Clin. exp. Immunol. (1984) **57**, 331–337.

Evidence of cells bearing interleukin-2 receptor at sites of disease activity in sarcoid patients

G. SEMENZATO, C. AGOSTINI, L. TRENTIN, R. ZAMBELLO, M. CHILOSI, A. CIPRIANI, E. OSSI, M. R. ANGI, L. MORITTU & G. PIZZOLO *Padua University School of Medicine, Department of Clinical Medicine, 1st Medical Clinic, Clinical Immunology, Pneumology and Eye Clinic; Verona University School of Medicine, Departments of Pathology and Haematology, Italy*

(Accepted for publication 29 February 1984)

SUMMARY

The frequency of cells reactive with anti-Tac monoclonal antibody (MoAb), which recognizes the interleukin-2 (IL-2) receptor, has been evaluated in cell suspensions from peripheral blood and bronchoalveolar lavage (BAL), and in frozen sections from involved tissues in 18 patients with active sarcoidosis. Peripheral blood lymphocytes of sarcoid patients do not bear Tac determinant and reduced numbers of Tac^+ cells are inducible following PHA stimulation. On the other hand, significant numbers of lymphocytes reactive with anti-Tac MoAb are present in the cells obtained from the BAL and a number of Tac^+ cells infiltrate the lung, lymph node and conjunctiva. The finding of Tac^+ cells in the BAL fluid and in other organs in patients with sarcoidosis provides evidence that some T cells in these involved tissues have the characteristics of IL-2 responder cells and thus the potential to absorb IL-2, supporting the hypothesis that T lymphocytes replicate *in situ* at sites of disease activity.

Keywords active sarcoidosis disease activity sites interleukin-2 receptor anti-Tac monoclonal antibody

INTRODUCTION

Sarcoidosis is currently defined as a multisystem disease of unknown aetiology characterized by enhanced immune responses at sites of disease activity. Evidence of activated cells has been provided in different involved tissues in these patients (James & Jones Williams, 1982; Semenzato & Pezzutto, 1982; Crystal *et al.*, 1983). Comparative evaluation of T cell subsets defined by monoclonal antibodies (MoAb) in the blood and at sites of disease activity revealed in patients with active sarcoidosis a redistribution of activated lymphocytes with helper related phenotype (Hunninghake & Crystal, 1981; Semenzato *et al.*, 1982, 1984; Modlin *et al.*, 1983), supporting the hypothesis that these cells continually migrate from the blood to different organs. The question is posed whether or not T lymphocyte in this disease are able to proliferate *in situ*.

The spontaneous production of interleukin-2 (IL-2) has been recently reported in the lung from patients with active sarcoidosis by Pinkston, Bitterman & Crystal (1983) and by Hunninghake *et al.* (1983). Since the reactivity of T cells to IL-2 relies on the presence of specific receptors for IL-2 (Robb, Munck & Smith, 1981), our approach to this problem is by evaluating cells reactive to anti-Tac MoAb, both in the blood and in different involved tissues of sarcoid patients. We also evaluated the ability of sarcoid peripheral blood lymphocytes to express Tac antigen after *in vitro*

Correspondence: Dr G. Semenzato, Istituto di Medicina Clinica, 35100 Padova, Italy.

activation. In fact this reagent, originally described as a marker of activation (Uchiyama, Broder & Waldmann, 1981), is expressed on proliferating T cells and has been found to recognize the membrane receptor for IL-2 on activated T cells (Leonard *et al.*, 1983). More specifically, direct demonstration of the identity of IL-2 binding protein and the Tac antigen has recently been provided by Robb & Greene (1983).

MATERIALS AND METHODS

Patients. Eighteen patients with active sarcoidosis (10 women and eight men; mean age 32 ± 7 years) were studied. In all cases the diagnosis was made by biopsy obtained from lungs, lymph nodes or liver showing non-caseating epithelioid cell granulomas, with no evidence of inorganic material known to cause granulomatous diseases. According to radiological pulmonary changes, the patients were classified into stage I (10 patients), stage II (seven patients), and stage III (one patient). Disease activity was evaluated according to (1) clinical features and laboratory findings including the percentage of T cells in the bronchoalveolar fluid; (2) the positivity of the ^{67}Gallium scan and (3) the level of serum angiotensin converting enzyme activity (SACE). Nine were studied at the time of diagnosis before any treatment. An additional nine cases had been previously treated with corticosteroids but they were out of treatment for at least 2 months at the time of the study.

