Clin. exp. Immunol. (1977) 28, 268–275.

Immunological studies of patients with asbestosis

II. STUDIES OF CIRCULATING LYMPHOID CELL NUMBERS AND HUMORAL IMMUNITY

*E. KAGAN, A. SOLOMON, J. C. COCHRANE, PETRA KUBA, PAULINE H. ROCKS & I. WEBSTER *Departments of Immunology, Occupational Medicine and Biostatistics, National Research Institute for Occupational Diseases of the South African Medical Research Council, Johannesburg and School of Pathology, University of the Witwatersrand, Johannesburg, South Africa*

(*Received* 28 *October* 1976)

SUMMARY

As part of an overall assessment of immunological function, several aspects of humoral immunity and circulating lymphocyte subpopulations were evaluated in a group of twenty-six patients with radiographic evidence of parenchymal asbestosis. Statistical comparisons were made between the patient group and a comparable group of forty-five controls. Both the percentages and absolute numbers of circulating T lymphocytes were significantly reduced in the patient group compared with controls. Significant elevations of salivary secretory IgA and of serum IgA, IgG, IgM and IgE were noted amongst the patients compared with the controls. Non-organ-specific autoantibodies and cold-reactive lymphocytotoxins were present in high frequency in the patients' sera. Neoplasms were detected in four of the patients. The possible significance of these findings is discussed.

INTRODUCTION

Asbestos exposure has been linked to the development of a variety of malignant neoplasms (Parkes, 1973; Report, 1973). A recommendation of the Advisory Committee on Asbestos Cancers was the use of immunological techniques for the screening of individuals who might be at risk of developing asbestos-related cancers (Report, 1973). It is in accordance with this recommendation that the present project was instituted.

The aim of the study was to investigate several aspects of humoral immunity in subjects with radiographic evidence of parenchymal asbestosis. This was considered pertinent, in view of the manifestations of impaired cell-mediated immunity which were previously observed in these patients (Kagan *et al.*, 1977). The numbers of T and B lymphocytes circulating in the peripheral blood of these patients were also determined with appropriate surface marker techniques.

MATERIALS AND METHODS

Selection of patients. The patients had all previously been investigated with respect to their general status of cell-mediated immunity and their criteria for selection have been stated elsewhere (Kagan *et al.*, 1977). The group comprised twenty-six males (age range = 36–75 years; mean = 49·8 years), all of whom had features consistent with parenchymal asbestosis on chest radiographs.

Control subjects. Blood was obtained from 45 male controls for immunological studies (age range = 28–74 years; mean = 45·0 years). The criteria for subject selection were as described previously (Kagan *et al.*, 1977).

Lymphocyte surface marker studies. Venous blood was obtained for leucocyte count estimations in a Coulter Counter® Model S. Lymphocyte counts were calculated from slide differential counts.

* Present address: American Red Cross Blood Program, National Headquarters, Washington D.C. 20006, U.S.A.

Correspondence: The Librarian, National Research Institute for Occupational Diseases of the South African Medical Research Council, P.O. Box 4788, Johannesburg, 2000, South Africa.

The numbers of circulating T lymphocytes were determined from the percentages of lymphoid cells forming spontaneous rosettes with sheep red blood cells (SRBC). Leucocytes were obtained after sedimentation of approximately 20 ml of defibrinated venous blood on 3% gelatin for 30 min at 37°C (3 parts blood to 1 part gelatin). After having been washed three times in Hanks's balanced salt solution (HBSS), the leucocytes were resuspended in HBSS (containing 10% inactivated AB serum) and incubated with 200 mg of carbonyl iron at 37°C for 30 min. Thereafter the iron-containing phagocytic cells were removed with a magnet. Residual red cells were lysed with 0·83% NH_4Cl and, after being washed with HBSS, the remaining lymphocytes were resuspended at a concentration of 4×10^6 cells per ml. Sheep erythrocytes were collected into Alsever's solution, washed 3 times in saline and used as a 0·5% suspension in HBSS.

