Circulating Levels of Cytokines and Soluble Cytokine Receptors in Various T-Cell Malignancies

Syed Raziuddin, Ph.D., M.R.C.Path., Anwar Sheikha, M.B., Ch.B., M.R.C.Path.,† Saeed Abu-Eshy, M.D.,‡ and Mansour Al-Janadi, M.D.§*

Background. **Cytokines, interleukin (IL)-4, IL-6, interferon-gamma (IFN-γ), tumor necrosis factor–alpha (TNF-α), soluble CD23 (sCD23), and soluble IL-2 receptors (sIL-2R) are mediators of inflammation and immune response. Alterations in immune status of patients with various cancers may result in release of cytokines in circulation. The authors measured the circulating levels of IL-4, IL-6, IFN-γ, TNF-α, sCD23, and sIL-2R from patients with T-cell chronic lymphocytic leukemia (T-CLL), T-cell acute lymphoblastic leukemia (T-ALL) and peripheral T-cell lymphoma (PTCL) to determine their importance in these T-cell disorders.**

Methods. **IL-4, IL-6, IFN-γ, TNF-α, sCD23, and sIL-2R levels were measured from the serum samples by enzyme-linked immunosorbent assay or bioassay methods.**

Results. **IL-4 levels were higher only in T-CLL, whereas, IFN-γ and sIL-2R levels were higher in T-CLL and T-ALL. However, IL-6, TNF-α, and sCD23 levels were higher in PTCL.**

Conclusions. **T-cell–derived IL-4 and IFN-γ in T-CLL may act as an autocrine growth factor for proliferation of neoplastic T-cells. The sIL-2R levels in T-CLL, T-ALL, and PTCL are an indication of the degree of T-cell or immune activation due to concomitant immunologic processes in these disorders. However, IL-6, TNF-α, and sCD23 levels may contribute to inflammatory response and provide evidence of monocyte/macrophage, T-cell, or B-cell aberrations in PTCL. *Cancer* 1994; 73:2426–31.**

Key words: T-cell malignancy, cytokines, interleukin-4, interferon-γ, interleukin-6, tumor necrosis factor–α, soluble CD23, soluble interleukin-2 receptors.

Cytokines are the major modulators of immune response and inflammatory process in human disease.[1,2] Interleukin (IL)-4, IL-6, interferon-gamma (IFN-γ), and tumor necrosis factor–alpha (TNF-α) are the prime cytokines that are secreted by T-cells, B-cells, monocytes/macrophages, and other cells that are involved in homeostasis and pathogenesis of various immune disorders, including malignancy.[3] Thus it is important to identify the exact role of individual cytokines in immunopathogenesis of various malignant diseases.

The pathogenesis of T-cell malignancy, T-cell chronic lymphocytic leukemia (T-CLL), T-cell acute lymphoblastic leukemia (T-ALL), and peripheral T-cell lymphoma (PTCL) is unknown. However, cellular proliferation of malignant T-cells is believed to be regulated by a network of cytokines with autocrine and pleiotropic activities. Patients with T-CLL, T-ALL, and PTCL demonstrate alteration of normal immune function.[4–11] However, evidence of abnormal cytokine secretion in circulation and their regulation in these T-cell neoplasms is less definite. As part of a continuing program to define immunoregulatory abnormalities in T-cell malignancy,[7–11] we measured the cytokine, IL-4, IL-6, IFN-γ, TNF-α, soluble CD23 (sCD23) and cytokine receptor, soluble IL-2 receptor (sIL-2R) levels from serum samples of patients with T-CLL, T-ALL, and PTCL. These studies showed that serum levels of IL-4 were higher in T-CLL than in T-ALL and PTCL. However, IL-6, TNF-α, and sCD23 levels were significantly elevated in PTCL than in other patient groups and in healthy control subjects. These studies should lead to a better understanding of the physiologic role of various cytokines in T-cell lymphoproliferative disorders.

Patients and Methods

Patients

Patients with a diagnosis of T-CLL, T-ALL, or PTCL disease were selected from those hospitalized in our in-

From the Departments of *Clinical Immunology, †Clinical and Laboratory Hematology (Pathology), ‡Surgery, and §Medicine, King Saud University, College of Medicine, and Asir Central Hospital, Abha, Saudi Arabia.