Ten normal non-smoking volunteers (six women and four men, with a mean age of 32 ± 5 years) were used as controls for the bood and bronchoalveolar lavage (BAL) studies. For ethical reasons controls from other tissues (lungs, lymph nodes, conjunctiva, skin) were studied only when available from subjects suspected of having different disorders (but which were found to be normal), or during cardiac surgery in patients with no clinical signs of sarcoidosis.

Studies on cell suspensions. Mononuclear cells obtained from freshly drawn heparinized peripheral blood by centrifugation on Ficoll-Hypaque (F/H) gradient, washed three times with phosphate-buffered saline, and resuspended in RPMI 1640 medium.

Peripheral blood mononuclear cells (1×10^6/ml) from normal donors and sarcoid patients were stimulated with 2 μg/ml PHA (Difco, Detroit, Michigan, USA) in RPMI 1640 culture medium supplemented with 15% FCS, 4 mM glutamine, 50 u/ml penicillin and 50 μg/ml streptomycin at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. The cultured cells were washed three times, resuspended in RPMI 1640 medium, and tested for the expression of Tac antigen. Cells recovered from BAL fluid were separated by centrifugation on F/H gradient, washed and resuspended in RPMI 1640 medium. Adherent cells were removed by incubation at 37°C for 90 min in plastic Petri dishes.

The ascitic fluid containing the anti-Tac MoAb was generously provided by Dr Takashi Uchiyama, from the University of Kyoto, Japan. The frequencies of Tac positive cells were determined by indirect immunofluorescence as previously described (Semenzato *et al.*, 1984). Since rosette assays are sometimes more sensitive than immunofluorescence for detecting antigens on cell suspensions, in some cases (Table 2) Tac positive cells were enumerated by techniques other than immunofluorescence. (i) By a rosette assay employing IgG anti-mouse antibodies coated ox red blood cells (OxRBC) prepared according to the $CrCl_3$ method by Ling, Bishop & Jefferis (1977). Fifty microlitres of 2% OxRBC were mixed with 50 μl of cell suspension (5×10^6 ml) that had been treated with anti-Tac MoAb (30 min at 4°C) and the mixture was centrifuged at 200*g* for 5 min. After standing for an additional 30 min the pellet was gently resuspended and percentages of rosetting cells were counted. (ii) By a rosette technique employing Staphylococcal protein A (SPA, Pharmacia Fine Chemicals, Upsala, Sweden) coated OxRBC. Since anti-Tac MoAb belongs to the mouse IgG2 subclass, it is able to bind SPA. One per cent SPA coated OxRBC were prepared by a modification of the method of Gronowicz, Continho & Melchers (1976) and used for rosetting as described above. In order to rule out the possibility that B cells directly bind SPA coated OxRBC via specific receptors, in these experiments a preliminary separation of T cells, performed as previously described in detail (Semenzato *et al.*, 1981), was necessary.

Controls were set up performing parallel rosette tests using anti-Tac untreated cell suspensions plus IgG anti-mouse antibodies coated OxRBC or SPA coated OxRBC. Percentages less than 1% were observed in all experiments.

Studies on tissues. Biopsy specimens were obtained from the lung in six patients by transbronchial lung broncoscopy, from the lymph nodes in five patients during mediastinoscopy, from the skin in three patients and from the conjunctiva in five patients. After excision the specimen was embedded in Ames OCT Compound (Miles Lab., Elkhart, Indiana, USA) and snap frozen in liquid N_2 within 1 h of excision. Five micrometre sections were cut on a cryostat, transferred into albuminized glass slides, air dried, and fixed in 1:1 chloroform–acetone mixture for 5 min at 4°C before staining.

Immunohistological studies with anti-Tac and UCHT-1 (kindly provided by Dr P. Beverly, London, UK) MoAbs were performed using immunofluorescence or immunoperoxidase procedures previously described (Chilosi *et al.*, 1983; Semenzato *et al.*, 1984). Briefly, sections were covered with MoAb in 5–10 μl fluid, incubated for 30 min, washed three times in PBS and stained with goat or rabbit anti-mouse immunoglobulins second layer coupled to fluoresceine isothiocynate (G-anti-MIg-FITC) or peroxidase (R-anti-MIg-Px) for 30 min. After further washing the sections were mounted with a drop of glycerol and coverslip, and then examined under a fluorescence microscope. For immunoperoxidase preparations the histochemical reaction for peroxidase was performed as previously described (Chilosi, Bonetti & Iannucci, 1981) using H_2O_2 and 3,3′-diaminobenzidine.