For rosetting, 0·25-ml vol. of lymphocytes and SRBC were mixed and incubated at 37°C for 30 min. After centrifugation at 200 *g* for 5 min, half the supernatant was removed and replaced with 0·3 ml absorbed foetal calf serum, whereupon the mixture was further incubated at 4°C overnight. Thereafter the supernatant was removed, 0·3 ml of 0·8% glutaraldehyde in HBSS was added, and the suspension was incubated for 20 min on ice. After the cells were resuspended, the number of T lymphocytes forming rosettes with SRBC (E rosettes) was determined by counting 200 consecutive lymphoid cells. Adherence of three or more SRBC to a central lymphoid cell constituted a rosette.

The numbers of circulating B lymphocytes were calculated from the percentages of lymphoid cells bearing surface immunoglobulin determinants. The method of Jondal, Holm & Wigzell (1972) was employed for the quantitation of lymphoid cells exhibiting surface immunoglobulin immunofluorescence, for which approximately 20 ml of defibrinated blood was taken. Commercial polyvalent and monospecific (anti-IgA, anti-IgG and anti-IgM) antisera to human immunoglobulin (Hyland) were used at a 1/5 dilution. Fluorescence was viewed on a Reichert Immunoplan® microscope. The results were expressed as the sum of the percentages of positive cells for IgA, IgG and IgM. The values obtained did not differ from values recorded with polyvalent antisera by more than 1–2% in individual instances.

Absolute numbers of T and B lymphocytes were calculated from the product of the percentage and the lymphocyte count.

Serum immunoglobulins. Serum IgA, IgG and IgM estimations were performed on radial immunodiffusion plates (Partigen® Behringwerke). The results were expressed in i.u./ml. Serum IgE was measured by radioimmunoassay using IgE test kits (Phadebas®, Pharmacia). The results were expressed in u/ml.

Secretory IgA. The 11 S dimer of secretory IgA was measured in specimens of saliva on Partigen® radial immunodiffusion plates. A correction factor was applied as described previously (Kagan et al., 1975) and the results were expressed in i.u./ml.

Serum lymphocytotoxicity studies. Sera were assayed for the presence of lymphocytotoxic antibodies by a macrotechnique (Dausset *et al.*, 1968), as modified previously (Kagan *et al.*, 1975). Target lymphocytes were obtained from a random panel of twenty donors, and also included autochthonous lymphocytes derived from the serum donor. Duplicate assays were performed, and the results were averaged. Parallel tests were run at 37°C and 15°C for the assessment of 'warm' and 'cold' antibody activity, respectively.

A reaction was regarded as positive if more than 25% of the target lymphocytes were lysed. A serum was classed as having demonstrable lymphocytotoxic antibodies if positive reactions were obtained with more than 10% of the donor lymphocyte panel (i.e. with lymphocytes from at least 3 panel members). As far as could be ascertained, none of the patients or controls had been previously transfused.

Non-organ-specific autoantibodies. Anti-nuclear factor (ANF) in serum was measured by a standard double-layer immunofluorescent technique, using rat liver as a substrate (Turner–Warwick & Parkes, 1970). Polyvalent, fluorescein-conjugated antiserum to human γ-globulin (Becton, Dickenson & Co.) was used at a 1/8 dilution. Test serum titres of 1/10 or higher were classed as positive.

Rheumatoid factors in serum were assessed by two methods. The human erythrocyte agglutination test (HEAT) and the latex fixation test (LFT) were performed as standard procedures (Valkenburg, 1963a, b). Serum titres of 1/32 or greater were scored as positive for the HEAT assay. In order to circumvent the difficulties produced by occasional low-intensity prozone reactions, serum titres of 1/1,280 or higher were regarded as positive for the LFT procedure.

RESULTS

Quantification of circulating T and B lymphocytes

The percentages of circulating T and B lymphocytes in the two groups of subjects are shown in Fig. 1. A highly significant reduction in the proportion of circulating T-lymphocytes was observed in the patient group, when compared with the controls, as reflected by the Wilcoxon–Mann–Whitney test ($P<0·001$). No significant differences were, however, observed in the percentages of circulating B lymphocytes amongst the two groups.

The absolute numbers of circulating T and B lymphocytes in both groups are illustrated in Fig. 2. Distinct differences were again noted between the numbers of circulating T cells in the patient group, when compared with the control group ($P<0·01$). No significant differences were noted between patients and controls with respect to the absolute numbers of circulating B cells.

