and streptomycin at 100 μg/ml. The purity of lymphocyte suspensions for SzS and ATL patients, 3A1 (anti-CD7, obtained from Dr. Barton Haynes) at 5 μg/ml was included in the above antibody combination to remove the nonleukemic mature T-lymphocytes. The antibody-coated cells were suspended in media containing 70% goat anti-mouse magnetic beads (Advanced Magnetics, Cambridge, MA) per anticipated target cell to be removed. Following incubation on ice for 20 min, the magnetic bead-cell suspension was exposed to a magnet. The residual bead-negative cells were collected and retrieved with the above antibody cocktail. Antibody-coated cells were subjected to another round of magnetic bead depletion using goat anti-mouse Dynabeads (Dynal, Great Neck, NY) at a ratio of 5 bead particles/target cell. This suspension was exposed to a magnetic field and free, bead-negative cells were collected. An aliquot of the purified lymphocyte fraction from each patient or donor was stained with fluorescein isothiocyanate or phycoerythrin-conjugated Leu3 (anti-CD4), Leu2 (anti-CD8), Leu11c (anti-CD16), LeuM3 (anti-CD14), and Leu16 (anti-CD20) purchased from Becton Dickinson (Mountain View, CA) and analyzed by flow cytometry. The resulting CD4+ T-cell populations were ≥95% Leu3+ (CD4+ with <1% CD8+), CD20+, CD14+, or CD16+ contaminating cells. Leukemic T-cells purified in this way were over 90% viable by trypan blue exclusion and had the characteristic morphological abnormalities of Sézary or ATL cells.

**Cell Culture Conditions.** CD4+ T-cells from patients and normal controls were cultured at a density of 2×10⁶/ml in RPMI 1640 containing l-glutamine, 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer (Biofluids, Inc., Rockville, MD), penicillin and streptomycin at 100 μg/ml each (GIBCO Laboratories, Grand Island, NY), and 10% heat-inactivated human AB serum (Advanced Biotecnologies, Columbia, MD). Lymphocyte cultures were incubated at 37°C, with or without PHA at 1 μg/ml (GIBCO) and PMA at 10 ng/ml (Calbiochem, La Jolla, CA), for 6 h (to obtain RNA) and 48 h (prior to harvest of supernatants), respectively. In a subset of patients, RNA was extracted from CD4+ T-cells following 12 h of mitogen stimulation.

**RNA Isolation and Northern Blot Analysis.** Total cellular RNA was isolated from both the resting (Time 0) and mitogen-activated (6-h cultures) purified CD4+ T-cell populations by the guanidium thiocyanate method (12). Fifteen μg of total RNA from stimulated patient and control CD4+ cells were electrophoresed through a 1% agarose gel containing 0.66 μM formaldehyde. Gels were photographed and blotted onto nitrocellulose filters. Filters were hybridized with the following 32P-labeled random-primer cDNA probes according to the method of Church and Gilbert (13): a 0.71-kilobase HindIII/PT fragment of human IL-2 (pLW55 obtained from the American Type Culture Collection, Rockville, MD); a human IFN-γ cDNA clone provided by Dr. Grace Ju (Hoffman LaRoche Inc., Nutley, NJ); and a 1.9-kilobase BamHI cDNA clone for human β-actin provided by Dr. Wayne Tsang (National Cancer Institute, Bethesda, MD). To assess IL-5 mRNA expression, a Nhel/EcorV fragment of 5'-IL-5 cDNA from Dr. Michael Sneller (National Institute of Allergy and Infectious Diseases, Bethesda, MD) was cloned into pGEM 3Z (Promega, Madison, WI) and an antisense riboprobe was synthesized with [32P]UTP (Amersham, Arlington Heights, IL) according to the vendor's directions (Promega). Northern blots for IL-2 and IFN-γ expression were also hybridized with the IL-5 riboprobe at 60°C for 16 h and then washed twice in 2X standard saline-citrate (1X is 150 mM sodium chloride, 15 mM sodium citrate) at room temperature and in 0.1X standard saline-citrate at 65°C for 20 min. Autoradiography was performed for 48 h at ~70°C with an intensifying screen.