The possibility that cells under study bind anti-Tac MoAb via Fc receptors was ruled out by evaluating cells suspensions and histological sections after incubation with gamma-globulins Cohn fraction II and further washing. Statistical analysis compared mean and standard error of the mean by the Student's *t*-test.

RESULTS

As shown in Fig. 1, peripheral blood lymphocytes (PBL) from patients with sarcoidosis do not react with anti-Tac MoAb (mean $1{\cdot}2 \pm 0{\cdot}6$ *vs* $0{\cdot}6 \pm 0{\cdot}2$ controls; P = not significant).

When PBL from healthy subjects are activated *in vitro* by PHA they were able to express Tac determinants (Table 1). In contrast, PBL from sarcoid patients, following PHA stimulation, failed to express Tac antigen both in treated and untreated patients (Table 1).

The proportion of BAL lymphocytes reacting with anti-Tac MoAb was higher in patients with active sarcoidosis when compared values obtained in corresponding samples from peripheral blood

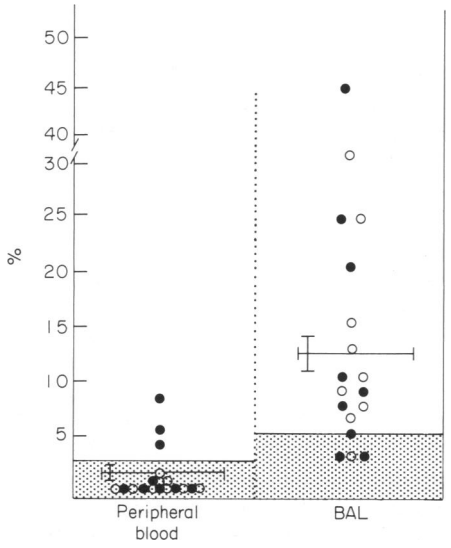

Fig. 1. Anti-Tac positive lymphocytes in cell suspensions from peripheral blood and BAL fluid. ● = untreated and ○ = treated sarcoid patients.

and BAL from controls (13·4 ± 2·6 *vs* 1·2 ± 0·6 PBL and 2·2 ± 0·5 BAL controls). Also in this case we did not find differences between patients who received therapy and those who did not (13·7 ± 4·9 untreated patients; 12·3 ± 2·8 treated patients).

Evaluation with techniques other than immunofluorescence using different indicators did not show major differences in the frequencies of Tac^+ cells obtained (Table 2). Even if rosetting assay using OxRBC coated with IgG anti-mouse antibody appears more sensitive than direct immunofluorescence method, differences between the two techniques were not statistically significant (P = not significant).

Immunohistological evaluation of involved lymph nodes revealed the presence of several Tac^+ cells. These exhibited a lymphoid appearance and were mainly located around and within epithelioid granulomas (Fig. 2b). A variable diffuse Tac positivity was also detectable in the majority of epithelioid macrophages. When present in the other parenchymas studied by immunohistology (lung five of six, conjunctiva four of five and skin none of three), Tac^+ cells were mainly scattered or clustered in small aggregates with no obvious correlation with epithelioid macrophages.

In reactive lymph nodes immunostained as controls Tac^+ lymphoid cells were usually scanty, less numerous than in sarcoid lymph nodes, and mainly scattered through the perivascular areas. On the contrary no Tac^+ cells were found on samples of normal skin ($n = 3$) and lung ($n = 3$).

Table 1. Percentages of Tac^+ cells in peripheral blood lymphocytes after 72 h culture

		No. of expt	Unstimulated lymphocytes	PHA stimulated lymphocytes
Controls		10	< 5	63·7 ± 3·9
Sarcoidosis	Untreated	8	< 5	35·5 ± 4·5
	treated	7	< 5	34·5 ± 4·2
P			NS	< 0·001

Table 2. TAC^+ cells as defined by different techniques in sarcoidosis patients

Source of cells	No. of expt	Indirect immunofluorescence	IgG anti-mouse antibody coated OxRBC	SPA coated OxRBC	P
Blood	5	< 3	< 3	< 3	NS
PHA stimulated blood cells	3	34·3 ± 4·0	38·7 ± 5·2	—	NS
BAL fluid	6	9·0 ± 3·7	11·1 ± 3·8	9·8 ± 3·4	NS

DISCUSSION

Our study demonstrated that (1) PBL of patients with sarcoidosis do not bear Tac determinant and reduced frequencies of Tac^+ cells are inducible following PHA stimulation; (2) significant numbers of Tac^+ lymphocytes are present in the cells obtained from BAL and (3) a discrete number of Tac positive cells infiltrate different sarcoid tissues (lung, lymph nodes, conjunctiva).