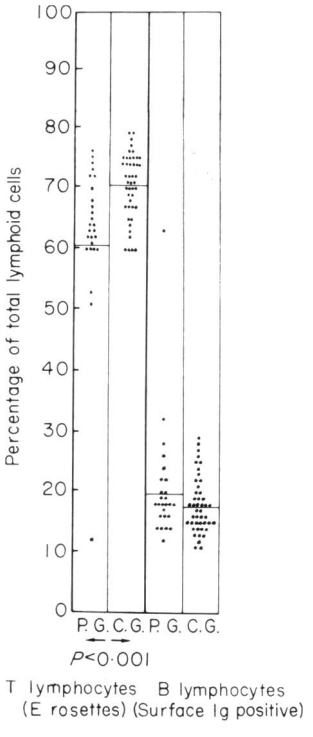

FIG. 1.

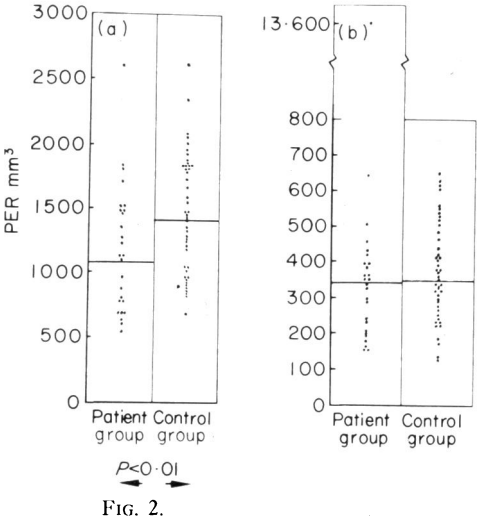

FIG. 2.

FIG. 1. Percentages of circulating T lymphocytes (forming E rosettes) and B lymphocytes (exhibiting surface immunoglobulin positive immunofluorescence). Horizontal lines indicate geometric means. The highest B-lymphocyte value was recorded in a patient with chronic lymphocytic leukaemia. P.G. = Patient group; C.G. = control group.

FIG. 2. Absolute numbers of circulating T and B lymphocytes. Values are derived from percentage values and lymphocyte counts of individual subjects. Horizontal lines indicate geometric means. The highest B-lymphocyte value was recorded in a patient with chronic lymphocytic leukaemia. (a) T lymphocytes; (b) B lymphocytes.

Serum immunoglobulins

The serum immunoglobulin concentrations are demonstrated in Fig. 3. Considerable degrees of scatter were noted within both groups for all parameters tested. Nonetheless, the patients exhibited a pronounced tendency for elevated serum immunoglobulin levels of all classes tested. Highly significant differences were recorded between the serum IgA and IgG concentrations of the patient group and the corresponding values for control subjects ($P<0{\cdot}001$). Distinct differences were also observed between patients and controls with respect to serum IgM ($P<0{\cdot}05$) and serum IgE ($P<0{\cdot}01$).

Secretory IgA

Levels of secretory IgA in saliva (Fig. 4) were also significantly higher in patients with asbestosis than in controls ($P<0{\cdot}05$).

Serum lymphocytotoxicity studies

'Warm' serum lymphocytotoxins were detected in only one patient and in none of the controls. However, low intensity reactions (15–25% target lymphocytes lysed) were noted at 37°C in sera from three other patients. Whether these reactions represent early evidence of 'warm' antibody production is unknown. No auto-reactive antibodies were detected at 37°C.

The incidence of 'cold' serum lymphocytotoxins in the two groups of subjects is shown in Table 1. The highest incidence was found amongst the patients (twenty-one of twenty-six). Similar antibody

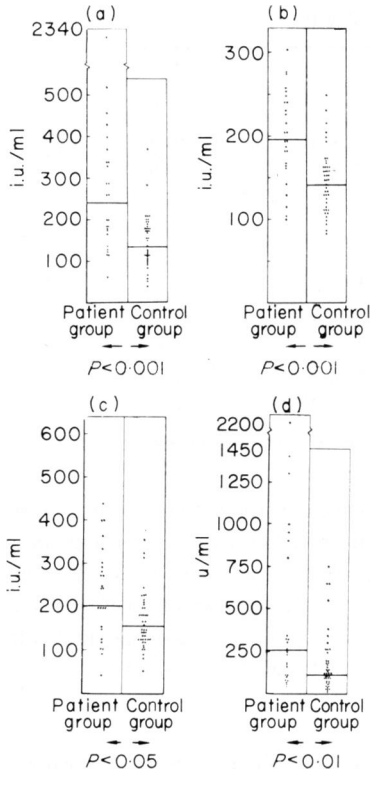

FIG. 3.