**IL-4 mRNA Expression by Reverse Transcribed PCR.** IL-4 cDNA synthesis was performed on 1 μg of resting CD4+ T-cell RNA from patients and normal controls using reverse transcriptase and oligodeoxythymidylate according to a protocol supplied with the GeneAmp RNA PCR kit (Perkin Elmer Cetus, Norwalk, CT). Reagent controls consisted of the reverse transcription reaction mixture without template RNA. RNA from a Chinese hamster ovary cell line stably transfected with an IL-4 cDNA was used as a positive control. Reverse transcription products were diluted to obtain a final PCR mixture containing 25 mM Tris-HCl, 50 mM KCl, 2.5 mM MgCl₂, all four deoxynucleotide triphosphates at 0.2 mM each, IL-4 sense and antisense primers at 0.2 mM each, and 2.5 units of Thermus aquaticus polymerase (Perkin Elmer Cetus) in 100 μl. An IL-4 sense primer, 5'-ATGGGGTCCTACCTCCCAACTGCT-3', and an IL-4 antisense primer, 5'-GTTTCTCAACGGTTCG-3', were kindly provided by Dr. Thomas Nutman (Laboratory of Parasitic Diseases, Bethesda, MD); these primers directed amplification of a 456-base pair fragment within the IL-4 mRNA. cDNA samples were subjected to 30 cycles of amplification consisting of denaturation for 1 min at 94°C, primer annealing for 1 min at 55°C, and polymerization for 2 min at 72°C. An actin-targeted sequence served as an internal control test and was defined by 5'-TCTTACAATGAGCTGCGTGT-3' and 5'-GCCGACCGACTGTGTTGCC-3', sense and antisense primers, respectively. The amplified 636-base pair actin signal was detected by an internal 40-base pair probe as described previously (14). PCR products following IL-4 mRNA amplification were analyzed by Southern blot hybridization as follows. Twenty μl of each PCR reaction were electrophoresed through a 1% agarose gel and then transferred onto a nitrocellulose filter. The filter was prehybridized in a previously described buffer (15) and hybridized overnight at 60°C with 4.5 ml of buffer containing a 32P-nick-translated (1×10⁶ dpm/ml) cDNA for human IL-4 (pHILA, kindly provided by Dr. Steven Clark, Genetics Institute, Cambridge, MA). The filter was washed (15) and autoradiographed at ~70°C.

**Cytokine Assays.** CD4+ T-cell supernatants were assayed for human IL-2 (DuPont New England Nuclear, Boston, MA) and IFN-γ (Centocor, Malvern, PA) by specific ELISAs according to the manufacturer's directions. IL-4 was measured by a commercially available ELISA (R&D Systems, Inc., Minneapolis, MN). The ELISA for IL-5 was kindly performed by Dr. Nutman (Laboratory of Parasitic Diseases) as described previously (16). Calculated sensitivity levels for the IL-2, IFN-7, IL-4, and IL-5 ELISAs were 1 unit/ml, 0.1 unit/ml, 3 pg/ml, and 20 pg/ml, respectively. The mean IL-2, IFN-γ, IL-4, and IL-5 T-cell supernatant concentrations were calculated for the SzS and ATL patient populations, as well as the normal controls. The distribution of values for the respective cytokines among the various patient groups was compared using the unpaired Student t test.

**Quantification of TGF-β Secreted by Circulating Leukemic T-Cells.** Purified CD4+ T-cell populations from 4 SzS and 4 ATL patients, as well as from 2 normal controls, were resuspended in serum-free Nutridoma at 3×10⁶ cells/ml and cultured for 72 h at 37°C. TGF-β levels in the conditioned media were measured by the specific sandwich ELISA for TGF-β, as described previously (17, 18).

**RESULTS**

**Isolation of CD4+ Sézary and ATL Cells.** CD4+ leukemic T-cells were purified from the circulating cells of SzS and ATL patients by magnetic bead depletion as described in "Materials and Methods." The results of immunophenotypic analyses of the cells from a representative SzS patient, prior to and after negative selection, are depicted in Fig. 1. As shown in Fig. 1, virtually all of the
CYTOKINE DYSFUNCTION IN MATURE T-CELL LEUKEMIAS