Tac antigen is primarily expressed on proliferating T cells (Uchiyama *et al.*, 1982). It is easily inducibile on the *in vitro* stimulated T cells, but Tac^+ T cells have not been identified in peripheral blood in normal and various disease states with the exception of malignant cells from patients with

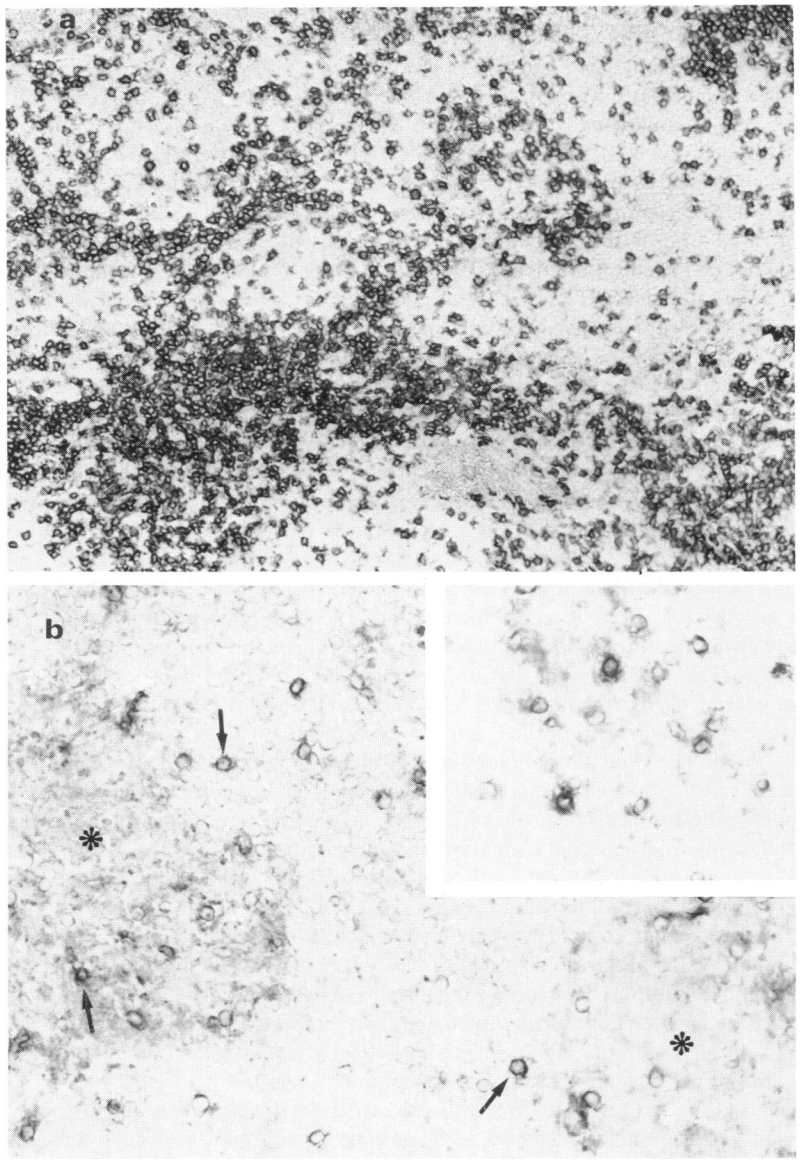

Fig. 2. Cryostat sections of a sarcoid lymph node immunostained with (a) a pan T MoAb (UCHT-1) and (b) anti-Tac MoAb, using a indirect immunoperoxidase technique. A large number of T cells infiltrate sarcoid tissue (a, magnification × 150) and several Tac^+ lymphoid cells (b, magnification × 250; the arrows indicate three of these cells) are scattered in the tissue with a preferential homing around and within (see inset, magnification × 400) the epithelioid granulomas (asterisks).