Address for reprints: Syed Raziuddin, Ph.D., M.R.C.Path., College of Medicine, Abha, P.O. Box 641, Abha, Saudi Arabia.

Accepted for publication December 30, 1993.

stitution's teaching hospital (Asir Central Hospital, Abha) between April 1989 and January 1993. The patients were diagnosed according to the characteristic clinical, hematologic, and histologic criteria.[7–11] The patients with T-CLL, age 54–69 years (9 men and 5 women) were diagnosed based on hypercellular bone marrow and high leukocyte counts in blood: 28.5–167.3 $\times 10^{-3}/\mu l$. Patients with T-CLL had moderate to massive splenomegaly without significant lymphadenopathy. The leukemic cells had abundant cytoplasm with azurophilic granules (in most cases) in variable amounts. Nonspecific esterase activity and alkaline phosphatase reaction were negative. The acid phosphatase and periodic acid-Schiff reaction were positive. Patients with T-ALL, age 7–19 years (13 males and 5 females) were diagnosed based on replacement of bone marrow with more than 70% of leukemic blasts and high leukocyte counts in blood: 87.0–210 $\times 10^{-3}/\mu l$. Most patients with T-ALL disease had characteristic clinical signs, such as fatigue, bone and joint pains, and anemia. Patients with PTCL, age 63–78 years (7 men and 4 women) had characteristic lymphadenopathy (axillary, inguinal, and cervical) and clinical signs (anorexia, asthenia, and weight loss). PTCL disease was histopathologically diagnosed (from biopsied lymph nodes) according to Working Formulation[12] into non-Hodgkin's lymphoma (6 patients had diffuse large cell lymphoma and the other 5 had diffuse, mixed small, and large cell lymphoma).

T-cell origin of T-CLL, T-ALL, and PTCL was established by documentation of more than 90% of the cells (peripheral blood mononuclear cells in T-CLL and T-ALL and lymph node–purified mononuclear cells in PTCL) staining with T-cell reagents expressing one or more markers identified by CD1 (OKT6), CD2 (OKT11), CD3 (OKT3), CD4 (OKT4), CD7 (Leu-9), CD8 (OKT8), CD25 (anti-IL-2R), CD38 (OKT10), CD71 (OKT9), TcR$\alpha\beta$ (T-cell receptor $\alpha\beta$, TcR-1, WT31+), and TcR$\gamma\delta$ (TcR-δ 1, WT31−). These reagents (monoclonal antibodies) were obtained either from Ortho Diagnostics (Raritan, NJ) or Becton-Dickinson (Mountain View, CA). Staining was done by a standard indirect immunofluorescence technique described previously.[7–11] There was no serologic evidence of infection by human T-cell leukemia/lymphoma virus I/II; Epstein–Barr virus; human immunodeficiency virus; or hepatitis A, B, or C virus in any of these patients with T-cell malignancy. Patients with evidence of bacterial, viral, and parasitic infection were not included in these studies. The serum samples obtained were aliquoted and stored at −80°C after the firm diagnosis of respective disease (T-CLL, T-ALL, or PTCL) and before commencement of any therapy. The samples were thawed once for each cytokine assay. The healthy control subjects (n = 20, age 8–60 years) had no history of recent illness.

Cytokine Assays

IL-4 and IL-6 levels were measured with commercially available enzyme-linked immunosorbent assay kits (ELISA, Inter-Test IT-4 and Inter-Test IT-6, Genzyme Corp., Boston, MA). We determined that the quantitation of IL-6 by ELISA gives almost identical results compared with a bioassay procedure using IL-6–responsive murine hybridoma cell line, MH60.BSF2 (a gift of Dr. T. Kishimoto, Institute for Molecular and Cellular Biology, Osaka University, Osaka, Japan). IFN-γ was measured in an ELISA in which two monoclonal antibodies recognizing different epitopes of IFN-γ (Genzyme; Hoffman-La Roche, Basel, Switzerland) were used as catcher and tracer antibodies, respectively.[13] The TNF-α and sIL-2R levels were measured by ELISA as described previously.[13,14] The sCD23 levels were measured with a commercially available ELISA kit (sCD23, The Binding Site, Institute of Research and Development, Birmingham, U.K.) according to the manufacturer's protocol. In each assay, the appropriate recombinant human cytokines were used to generate the standard curves.