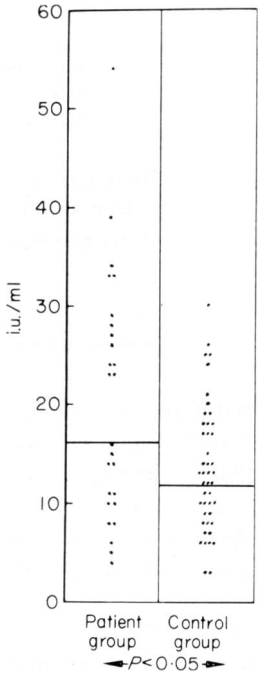

FIG. 4.

FIG. 3. Serum IgA, IgG, IgM and IgE levels. Horizontal lines indicate geometric means. The highest serum IgA value was recorded in a patient with an IgA-secreting myeloma. (a) Serum IgA levels; (b) serum IgG levels; (c) serum IgM levels; (d) serum IgE levels.

FIG. 4. Secretory IgA levels in saliva. Horizontal lines indicate geometric means.

TABLE 1. Comparative incidence of 'cold' serum lymphocytotoxins amongst patients with asbestosis and control subjects

Target lymphocytes used	Incidence of lymphocytotoxins	
	Patient group (total = 26)	Control group (total = 45)
Allogeneic panel	21 (80·8%)	10 (22·2%)
	$P<0·01$	
Autochthonous	7 (26·9%)	3 (6·7%)
	$P<0·05$	

TABLE 2. Comparative incidence of non-organ-specific autoantibodies in the sera of patients with asbestosis and control subjects

Nature of test	Incidence of autoantibodies	
	Patient group (total = 26)	Control group (total = 45)
Anti-nuclear factor	2 (7·7%)	0
Human erythrocyte agglutination test	4 (15·4%)	0
Latex fixation test	9 (34·6%)	5 (11·1%)
	$P<0·05$	

activity was recorded in the sera of only ten of forty-five control subjects. The χ^2 test revealed that the incidence of these 'cold' lymphocytotoxins was significantly lower in control sera compared with that in patients' sera ($P<0·01$). The frequency of 'cold' auto-reactive antibodies was also significantly lower in control than in patients' sera ($P<0·05$). Comparison of the number of positive reactions exhibited by individual serum specimens showed a significantly higher rate of such reactions amongst the patient group, when compared with the control group ($P<0·001$; results not shown).

Non-organ-specific autoantibodies

The comparative incidence of rheumatoid factors in sera obtained from the two subject categories is evident from Table 2. Positive reactions were recorded only in four patients' sera (but in none of the control sera) by the HEAT procedure. Using the LFT assay, however, positive results were demonstrated in nine patients' sera (three of which gave positive HEAT reactions) and in five control sera. The frequency of positive LFT reactions in patients' sera was significantly greater than that in control sera ($P<0·05$), as shown by the χ^2 test. Higher positive LFT titres were generally noted in the patients' sera (range 1/1280–1/10,240) than in control sera (range 1/1280–1/2560).

Anti-nuclear factor was found only in 2 sera, both from patients. One of these sera also gave a positive HEAT reaction.

DISCUSSION

The present study has revealed a reduction in the proportion and absolute numbers of circulating T lymphocytes in a group of patients with radiographic evidence of parenchymal asbestosis. This finding may reflect a quantitative T-cell deficit in such individuals. Another study has provided possible evidence of a qualitative T-cell defect in the same group of patients (Kagan *et al.*, 1977). Several of the patients had reduced numbers of circulating T lymphocytes in association with impaired mitogen-induced lymphocyte proliferative assays and with cutaneous anergy to DNCB. It is thus conceivable that both qualitative and quantitative T-lymphocyte deficiencies may act in concert and, in some instances, may be responsible for the defective expressions of cell-mediated immunity which were noted in the patients with asbestosis.