Reduced Expression of IFN-γ, IL-2, and IL-5 mRNA by Leukemic Cells from Sézary and ATL Patients. We evaluated the pattern of mitogen-induced lymphokine transcription in the purified CD4+ T-cells from Sézary and ATL patients, as well as normal controls. Total RNA was isolated from PHA/PMA-stimulated patient and control CD4+ T-cells. Utilizing Northern blot analysis, we compared the mRNA levels of IFN-γ, IL-2, and IL-5 in these T-cell lymphoproliferative patient populations. The mRNA expression of IFN-γ, IL-2, and IL-5 was markedly reduced in the purified leukemic cells isolated from Sézary and ATL patients (with the exception of Sézary patient 3) as compared to similarly activated normal CD4+ cells (Fig. 2). There was no evidence of cytokine expression by the Sézary or ATL cells of the patients when cultured in media alone or when stimulated for 12 h with PHA/PMA (data not shown). These results reflect a profound abnormality of T-cell cytokine expression in patients with these T-lymphoproliferative disorders.

Reduced Production of IL-2, IFN-γ, and IL-5 by Leukemic Cells from Sézary and ATL Patients. To correlate the abnormal mRNA expression of IL-2, IFN-γ, and IL-5 with their respective gene products, we measured the protein levels of these cytokines in supernatants of mitogen-induced CD4+ T-cells from patients and normal controls (Fig. 3). IL-2 production by PHA/PMA-stimulated ATL (0.7 ± 1.57 (SD) units/ml) and Sézary cells (28.8 ± 7.2 units/ml) was significantly less (P < 0.01) than that of similarly activated normal CD4+ cells from normal controls (95.8 ± 13.6 units/ml). To ascertain if there was a concomitant decrease in IFN-γ production by the leukemic T-cells from Sézary and ATL patients which were deficient in IL-2 secretion, we measured the levels of IFN-γ in these same supernatant samples by ELISA. IFN-γ levels in ATL (0.8 ± 1.9 units/ml) and Sézary (144 ± 164.4 units/ml) were significantly decreased (P < 0.01) below that of normal controls (489.5 ± 143.5 units/ml). Paralleling the decreased mRNA expression of IL-5 in the PHA/PMA-activated leukemic T-cells, IL-5 production was significantly reduced (P < 0.01) in the stimulated ATL (16.8 ± 37.6 pg/ml) and Sézary cells (78.4 ± 175.3 pg/ml) as compared to mitogen-induced CD4+ cells from normal controls (393.4 ± 104.8 pg/ml).

contaminating B-cells (CD20+), monocytes (CD14+), natural killer cells (CD16+), and normal T-cells (CD7+) were effectively removed from the fresh PBMC population following immunomagnetic selection. The purification technique resulted in an enriched population of CD4+ T-cells, indicated by enhanced Leu3 expression in the cells of the patient after negative selection. Similar cell surface expression results were obtained on purified CD4+ T-cell populations isolated from the remaining Sézary and ATL patients, as well as from normal controls (data not shown). These highly purified malignant T-cell populations were studied for their ability to express cytokines on induction.

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Increased IL-4 Expression by Leukemic T-Cells from SzS but Not ATL Patients. Since IL-4 is capable of down-regulating the expression of IL-2 and IFN-γ (19–21), as well as inducing IgE synthesis (22), we wished to evaluate the possibility that increased IL-4 production by the malignant T-cells was responsible for these immune abnormalities in SzS patients. IL-4 mRNA was barely detected by Northern blotting in PHA/PMA-stimulated CD4+ T-cells from normal controls (data not shown). Therefore, we utilized the more sensitive technique of mRNA amplification by PCR to quantitatively analyze the ex vivo mRNA expression of IL-4 from the resting, purified CD4+ cells of patients with SzS or ATL and normal controls. IL-4 transcription, assessed by amplification of a specific 456-base pair sequence, was markedly up-regulated in the leukemic T-cells in 4 of 5 SzS patients as compared to the constitutive low level of expression in ATL and normal controls (Fig. 4).

Dysregulation of IL-4 mRNA expression in the leukemic T-cells from SzS patients was correlated with its gene product by measuring IL-4 levels in the conditioned media from mitogen-activated patient and normal CD4+ T-cells (Table 1). Paralleling the up-regulation of IL-4 transcription in Sézary cells, IL-4 production in PHA/PMA-activated CD4+ T-cells from SzS patients (83.5 ± 15.4 pg/ml) was significantly greater (P < 0.01) than similarly cultured CD4+ cells from ATL patients (5.2 ± 8.4 pg/ml) and normal controls (36.1 ± 9.6 pg/ml). In addition, modest but significantly decreased IL-4 levels were detected in the stimulated ATL cells as compared to the mitogen-induced normal CD4+ cells (P < 0.01). This disparity in IL-4 expression between the leukemic T-cells of SzS and ATL patients suggests that phenotypically similar CD4+ T-cells have the capacity to produce diverse cytokine profiles, leading to altered immunoregulatory functions.