Japanese adult T cell leukaemia (ATL) (Hattori *et al.*, 1981) or following tetanus toxoid inoculation (Yachie *et al.*, 1983). In addition, Tac antigen is expressed on about 10% of cerebrospinal fluid cells in mumps meningitis (Taniguchi *et al.*, 1983), and on a discrete proportion of T cells obtained from the inflamed synovial tissues or fluids from patients with inflammatory rheumatic diseases (Burmester *et al.*, 1983).

The presence of Tac determinant on lymphocytes from BAL of sarcoid patients indicates that these cells in the lung are activated. In line with the presence of Tac^+ cells in BAL fluid on the basis

of the flow cytometry analysis, Pacheco *et al.* (1982) demonstrated that alveolar T lymphocytes in sarcoidosis are in the G_1 phase, which is the cycle phase where Tac antigen is primarily expressed at the cellular surface (Taniguchi *et al.*, 1983).

More interestingly, anti-Tac MoAb appears to recognize the membrane receptors for IL-2 on activated T cells (Leonard *et al.*, 1983; Robb & Greene, 1983). Following mitogen or antigen activation, a subset of T cells (IL-2 producer cells) co-operates with adherent cells and releases IL-2. Another T cell subset (IL-2 responder cells) simultaneously acquires the capacity to react to IL-2, expressing specific receptors (Smith *et al.*, 1979). The finding of Tac^+ cells in the BAL fluid from sarcoid patients suggests that some lung T cells in this disease have the characteristic of IL-2 responder cells and thus the potential to absorb IL-2. Consistent with the present study, Pinkston *et al.* (1983) and Hunninghake *et al.* (1983) independently described increased production of IL-2 by lung T cells in active sarcoidosis, providing evidence that IL-2 producer cells are increased in these patients. Taken together, these observations favour the hypothesis that T cells replicate *in situ* at sites of disease activity. The question now arises as to whether IL-2, once generated at the site of involvement, can raise a systemic T cell response by being distributed via the blood, or whether IL-2 is cleared rapidly and thus functions only as a local mediator. The findings presented in this study detailing that PBL of these patients do not express Tac determinant and that low frequencies of PHA inducible Tac positive cells are present in their blood seem to suggest a compartmentalization of T cell responses in sarcoidosis and thus further support the hypothesis of a T cell redistribution to the sites of disease activity. Another possibility rests on the fact that the expression of Tac antigen in PBL might be suppressed by antigens which counteract the activity of IL-2 (Hardt *et al.*, 1981).

Much interest has been recently focused on Tac determinant. In particular, the strong correlation between the expression of cell surface Tac antigens and the presence of the human T cell leukemia virus (HTLV) suggested that abnormalities of the IL-2 receptor system may partially explain the uncontrolled growth of ATL cells (Gallo & Wong-Staal, 1982). At present there is no evidence that a similar uncontrolled cellular replication mediated by viruses is involved in sarcoidosis. We do not know the aetiology of this disease, nor have antibodies against HTLV been detected in the serum of these patients (Gallo *et al.*, 1983). However, we suggest that the presence of Tac^+ cells, potentially able to replicate at different sites of disease activity, might be crucial for the mechanism leading to granuloma formation and the evolution of the disease.

Steroid therapy seems to interfere with the IL-2 production (Gillis, Crabtree & Smith, 1979). In particular, corticosteroids inhibit the release of IL-2 induced by lectin stimulation. Since sarcoid patients are treated with steroid therapy, we would have anticipated differences in the frequencies of Tac^+ cells in untreated and treated cases. On the contrary, we were unable to find any differences between the above groups. However, we must point out that our group of treated patients at the time of the study had been out of therapy for at least 2 months. Since IL-2 receptor is a transient structure (Cantrel & Smith, 1983), it seems better to investigate these patients during the administration of corticosteroids to clarify this point.

Provided that Tac antigen or IL-2 receptor is essential for T cell growth, and in doing so is able to modulate the human immune response, some authors have begun to evaluate the efficacy of the anti-Tac MoAb in the therapy of patients with positive ATL (Waldman, 1983). The presence of cells bearing IL-2 receptor described in this paper together with the evidence of increased IL-2 production at sites of involvement in active sarcoidosis (Pinkston *et al.*, 1983; Hunninghake *et al.*, 1983) may provide the basis for a new approach in the therapy of these patients.