Reductions in the percentages and absolute numbers of circulating T lymphocytes have also been shown by other workers in patients with asbestosis (Kang *et al.*, 1974) and pleural mesotheliomas (Ramachandar *et al.*, 1975). It is of interest that the decreased proportion of circulating T cells in patients with asbestosis was not matched by a corresponding increase in the percentage of circulating B-cells in the present study. Whether this could be attributed to a relative increase in the number of

circulating non-T, non-B ('null') cells is not known, since surface marker studies were not performed on enriched cell populations.

Striking increases in the serum concentrations of IgA, IgG, IgM and IgE were noted in the group of patients with asbestosis, when compared with corresponding values for the group of control subjects. These findings support those of another study (Lange *et al.*, 1974) where significantly raised serum levels of IgA, IgG and IgM were detected in patients with asbestosis. The reason for the polyclonal increase of serum immunoglobulins in patients with asbestosis was not evident from our own study. Although associated pulmonary infection was a possibility, there was no clinical or radiographic evidence of active pulmonary infection in any patient at the time of being studied. There was also no obvious explanation for the elevated serum IgE levels amongst the patients, since definite allergic histories were only obtained in a couple of instances.

A generalized increase in immunoglobulin production could, alternatively, be related to the pathological processes in the lung which result in asbestotic fibrosis. An adjuvant-like action was been ascribed to asbestos, in that injections of crystalline asbestos into experimental animals have been shown to boost serum γ-globulin levels (cited by Lange *et al.*, 1974). This might also explain, in the present study, the significant increase of salivary secretory IgA concentrations in patients with asbestosis compared with controls. An adjuvant-like effect has also been demonstrated on macrophage surface membrane ultrastructure following the ingestion of crocidolite dust by macrophages *in vivo* (Miller & Kagan, 1976).

The detection of cold-reactive serum lymphocytotoxins in 81% of patients with asbestosis, but in only 22% of controls, provides further evidence of stimulation of humoral immune mechanisms in asbestosis. Similar serum activity has been described in association with a variety of diseases states (Terasaki, Mottironi & Barnett, 1970; Naito *et al.*, 1971; Goldberg, Cunningham & Terasaki, 1972; Steffen *et al.*, 1973; Mayer, Falkenrodt & Tongio, 1973; Strickland *et al.*, 1975; Kagan *et al.*, 1975).

Although the significance of these cold-reactive lymphocytotoxins is unknown (Kagan *et al.*, 1975), they appear to reflect a humoral immunological disturbance in response to a variety of antigenic stimuli. The demonstration of autoreactive lymphocytotoxins in approximately 25% patients with asbestosis is of particular interest, in view of the reported association of such autoreactivity with various autoimmune disorders. (Terasaki *et al.*, 1970; Naito *et al.*, 1971; Goldberg *et al.*, 1972; Steffen *et al.*, 1973.)

Non-organ-specific autoantibodies (rheumatoid factors or ANF) were detected in 42% of sera from patients with asbestosis. The incidence of rheumatoid factors (38%) was comparable with that noted in other surveys (Turner–Warwick, 1973; Lange *et al.*, 1974), although ANF was detected at a lower frequency (8%). The high frequency of non-organ-specific autoantibodies in asbestosis patients provides further evidence of disturbed humoral immunity in association with asbestosis.

We have found a remarkable imbalance between the cellular and humoral components of the immune response in patients with asbestosis, as evidenced by a depression of cell-mediated immunity, coupled with a heightened propensity towards the production of immunoglobulins and a variety of antibodies. Although no simple explanation for the paradox seems likely, it could be a manifestation of abnormal T-cell–B-cell interaction.

Suppressor T cells can exert specific feedback control on antibody synthesis by B cells (Allison, Denman & Barnes, 1971; Basten, 1974). Relaxation of this immuno-regulatory function is believed to be an important factor in the development of autoimmune diseases (Allison, 1974). It is conceivable that a similar loss of suppressor T-cell modulation could explain the disturbances of humoral immunity in patients with asbestosis. Altered T-cell–B-cell interaction might also account for the postulated adjuvant-like action of asbestos, since adjuvants may augment the helper role of T-lymphocytes in a non-antigen-specific fashion (Allison *et al.*, 1971).