Increased TGF-β1 Secreted by the Leukemic T-Cells from ATL but Not SzS Patients. We had previously reported that freshly isolated PBMC from ATL patients constitutively expressed high levels of TGF-β1 mRNA and secreted TGF-β1 protein into the culture media (9, 18). To determine whether variations in TGF-β1 production may play a role in the immunological differences observed in patients with T-cell malignancies, we compared the level of TGF-β1 protein secreted by purified populations of CD4+ T-cells isolated from ATL and SzS patients, as well as normal controls (Table 2). The resting leukemic T-cells from all ATL patients studied secreted at least 10 times more TGF-β1 protein (mean, 15.4 pm) as measured by a specific sandwich ELISA, than similarly cultured T-cells from SzS patients and normal controls (mean, <0.4 pm).

DISCUSSION

The soluble cytokines secreted by T-cells after antigenic stimulation are the major determinants of T-cell function. Aberrant cytokine production by human T-cells results in an altered immune response and may contribute to the clinical features of these T-lymphoproliferative disorders. In this study we have investigated the cytokine patterns of leukemic T-cells from SzS and ATL patients. We chose to examine such neoplastic T-cells because they represent clonal expansions of cells arrested at specific stages of differentiation that retain distinct and specific immunoregulatory functions. Such an analysis could thus provide insight into the T-cell network that regulates

![Fig. 4. Expression of IL-4 mRNA in the T-lymphoproliferative disorders. Total RNA from the freshly purified CD4+ cells of ATL and SzS patients, as well as from normal controls, was subjected to reverse transcription followed by PCR amplification as described in "Materials and Methods." PCR reaction mixtures were transferred to a nitrocellulose filter and the amplified IL-4 mRNA (456 base pairs [bp]) was detected by hybridization of the filter with a 32P-nick-translated IL-4 cDNA probe. An actin sequence was also targeted for amplification in the same reaction vial as IL-4 using primer pairs homologous to regions within the actin mRNA.](image-url)
normal immune responses. In addition, the functional and clinical differences between these T-lymphoproliferative disorders suggested the existence of alternate patterns of cytokine expression in these leukemic cells with similar mature T-cell surface antigens (CD4+, CD7−, and CD8−). We found that the malignant T-cells from SzS patients produced significantly greater levels of IL-4 than did similarly stimulated normal CD4+ T-cells from ATL patients and normal controls, while exhibiting a profound deficiency in IL-2 and IFN-γ expression. Paralleling this impairment of IL-2 and IFN-γ production in Sézary cells, expression of IL-2 and IFN-γ was markedly reduced in the purified leukemic T-cells from ATL patients as well. However, in contrast to the up-regulation of IL-4 production demonstrated in SzS, we found that the enriched ATL cells secreted diminished amounts of IL-4 but large quantities of TGF-β1. This variation in the cytokine secretion patterns of SzS versus ATL cells identified in our study correlates well with the diverse pathophysiological features associated with these respective T-cell malignancies.

The in vivo immunological abnormalities described in SzS patients are quite similar to the known biological effects of IL-4. Patients with SzS often display decreased T-cell responses to mitogens and antigens, decreased natural killer cell activity, decreased IL-2 and IFN-γ production, and increased levels of IgE (11). Although the mechanism by which Sézary cells directly contribute to the immune status of the patient is unclear, an excessive production of IL-4 by the malignant T-cells would reproduce all of the observed immune abnormalities in SzS patients. IL-4 is a multifunctional cytokine which can deliver stimulatory or inhibitory signals to various cell types (19). It has been shown to inhibit the activation and proliferation of natural killer cells (20), suppress the production of IL-2 and IFN-γ by normal CD4+ T-cells (21), and induce the synthesis of IgE (22). In addition, recent studies have demonstrated that IL-4 may act as a cofactor in the stimulation of Sézary cells (23). Moreover, the quantity of IL-4 produced by the mitogen-activated Sézary cells in this study is of a magnitude where suppression of IL-2 and IFN-γ synthesis has been observed (19, 21). Thus, the overexpression of IL-4 by the leukemic T-cells from SzS patients not only inhibits IL-2 and IFN-γ production but may also stimulate the malignant cells to proliferate. This would result in a selective advantage for the leukemic Sézary cells, enabling them to proliferate while escaping host immune-mediated destruction.