Results

The immunophenotypic results of the circulating peripheral blood mononuclear cells from T-CLL and T-ALL cases and lymph node–purified mononuclear cells from PTCL cases are shown in Table 1. This immunophenotype was established by staining of mononuclear cells between 91% and 98% with CD3+, CD4+, CD8+, TcR$\alpha\beta$+, and TcR$\gamma\delta$+ monoclonal antibodies. None of the cells from T-CLL, T-ALL, or PTCL cases showed significant reactivity for anti-kappa, anti-lambda immunoglobulin light chains or for B-cell reagents (CD19 and CD20). However, expression of activation antigen markers defined by CD25, CD38, and CD71 varied case by case, from 11% to 73% (data not shown). Of interest, 3 of 14 cases from T-CLL, 5 of 18 cases from T-ALL, and 4 of 11 cases from PTCL expressed TcR$\gamma\delta$. In addition, all T-ALL cases expressed 78–96% CD7+ antigens.

The serum levels of IL-4, IL-6, IFN-γ, and TNF-α from patients with T-CLL, T-ALL, and PTCL and healthy control subjects are summarized in Figures 1 and 2. The IL-4 levels were found to be elevated only from T-CLL. However, IFN-γ levels were higher in T-CLL and T-ALL than in PTCL. Of interest, IL-6 and TNF-α levels were low in T-CLL and T-ALL but high in PTCL ($P < 0.001$). IL-4 and IFN-γ levels were significantly higher ($P < 0.001$) in T-CLL than in PTCL.

Table 1. Immunophenotype of T-CLL, T-ALL and PTCL Cases*

Immunophenotype	No. of cases
T-CLL	
CD3+ CD4+ CD8− TcR$\alpha\beta$+	6
CD3+ CD4− CD8− TcR$\gamma\delta$+	3
CD3+ CD4+ CD8+ TcR$\alpha\beta$+	2
CD3− CD4+ CD8− TcR$\alpha\beta$+	1
CD3+ CD4− CD8+ TcR$\alpha\beta$+	2
T-ALL	
CD3+ CD4+ CD8− TcR$\alpha\beta$+	7
CD3+ CD4− CD8+ TcR$\alpha\beta$+	3
CD3+ CD4− CD8− TcR$\gamma\delta$+	5
CD3+ CD4+ CD8+ TcR$\alpha\beta$+	3
PTCL	
CD3+ CD4+ CD8− TcR$\alpha\beta$+	5
CD3+ CD4− CD8− TcR$\gamma\delta$+	3
CD3+ CD4− CD8− TcR$\alpha\beta$+	2
CD3− CD4− CD8+ TcR$\gamma\delta$+	1

T-CLL: T-cell chronic lymphocytic leukemia; T-ALL: T-cell acute lymphoblastic leukemia; PTCL: peripheral T-cell lymphoma.

* The immunophenotyping data of circulating blood mononuclear cells from T-CLL and T-ALL cases and lymph node purified mononuclear cells from PTCL cases are given.

The serum levels of sIL-2R and sCD23 are shown in Figure 3. The sIL-2R levels were significantly elevated ($P < 0.0001$) from T-CLL and T-ALL compared with healthy control subjects. However, sIL-2R levels were significantly lower ($P < 0.001$) in PTCL than in T-CLL and T-ALL.

Elevated levels of sCD23 were found in 9 of 11 patients with PTCL and only 2 of 14 patients with T-CLL but in none of the patients with T-ALL or healthy control subjects.