It has been postulated that lymphoproliferation evoked by continuous antigenic stimulation, if unchecked by conventional feedback mechanisms, may ultimately terminate in lymphoid malignancy (Gershwin & Steinberg, 1973; Schwartz, 1975). Some experimental support for this concept is provided by the graft-versus-host model (Gleichmann, Gleichmann & Schwartz, 1972). The remarkable incidental detection of two neoplasms of B-cell lineage amongst the twenty-six patients with asbestosis may thus give added significance to the immunological findings in this study. Chronic lymphocytic leukaemia was

diagnosed incidentally in one patient, in whom serial blood counts showed a persistent lymphocytosis ($21–30 \times 10^3$ leucocytes per μl; 79–92% lymphocytes, mainly mature forms, occasional primitive types). An IgA-secreting myeloma was also diagnosed incidentally in one patient, in whose serum a persistent M-component (ranging in concentration from 1·9–2·7 g%) was detected over a period of 2 years.

Two other patients developed pleural mesotheliomas (diagnosed histologically at thoracotomy within 3 months and 12 months, respectively, after initial referral), giving a total incidence of neoplasia of 15·4% in this small group of patients. This figure is greatly in excess of the combined expected incidence rates for these tumour categories (approximately 3·2 per 100,000) in the general population (Waterhouse, 1974). Although this finding must be interpreted with caution, in view of the small numbers and selected basis of the populations studied, this may be more than a chance occurrence.

The results of this study clearly support the recommendation of the Advisory Committee on Asbestos Cancers (Report, 1973). However, long-term studies on larger numbers of patients at risk will ultimately determine whether these procedures have value in predicting the subsequent development of asbestos-related neoplasms.

We wish to thank Dr L. Irwig and Dr B. Goldstein for helpful advice and criticism. Assays of serum immunoglobulins were performed by Dr T. Ipp, secretory IgA was measured by Dr G. Shulman, leucocyte counts were assessed by Dr J. Katz, and Dr I. Prinsloo performed the assays for ANF and rheumatoid factors. Mr R. I. M. Handfield prepared the illustrations.

REFERENCES

ALLISON, A.C., DENMAN, A.M. & BARNES, R.D. (1971) Cooperating and controlling functions of thymus-derived lymphocytes in relation to autoimmunity. *Lancet*, ii, 135.

ALLISON, A.C. (1974) Interactions of T and B lymphocytes in self-tolerance and autoimmunity. *Immunological Tolerance; Mechanisms and Potential Therapeutic Applications* (ed. by D.H. Katz and B. Benacerraf), p. 25. Academic Press, New York.

BASTEN, A. (1974) Specific suppression of the immune response by T cells. *Immunological Tolerance; Mechanisms and Potential Therapeutic Applications* (ed. by D.H. Katz and B. Benacerraf), p. 107. Academic Press, New York.

DAUSSET, J., KOURILSKY, F., LEGRAND, L. & IVANYI, P. (1968) Lymphocytotoxicity. *Manual of Tissue Typing Techniques* (compiled by Transplantation Immunology Branch, Collaborative Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health), p. 1. Bethesda, Maryland.

GERSHWIN, M.E. & STEINBERG, A.D. (1973) Loss of suppressor function as a cause of lymphoid malignancy. *Lancet*, ii, 1174.

GLEICHMANN, E., GLEICHMANN, H. & SCHWARTZ, R.S. (1972) Immunologic induction of malignant lymphoma: Genetic factors in the graft-versus-host model. *J. nat. Cancer Inst.* **49**, 793.

GOLDBERG, L.S., CUNNINGHAM, J.E. & TERASAKI, P.I. (1972) Lymphocytotoxins and pernicious anemia. *Blood*, **39**, 862.

JONDAL, M., HOLM, G. & WIGZELL, H. (1972) Surface markers on human T and B lymphocytes. I. A large population of lymphocytes forming nonimmune rosettes with sheep red blood cells. *J. exp. Med.* **136**, 207.

KAGAN, E., SOSKOLNE, C.L., ZWI, S., HURWITZ, S., MAIER, G.M.G., IPP, T. & RABSON, A.R. (1975) Immunologic studies in patients with recurrent bronchopulmonary infections. *Amer. Rev. resp. Dis.* **111**, 441.