Since the leukemic cells of the SzS patients studied produced significantly greater quantities of IL-4 than did similarly stimulated normal CD4+ T-cells, one may have expected increased serum IgE levels in this population. However, there was no correlation between in vitro production of IL-4 by activated Sézary cells and serum IgE levels (data not shown). Although elevated IgE levels have been detected in about 50% of SzS patients, Vowels et al. (11) could not identify a direct relationship between serum IL-4 and IgE levels. Our results are similar and are probably explained by the fact that neither the serum IL-4 level nor in vitro production of IL-4 are reliable indicators of the IL-4 concentration in the microenvironment of the bone marrow where B-cell maturation occurs.

A similar pattern of decreased IL-2 and IFN-γ production was observed in the purified ATL cells; however, this was not secondary to an induction of IL-4 as seen in the SzS patients but could theoretically be a consequence of the high levels of TGF-β1 secreted by the leukemic T-cells from ATL patients. TGF-β protein begins to accumulate in activated normal T-cells approximately 24 h after IL-2 induction, sending an inhibitory signal to the proliferating T-cell (24). This balance between IL-2 and TGF-β is essential to the regulation of the T-cell response. In the presence of excess TGF-β, IL-2-dependent T-cell proliferation, activation of natural killer cells, and cytotoxic T-cell induction are all inhibited (24-26). In addition, TGF-β interferes with the action of B-cell growth and differentiation factors, leading to inhibition of immunoglobulin secretion by normal stimulated B-cells (27). The biological effects of TGF-β on the cells of the immune system bear a strong similarity to the observed immune abnormalities in patients with ATL. For example, all of our ATL patients displayed impaired cell-mediated immunity as manifested by cutaneous anergy and some acquired opportunistic infections. In fact, ATL patient 1, who secreted at least 5 times more TGF-β1 than the other ATL study patients, had a disseminated cutaneous varicella infection consistent with impaired cell-mediated immunity. In addition, both ATL cells and conditioned media from ATL cell lines are capable of suppressing immunoglobulin synthesis in mitogen-stimulated PBMC from normal controls (8, 28, 29). Thus, increased protein levels of TGF-β1 produced by the ATL cells in vivo may contribute to the immunosuppression observed in these patients.

The leukemic T-cells of both SzS and ATL patients are capable of contributing to T-cell suppression via an induction of IL-4 and TGF-β1, respectively. However, these malignant T-cells differ functionally in ways that parallel the different effects of IL-4 and TGF-β1 on the immune system. Specifically, in immunoglobulin biosynthesis assays, the leukemic T-cells of SzS patients function as helper T-cells when cocultured with normal pokeweed mitogen-stimulated B-cells, whereas ATL cells effectively suppress immunoglobulin production in the same assay (8). This functional difference between the two mature T-cell leukemias is paralleled by the differences in IL-4 and TGF-β1 production between these two T-cell malignancies. Thus, the increased levels of IL-4 produced by Sézary cells, in contrast to ATL, may contribute to their ability to mediate helper function for immunoglobulin biosynthesis. In contrast, the large amounts of TGF-β1 secreted by the leukemic T-cells in ATL, but not SzS, may be one of the important negative regulators of immune function-inhibiting immunoglobulin production in these patients, rendering them extremely vulnerable to life-threatening infections. In fact, in a recent review of infectious complications in ATL patients, White et al. (5) reported an incidence of 0.70 serious infection/patient year, almost 10-fold higher.