Discussion

We examined the levels of various cytokines in the circulation of patients with T-cell lymphoproliferative disorders. These studies were designed to evaluate the abnormalities present in T-CLL, T-ALL, and PTCL disease regarding the secretion of T-cell–derived, IL-4, IFN-γ, and sIL-2R cytokines. The results demonstrate distinct abnormalities in secretion of IL-4, IFN-γ, and sIL-2R levels between T-CLL, T-ALL, and PTCL. Increased levels of circulating IL-4 were found only in T-CLL, whereas IFN-γ and sIL-2R levels were higher in T-CLL and T-ALL. Thus, patients with PTCL had significantly low levels of all three T-cell–derived cytokines, IL-4, IFN-γ, and sIL-2R compared with patients with T-CLL and T-ALL. However, IL-6 and TNF-α, believed to be the proinflammatory cytokines secreted normally by monocytes/macrophages and activated T-cells, were elevated from PTCL. Moreover, sCD23, a factor released by B-cells, was also found in higher concentrations in the circulation of patients with PTCL compared with patients with T-CLL or T-ALL or in healthy control subjects. Several factors may contribute to the abnormality in soluble cytokine receptor levels, including abnormal increased secretion or a decreased turnover metabolism in these disorders. The immunoregulatory abnormalities of the immune system and the mechanisms by which these factors are secreted and released in circulation in T-cell malignancy remains to be elucidated. However, based on earlier observations of cytokine influence in pathogenesis[1–3] and induction of inflammatory response, it is likely that cytokines secreted in T-cell malignancies may be the important mediators of clinical disease.

T-cell activation represents a novel feature of T-cell malignancy.[9,10,15] Evidence of T-cell activation is the

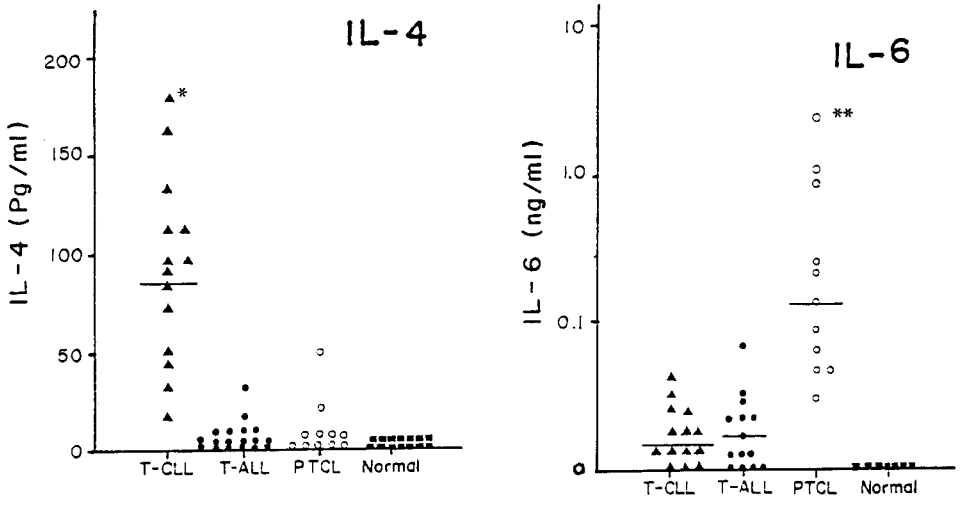

Figure 1. Serum levels of IL-4 and IL-6 in patients with T-CLL, T-ALL, PTCL and normal controls. *$P < 0.001$ versus T-ALL and PTCL; **$P < 0.001$ versus T-CLL and T-ALL.

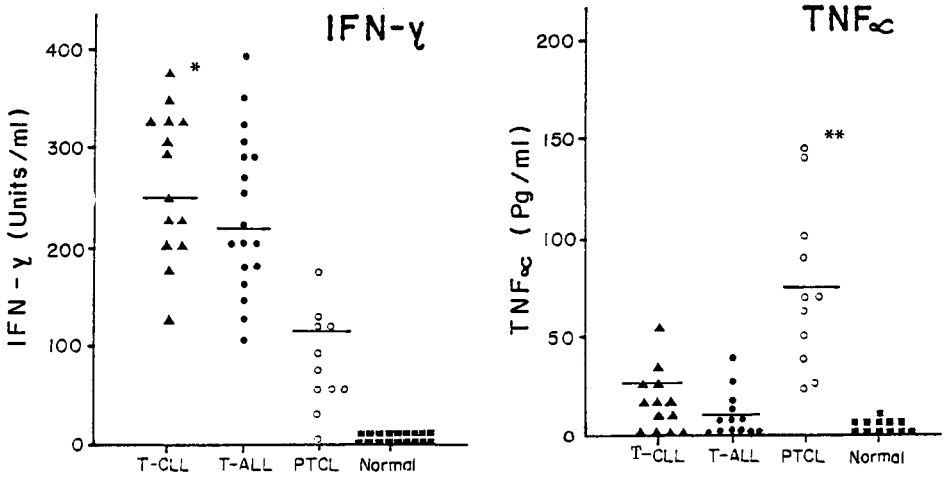

Figure 2. Serum levels of IFN-γ and TNF-α in patients with T-CLL, T-ALL, and PTCL and in healthy control subjects. *$P < 0.001$ versus PTCL; **$P < 0.001$ versus T-CLL and T-ALL.