We thank Dr T. Uchiyama (Kyoto, Japan) for providing anti-Tac MoAb and Dr P. Beverley (London, UK) for providing UCHT-1 MoAb. We are also indebted to Mr C. Vincenzi for his expert technical assistence, to Miss I. Caridakis, Mr M. Polisin and Dr M. Porcelli for the excellent editorial assistence. This work was supported by a Grant from University of Padua (No. 12.01.44).

REFERENCES

BURMESTER, G.R., JAHN, B., GRAMATZKI, M. & KALDEN, J.R. (1983) Activated T cells *in vivo* and *in vitro*: different stimulation requirements for the expression of Ia antigens and the receptor for Interleukin-2 ('Tac' antigen). *Immunobiology*, **165**, 250.

CANTRELL, D.A. & SMITH, K.A. (1983) Transient expression of interleukin-2 receptors. Consequences for T cell growth. *J. exp. Med.* **158,** 1895.

CHILOSI, M., BONETTI, F. & IANNUCCI, A. (1981) Carcinogenic 3,3′-diaminobenzidine. *Am. J. clin. Pathol.* **75,** 638.

CHILOSI, M., PIZZOLO, G., FIORE-DONATI, L., BOFILL, M. & JANOSSY, G. (1983) Routine immunofluorescent and histochemical analysis of bone marrow involvement of lymphoma/leukaemia: the use of cryostat sections. *Br. J. Cancer*, **48,** 763.

CRYSTAL, R.G., HUNNINGHAKE, G.W., GADEK, J.E., KEOGH, B.A., RENNARD, S.I. & BITTERMAN, P.B. (1983) State of the art: the pathogenesis of sarcoidosis. In *Sarcoidosis 1981* (ed. by J. Chretien, J. Marsac & J. C. Saltiel) p. 13. Pergamon Press, Paris.

GALLO, R.C., KALYANARAMAN, V.S., SARNGADHARAN, M.G., SLISKI, A., VONDERHEID, E.C., MAEDA, M., NAKAO, Y., YAMADA, K., ITO, Y., GUTENSHON, N., MURPHY, S., BUNN, P.A., CATOVSKY, D., GREAVES, M.F., BLAYNEY, D.W., BLATTNER, W., HARRET, W.F.H., ZUR HAUSEN, H., SELIGMANN, M., BROUET, J.C., HAYNES, B.F., JEGASOTHY, B.V., JAFFE, E., COSSMAN, J., BRODER, S., FISHER, R.I., GOLDE, D.W. & ROBERT-GUROFF, M. (1983) Association of the human type C retrovirus with a subset of adult T-cell cancers. *Cancer Res.* **43,** 3892.

GALLO, R.C. & WONG-STAAL, F. (1982) Retrovirus as etiologic agents of some animal and human leukemias and lymphomas and as tools for elucidating the molecular mechanism of leukemongenesis. *Blood*, **60,** 545.

GILLIS, S., CRABTREE, G.R. & SMITH, K.A. (1979) Glucocorticoid-induced inhibition of T cell growth factor production. I. The effect on mitogen-induced lymphocyte proliferation. *J. Immunol.* **123,** 1624.

GRONOWICZ, E., COUTINHO, A. & MELCHERS, F. (1976) A plaque assay for all cells secreting Ig of a given type or class. *Eur. J. Immunol.* **6,** 588.

HARDT, C., ROLLINGHOFF, M., PFIZENMAIER, K., MOSMANN, H. & WAGNER, H. (1981) Ly-23$^+$ cyclophosphamide-sensitive T cells regulate the activity of an interleukin-2 inhibitor *in vivo*. *J. exp. Med.* **154,** 262.

HATTORI, T., UCHIYAMA, T., TOIBANA, T., TAKATSUKI, K. & UCHINO, H. (1981) Surface phenotype of Japanese adult T-cell leukemia cells characterized by monoclonal antibodies. *Blood*, **58,** 645.

HUNNIGHAKE, G.W., BEDELL, G.N., ZAVALA, D.C., MONICK, M. & BRADY, M. (1983) Role of Interleukin-2 release by lung T-cells in active pulmonary sarcoidosis. *Am. Rev. Respir. Dis.* **128,** 634.