KAGAN, E., SOLOMON, A., COCHRANE, J.C., BEISSNER, E.I., GLUCKMAN, J., ROCKS, P.H. & WEBSTER, I. (1977) Immunological studies of patients with asbestosis. I. Studies of cell-mediated immunity. *Clin. exp. Immunol.* **28**, 261.

KANG, K.-Y., SERA, Y., OKOCHI, T. & YAMAMURA, Y. (1974) T lymphocytes in asbestosis. *New Engl. J. Med.* **291**, 735.

LANGE, A., SMOLIK, R., ZATOŃSKI, W. & SZYMAŃSKA, J. (1974) Autoantibodies and serum immunoglobulin levels in asbestos workers. *Int. Arch. Arbeitsmed.* **32**, 313.

MAYER, S., FALKENRODT, A. & TONGIO, M.M. (1973) Cold lymphocytotoxins in infections and parasitic infestations. *Tissue Antigens*, **3**, 431.

MILLER, K. & KAGAN, E. (1976) The *in vivo* effects of asbestos on macrophage membrane structure and population characteristics of macrophages: A scanning electron microscope study. *J. Reticuloendothel. Soc.* **20**, 159.

NAITO, S., MICKEY, M.R., HIRATA, A. & TERASAKI, P.I. (1971) Autolymphocytotoxins following immunization by pregnancy, transplantation, and disease. *Tissue Antigens*, **1**, 219.

PARKES, W.R. (1973) Asbestos-related disorders. *Brit. J. Dis. Chest*, **67**, 261.

RAMACHANDAR, K., SCHECHTER, R., PHELPS, B. & CHAHINIAN, PH. (1975) Immunocompetence in patients with pleural mesothelioma. *Proc. Amer. Ass. Cancer Res.* **16**, 142.

REPORT OF THE ADVISORY COMMITTEE ON ASBESTOS CANCERS TO THE DIRECTOR OF THE INTERNATIONAL AGENCY FOR RESEARCH ON CANCER. (1973) Biological effects of asbestos. *Ann. occup. Hyg.* **16**, 9

SCHWARTZ, R.S. (1975) Current concepts: Another look at immunologic surveillance. *New Engl. J. Med.* **293**, 181.

STEFFEN, C., THUMB, N., FRANK, O., EBERL, R., TAUSCH, G. & BOROS, M. (1973) Demonstration of lymphocytotoxins in rheumatoid arthritis in comparison with clinical course and other antibody activities. *Z. Immun.-Forsch.* **145**, 303.

STRICKLAND, R.G., FRIEDLER, E.M., HENDERSON, C.A., WILSON, I.D. & WILLIAMS, R.C. (1975) Serum lymphocytotoxins in inflammatory bowel disease: studies of frequency and specificity for lymphocyte subpopulations. *Clin. exp. Immunol.* **21**, 384.

TERASAKI, P.I., MOTTIRONI, V.D. & BARNETT, E.V. (1970) Cytotoxins in disease: Autocytotoxins in lupus. *New Engl. J. Med.* **283**, 724.

TURNER-WARWICK, M. & PARKES, W.R. (1970) Circulating

rheumatoid and anti-nuclear factors in asbestos workers. *Brit. med. J.* iii, 492.

TURNER-WARWICK, M. (1973) Immunological mechanisms in occupational disorders. *Proc. roy. Soc. Med.* **66**, 927.

VALKENBURG, H.A. (1963) Human erythrocyte agglutination test (HEAT). *The Epidemiology of Chronic Rheumatism* (ed. by J.H. Kellgren, M.R. Jeffrey and J. Ball), volume 3, p. 330. Blackwell Scientific Publications, Oxford.

VALKENBURG, H.A. (1963) Latex fixation test (LFT) *The Epidemiology of Chronic Rheumatism* (ed. by J.H. Kellgren, M.R. Jeffrey and J. Ball), volume 3, p. 337. Blackwell Scientific Publications, Oxford.

WATERHOUSE, J.A.H. (1974) *Cancer Handbook of Epidemiology and Prognosis*, pp. 32, 34, 76, 78, 80. Churchill Livingstone, Edinburgh.