presence of increased levels of circulating sIL-2R.[13] Small amounts of sIL-2R are present in the serum of healthy persons, and increased levels were observed in different T-cell disorders as a consequence of T-cell activation.[15] Moreover, sIL-2R production is not unique to diseases of T-cell disorders; occasionally, elevated sIL-2R levels were observed in patients with B-cell malignancy,[16] hairy cell leukemia,[17] and autoimmune and rheumatic diseases.[18] Comparing serum sIL-2R levels between T-CLL, T-ALL, and PTCL suggests a difference that allows the categorization of patients based on sIL-2R levels. Production of sIL-2R is also subject to significant interpatient variations. It is possible that neoplastic T-cells in the blood of patients with T-CLL and T-ALL disease express widely different amounts of cell surface receptors, IL-2R, and secrete different amounts of sIL-2R into circulation.

IL-2R expression can also occur on monocytes and natural killer cells, both of which may be abnormal in PTCL. Therefore, the higher levels of sIL-2R in patients with PTCL than in healthy control subjects may be derived from IL-2R–bearing monocytes, natural killer cells, B-cells, or T-cells. Because it is impossible to discriminate malignant cell–derived sIL-2R from sIL-2R

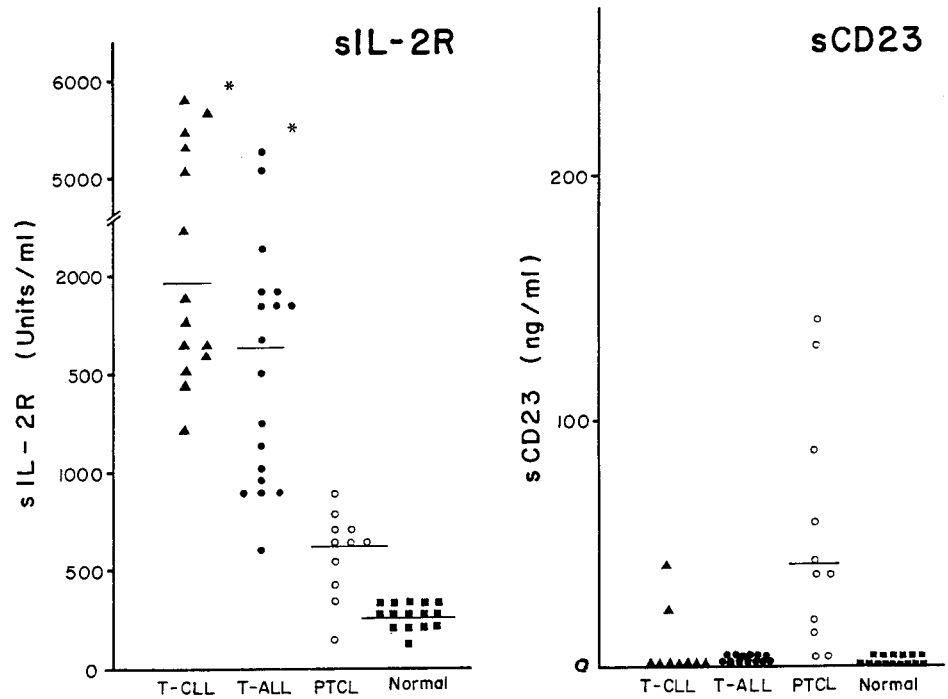

Figure 3. Serum levels of sIL-2R and sCD23 in patients with T-CLL, T-ALL, and PTCL and in healthy control subjects. *$P < 0.0001$ versus healthy control subjects.