<table>
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<th>Patient</th>
<th>ATL</th>
<th>SzS</th>
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<td>Mean ± SD</td>
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<td>83.5 ± 15.4</td>
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*CD4+ T-cells from each of the patients and normal controls were incubated with PHA/PMA for 48 h. Supernatants were then harvested and assayed for IL-4 by ELISA. Values represent the mean IL-4 levels in pg/ml of duplicate samples.*

<table>
<thead>
<tr>
<th>Patient</th>
<th>TGF-β1 (pg)</th>
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<tbody>
<tr>
<td>ATL 1</td>
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<tr>
<td>SzS 3</td>
<td>&lt;0.3</td>
</tr>
<tr>
<td>SzS 4</td>
<td>&lt;0.3</td>
</tr>
<tr>
<td>SzS 5</td>
<td>&lt;0.3</td>
</tr>
<tr>
<td>Normal 4</td>
<td>&lt;0.3</td>
</tr>
<tr>
<td>Normal 5</td>
<td>&lt;0.3</td>
</tr>
</tbody>
</table>

*Patient or normal CD4+ T-cells were cultured for 72 h in serum-free media as described in ‘Materials and Methods.’ TGF-β1 levels were assayed by a specific sandwich ELISA for TGF-β1. The results represent the mean ± SD of three measurements.*

### Table 1 Production of IL-4 (pg/ml) by stimulated CD4+ T-cells from patients and controls

<table>
<thead>
<tr>
<th>Patient</th>
<th>ATL</th>
<th>SzS</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>19.4</td>
<td>108.3</td>
<td>32.6</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>68.2</td>
<td>45.3</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>85.7</td>
<td>21.2</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>81.1</td>
<td>38.7</td>
</tr>
<tr>
<td>5</td>
<td>6.7</td>
<td>74.0</td>
<td>43.2</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>5.2 ± 8.4</td>
<td>83.5 ± 15.4</td>
<td>36.1 ± 9.6</td>
</tr>
</tbody>
</table>

*CD4+ T-cells from each of the patients and normal controls were incubated with PHA/PMA for 48 h. Supernatants were then harvested and assayed for IL-4 by ELISA. Values represent the mean IL-4 levels in pg/ml of duplicate samples.*

*Significantly less than normal controls (P < 0.01).*

*Significantly greater than normal controls (P < 0.01).*
than that observed in SzS. Aberrant expression of IL-4 in SzS and TGF-β in ATL are most consistent with the functional differences between these T-lymphoproliferative disorders and may therefore play roles in the pathogenesis of the immunological disorders associated with these leukemias of immunoregulatory cells.

Finally, the Sézary cytokine secretion pattern demonstrated in this study, namely the up-regulation of IL-4 with a concomitant decrease in IL-2 and IFN-γ production, is reminiscent of the murine Th2 cell. Studying the mouse, Mossmann et al. (30) demonstrated two patterns of cytokine production from CD4+ expressing T-cell clones that parallel the functional differences among these cell populations. Initially all the cell lines made IL-2; then at a later phase they divided into two populations. The Th1 population expressed IL-2 and IFN-γ and mediated a cytotoxicity response, whereas the Th2 population expressed IL-4, IL-5, IL-6, and IL-10 and was associated with antibody production. Although this dichotomy of CD4+ T-cells based on their respective cytokine profiles was initially described in mice, Th2-like clones were soon isolated from humans with severe atopic disease (31). Our demonstration of enhanced IL-4 production, accompanied by suppression of IL-2 and IFN-γ in the clonal T-cells of SzS patients, suggests that SzS may represent a human Th2-like proliferative disease. Moreover, the Th1 and Th2 responses are reciprocally controlled such that induction of one response normally leads to suppression of the other. For example, IFN-γ from Th1 cells suppresses the induction of IL-4 and IL-5 from Th2 cells (32). This has therapeutic implications for the treatment of SzS. Thus, the exogenous administration of IFN-γ could inhibit the enhanced production of IL-4 and thereby achieve augmented natural killer cell activity and decreased proliferation of leukemic Sézary cells. In fact, in a recent Phase I trial some SzS patients manifested a significant clinical response when treated with recombinant IFN-γ (33). As our understanding of the immunopathogenesis of the T-cell leukemias and their paraneoplastic clinical features becomes clearer, we should be able to design more rational and highly specific therapeutic approaches for these aggressive hematological malignancies. In addition, we may gain insight into the role of Th2-like CD4+ T-cells in other human immunological processes.

REFERENCES

25. David, P. A. Phase II study of recombinant human interferon γ for treatment of cutaneous
Abnormal Cytokine Expression in Sézary and Adult T-Cell Leukemia Cells Correlates with the Functional Diversity between These T-Cell Malignancies


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