HUNNIGHAKE, G.W. & CRYSTAL, R.G. (1981) Pulmonary sarcoidosis. A disorder mediated by excess helper T-lymphocyte at sites of disease activity. *N. Engl. J. Med.* **305,** 429.

JAMES, D.G. & JONES WILLIAMS, W. (1982) Immunology of sarcoidosis. *Am. J. Med.* **72,** 5.

LEONARD, W.J., DEPPER, J.M., UCHIYAMA, T., SMITH, K.A., WALDMANN, T.A. & GREENE, W.C. (1982) A monoclonal antibody that appears to recognize the receptor for human T-cell growth factor; partial characterized of the receptor. *Nature*, **300,** 267.

LING, N.R., BISHOP, S. & JEFFERIS, R. (1977) Use of antibody-coated red cells for the sensitive detection of antigen and in rosette tests for cells bearing surface immunoglobulins. *J. Immunol. Meth.* **15,** 279.

MODLIN, R.L., HOFMAN, F.M., MEYER, P.R., SHARMA, O.P., TAYLOR, C.R. & REA, T.H. (1983) *In situ* demonstration of T lymphocyte subsets in granulomatous inflammation: leprosy, rhinoscleroma and sarcoidosis. *Clin. exp. Immunol.* **51,** 430.

PACHECO, Y., CORDIER, G., PERRIN-FAYOLLE, M. & REVILLARD, J.P. (1982) Flow cytometry analysis of T lymphocytes in sarcoidosis. *Am. J. Med.* **73,** 82.

PINKSTON, P., BITTERMAN, P.B. & CRYSTAL, R.G. (1983) Spontaneous release of Interleukin-2 by lung T lymphocytes in active pulmonary sarcoidosis. *N. Engl. J. Med.* **308,** 793.

ROBB, R.J. & GREENE, W.C. (1983) Direct demonstration of the identity of T cell growth factor binding protein and the Tac antigen. *J. exp. Med.* **158,** 1332.

ROBB, R.J., MUNCK, A. & SMITH, K.A. (1981) T cell growth factor receptors. Quantitation, specificity and biological relevance. *J. exp. Med.* **154,** 1455.

SEMENZATO, G. & PEZZUTTO, A. (1982) Insights into the immunopathogenesis of sarcoidosis. *Immunol. Clin. Sper.* **1,** 35.

SEMENZATO, G., PEZZUTTO, A., AGOSTINI, C., GASPAROTTO, G. & CIPRIANI, A. (1981) Immunoregulation in sarcoidosis. *Clin. Immunol. Immunopathol.* **19,** 416.

SEMENZATO, G., PEZZUTTO, A., CHILOSI, M. & PIZZOLO, G. (1982) T lymphocyte redistribution in the lymph nodes of sarcoidisis patients. *N. Engl. J. Med.* **306,** 48.

SEMENZATO, G., PEZZUTTO, A., PIZZOLO, G., CHILOSI, M., OSSI, E., ANGI, M.R. & CIPRIANI, A. (1984) Immunohistological study in sarcoidosis: evaluation at different sites of disease activity. *Clin. Immunol. Immunopathol.* **30,** 29.

SMITH, K.A., GILLIS, S., BAKER, P.E. & MCKENZIE, D. (1979) T-cell growth factor-mediated T-cell proliferation. *Ann. N.Y. Acad. Sci.* **332,** 423.

TANIGUCHI, N., MIYAWAKI, T., YACHIE, A., IKUTA, N. & OHZEKI, S. (1983) Kinetics of expression of T-cell 'activation' antigens on in vivo- and in vitro-stimulated T cells. *Diagn. Immunol.* **1,** 104.

UCHIYAMA, T., BRODER, S. & WALDMANN, T.A. (1981) A monoclonal antibody (anti-Tac) reactive with activated and functionally mature human T cells. I. production of anti-Tac monoclonal antibody and distribution of Tac (+) cells. *J. Immunol.* **126,** 1393.

WALDMAN, T. (1983) The arrangement of immunoglobulin genes in leukemias of different human lymphocyte subsets. *5th International Congress of Immunology, Kyoto, Japan. 21–27 August 1983.*

YACHIE, A., MIYAWAKI, T., UWADANA, N., OHZEKI, S. & TANIGUCHI, N. (1983) Sequential expression of T cell activation (Tac) antigen and Ia determinants of circulating human T cells after immunization with tetanus toxoid. *J. Immunol.* **131,** 731.