released by mononuclear cells in malignancy, the source of sIL-2R in the patients investigated remains unclear. Moreover, the elevations in IFN-γ and sIL-2R seen in T-CLL and T-ALL but not in PTCL may be related to the fact that these two (T-CLL and T-ALL) are disorders of circulating cells, whereas PTCL tends to be a node-based disease.

sCD23 and IL-4 are implicated in B-cell regulation/differentiation and IgE synthesis.[19–22] sCD23 levels were present in the circulation of PTCL. However, sCD23 elevation was not related to IL-4 levels in PTCL. Similarly, we did not observe higher levels of serum IgE in T-CLL (where IL-4 levels were higher) or in PTCL (where sCD23 levels were higher) (data not shown). IL-4 is a T-cell–derived cytokine,[21,22] therefore, the major source of IL-4 in T-CLL may be neoplastic T-cells. Conversely, CD23 is not expressed in T-cells, natural killer cells, or monocytes/macrophages.[23] But CD23 was predominantly present in B-cells.[23] Therefore, circulating sCD23 observed in PTCL may be derived from B-cells. Thus, B-cell aberrations seem clear in PTCL, as evidenced by sCD23 release in circulation. Alternatively, this may be a normal response, and sCD23 may be released by B-cells in response to a T-cell neoplasm.

IL-6 and TNF-α levels differed significantly between T-cell leukemia (T-CLL and T-ALL) and T-cell lymphoma (PTCL). The highly elevated IL-6 and TNF-α levels in PTCL may contribute to the known pathologic behavior of this disease and may have important clinical and immunologic implications. Moreover, the patients with high levels of IL-6 also have high levels of TNF-α, suggesting the synergistic activity between the two proinflammatory cytokines in the biologic activity and immunopathologic process. IL-6 and TNF-α are the prime inflammatory mediators, which produce severe damage to endothelial cells and are implicated in acute phase response, fever, and septicemia.[2,24,25] Therefore, it is likely that typical clinical presentations of patients with PTCL disease, such as fever, asthenia, anorexia, weight loss, and lymphadenopathy, is a function of IL-6 and TNF-α. However, the biologic relevance of these cytokines, IL-6, TNF-α, and sCD23 to the pathogenesis of PTCL needs to be elucidated.

The immunologic basis for the appearance of such cytokines in T-cell malignancy is unknown. Although studies of in vitro cytokine production and release from cell cultures are important, they may provide information only on the potential cell responses to a stimulus that is irrelevant in vivo, and they cannot take into account the influence of multiple cytokines and other mediator combinations. The possibility that the rate of change in circulating cytokine levels in T-cell malignancy after treatment may have prognostic value could not be tested with our retrospective study but will be addressed in combination with other reliable parameters.

In summary, it appears that a distinct and recognizable cytokine profile may be present in T-CLL, T-ALL, and PTCL for various reasons. The high circulating levels of T-cell–derived factors, IL-4, and IFN-γ in T-CLL may influence T-cell function and contribute to proliferation of neoplastic T-cells. The levels of sIL-2R probably are an indication of the variable degree of T-cell or mononuclear cell activation in T-CLL, T-ALL, and PTCL, whereas elevated IL-6, TNF-α, and sCD23 levels in PTCL may be important in eliciting alteration in the physiologic, biochemical, and immunologic status in this disease. Thus, these cytokines may play a key role in a cytokine network, and a malignant disease may be characterized by unbalanced and unregulated cytokine production by the immune system released in circulation.

References

1. Dinarello CA, Mier JM. Current concepts: lymphokines. *N Engl J Med* 1987; 317:940–5.
2. Beutler B, Cerami A. The biology of catechin/TNF: a primary mediator of the host response. *Ann Rev Immunol* 1989; 7:625–55.
3. Malkovsky M, Sondel PM, Strober W, Dalgleish AG. The interleukins in acquired disease. *Clin Exp Immunol* 1988; 74:151–61.
4. Foon KA, Todd RF. Immunologic classification of leukemias and lymphomas. *Blood* 1986; 68:1–23.
5. Knowles DM, Halper JP. Human T cell malignancies: correlative clinical, histopathologic, immunologic and cytologic analysis of 23 cases. *Am J Pathol* 1982; 106:187–203.
6. Weiss LM, Crabtree GS, Rouse RV, Warnke RA. Morphologic and immunologic characterization of 50 peripheral T cell lymphomas. *Am J Pathol* 1985; 118:316–24.
7. Raziuddin S, Hussain NK, Latif AB. A monoclonal antibody and functional study of malignant T-cells of a patient with suppressor T-cell lymphoma. *Clin Immunol Immunopathol* 1987; 45:230–4.
8. Raziuddin S, Sheikha A, Teklu B. Humoral immunodeficiency in T-cell chronic lymphocytic leukemia: an immunologic assessment. *Cancer* 1991; 67:2518–22.
9. Raziuddin S, Sheikha A, Latif AB. T-cell chronic lymphocytic leukemia: T-cell function and lymphokine secretion. *Cancer* 1992; 69:1146–52.
10. Raziuddin S, Malatani T, Al-Sedairy S, Al-Saigh A. Peripheral T-cell lymphomas: immunophenotype, lymphokine production and immunologic functional characterization of the lymph node malignant T-cells. *Am J Pathol* 1991; 139:1181–9.
11. Sheikha A, Al-Janadi M, Al-Saigh A, Malatani T, Raziuddin S. Peripheral T-cell lymphoma with unique immunologic features. *Cancer* 1993; 71:831–8.
12. National Cancer Institute sponsored study of classification of non-Hodgkin's lymphomas: summary and description of a working formulation for clinical usage. *Cancer* 1982; 49:2112–35.
13. Al-Janadi M, Al-Balla S, Al-Dalaan A, Raziuddin S. Cytokine

profile in systemic lupus erythematosus, rheumatoid arthritis and other rheumatic diseases. *J Clin Immunol* 1993; 13:58–67.
14. Raziuddin S, Shetty S, Ibrahim A. Soluble interleukin-2 receptor levels and immune activation in patients with schistosomiasis and carcinoma of urinary bladder. *Scand J Immunol* 1992; 35: 637–41.
15. Zambello R, Pizzolo G, Trentin L, Agostini C, Chisesi T, Vinante F, et al. Evaluation of serum soluble interleukin-2 receptor in patients with chronic lymphoproliferative disorders of T cells. *Cancer* 1989; 64:2019–23.
16. Chilosi M, Semenzato G, Vinante F, Menestrina F, Piazzola E, Focchiatti V, et al. Increased levels of soluble interleukin-2 receptor in non-Hodgkin's lymphomas. Relationship with clinical, histologic and phenotypic features. *Am J Clin Pathol* 1989; 92: 186–91.
17. Richards JM, Mick R, Latta JM, Daly K, Ratain MJ, Vardiman JW, et al. Serum soluble interleukin-2 receptor is associated with clinical and pathologic disease status in hairy cell leukemia. *Blood* 1990; 76:1941–5.
18. Semenzato G, Bambara LM, Biaso D. Increased serum levels of soluble interleukin-2 receptor in patients with systemic lupus erythematosus and rheumatoid arthritis. *J Clin Immunol* 1988; 8: 447–52.
19. Guy GR, Gordon J. Coordinated action of IgE and a B-cell stimulatory factor on the CD23 receptor molecule upregulates B lymphocyte growth. *Proc Natl Acad Sci USA* 1987; 84:6239–43.
20. Gordon J, Flores-Romo L, Cairns JA. CD23—a multifunctional receptor/lymphokine. *Immunol Today* 1989; 10:153–7.
21. Paul WE. Interleukin-4: a prototypic immunoregulatory lymphokine. *Blood* 1991; 71:1859–70.
22. Callard RE. Immunoregulation by interleukin-4 in man. *Br J Haematol* 1991; 78:293–9.
23. Bengtsson M, Gordon J, Flores-Romo L, Cairns JA, Smedmyr B, Oberg G, et al. B-cell reconstitution after autologous bone marrow transplantation: increase in serum CD23 (IgE binding protein) precedes IgE and B-cell regeneration. *Blood* 1989; 73:2139–44.
24. Kishimoto T. The biology of interleukin-6. *Blood* 1989; 74:1–10.
25. Van Snick J. Interleukin-6: an overview. *Ann Rev Immunol* 1990; 8:253–78.