Clin. exp. Immunol. (1977) 28, 261–267

Immunological studies of patients with asbestosis

I. STUDIES OF CELL-MEDIATED IMMUNITY

E. KAGAN,* A. SOLOMON, J. C. COCHRANE, E. ISOBEL BEISSNER, JANE GLUCKMAN, 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 *July* 1976)

SUMMARY

A variety of cancers have been documented in patients exposed to asbestos dust. Since a deranged immune system may play a rôle in cancer development, the general level of immunocompetence was studied in a group of twenty-six patients with radiographically defined asbestosis, who might be at risk of developing asbestos-related neoplasms. Statistical comparisons were made with a comparable control group. A disproportionate number of the patients displayed cutaneous anergy to certain recall antigens and to 2,4-dinitrochlorobenzene. *In vitro* studies of cellular immunity, as evaluated by phytohaemagglutinin-induced proliferative and cytotoxicity assays, showed significantly lower values amongst the patient group. Serum inhibitors of mitogen-induced lymphocyte transformation were also detected in several of the patients. The possible significance of these findings is discussed.

INTRODUCTION

The development of malignant mesotheliomas has been well documented in association with previous occupational or environmental exposure to asbestos dust (Wagner, Sleggs & Marchand, 1960; Magner & McDonald, 1972; Webster, 1973). Attention has also recently been focused on further possible carcinogenic hazards of asbestos dusts in relation to various other human malignancies, especially lung cancers (Berry, Newhouse & Turok, 1972; Selikoff, Hammond & Churg, 1972), gastro-intestinal malignancies (Enterline & Kendrick, 1967; Selikoff, Hammond & Seidman, 1973), carcinoma of the larynx (Stell & McGill, 1973; Libshitz *et al.*, 1974) and neoplasias of the haematopoietic system (Lieben, 1966; Gerber, 1970).

The mode of action of asbestos dusts in exerting their fibrogenic and possibly carcinogenic effects has aroused considerable debate. Interest has centred largely on the structural and aerodynamic properties of the fibres (Stanton, 1973; Timbrell, 1973). However, other factors, particularly the body's immune defences, might also play a rôle. Opposing views have been advanced concerning the homeostatic rôle of the immune system during the early stages of oncogenesis (Burnet, 1970; Prehn & Lappé, 1971; Möller & Möller, 1976).

Reports of immunological studies on patients with asbestos-related disorders have been comparatively infrequent. Emphasis has been placed largely on disturbances of humoral immunity in patients with pleural thickening (Stansfield & Edge, 1974) and asbestosis (Turner-Warwick, 1973; Lange *et al.*, 1974). Investigations of cellular immunity relate to preliminary reports on small numbers of cases of asbestosis (Kang *et al.*, 1974) and mesotheliomas (Ramachandar *et al.*, 1975).

The present study was undertaken in accordance with a recommendation of the Advisory Committee

* Present address: National Headquarters, American National Red Cross Blood Program, 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.

on Asbestos Cancers (Report, 1973), in order to evaluate the general level of immunocompetence amongst a group of patients with parenchymal asbestosis, who could be at risk of developing asbestos-related neoplasms. Several parameters of cell-mediated immunity were studied *in vivo* and *in vitro*.

MATERIALS AND METHODS

Selection of patients. The twenty-six patients chosen for this study were all referred to the Department of Occupational Medicine of the National Research Institute for Occupational Diseases (NRIOD). An unequivocal history of exposure to asbestos dust was elicited in every instance. The patients, all males, ranged in age from 36–75 years (mean = 49·8 years). Most were employed in the asbestos cement industry, where mixed occupational exposure to chrysotile, Cape crocidolite and amosite asbestos had occurred. One person, a welder, worked with asbestos lagging, whilst another, a carpenter, cut asbestos sheeting. A history of asbestos mining was obtained in two instances: one in association with Cape crocidolite and one with chrysotile and Cape crocidolite.

Chest roentgenograms were performed on all patients. These comprised routine (60 kV) and high kilovoltage (200 kV) postero-anterior exposures, as well as high kilovoltage right and left oblique views. The radiographs were assessed according to the ILO/UC 1971 classification of radiographs of the pneumoconioses (International Labour Office, 1972).

Roentgenographic changes consistent with parenchymal asbestosis were apparent in all the patients included in the study. The pathological findings were predominantly basal in situation. Apically distributed stellate opacities, suggestive of parenchymal scarring, were recorded in seven instances. These were attributed to previous tuberculous infection. No other significant radiographic lung pathology was noted. The duration of asbestos exposure in individual cases varied from 9–39 years (mean = 21·9 years). No attempt was made to correlate the extent of radiographic changes with any quantitative immunological assessment, since advanced pathological changes were noted in the majority of patients and only four subjects demonstrated roentgenographic features which could be interpreted as slight. At the time of being studied, all the patients were afebrile, and none were receiving steroids or immunosuppressive therapy.

Control subjects. These were derived from the professional, scientific, clerical and maintenance workers of the NRIOD and neighbouring institutions. Care was taken to exclude from the study anyone having a possible or definite history of occupational or environmental exposure to asbestos dust. Postero-anterior chest radiographs were obtained on all individuals in this group and oblique views were taken of all except five individuals. Roentgenographic changes consistent with parenchymal asbestosis were not observed in any of the control subjects, although features consistent with lung scarring were found in five individuals. Delayed hypersensitivity skin tests were performed on thirty male control subjects (age range = 29–65 years; mean = 48·5 years) whereas *in vitro* immunological studies were performed on blood obtained from forty-five male controls (age range = 28–68 years; mean = 45·9 years).

Delayed hypersensitivity skin tests. A battery of delayed hypersensitivity skin test antigens were employed for the assessment of immunological recall: (1) streptokinase–streptodornase (Varidase®, Lederle) containing 4 u of streptokinase and 1 u of streptodornase per 0·1 ml; (2) *Candida albicans* allergenic extract (Hollister–Stier) diluted 1/100; tuberculin purified protein derivative (PPD), kindly supplied by Dr H. H. Kleeberg, containing 10 u per 0·1 ml of Tween® 80 solution. The presence or absence of delayed hypersensitivity cutaneous reactivity was noted 48 hr after i.d. injection of 0·1 ml of antigen solution. A positive reaction was recorded when there was induration of at least 5 mm diameter at the test site. If reactions of less than 5 mm induration were obtained with any of the antigens, the procedure was repeated with a 10-fold greater concentration of the antigen solution. A reaction was classed as negative if there was no cutaneous reactivity to either antigen strength. Positive reactions were scored as follows: + = positive reaction only to the stronger concentration of antigen; ++ = positive reaction to the weaker concentration of antigen.

The contact allergen, 2,4-dinitrochlorobenzene (DNCB), was used to evaluate sensitization to new antigens. The technique used for DNCB contact sensitization was based on the method of Catalona, Sample & Chretien (1972). Reactions were scored as follows: +++ = positive spontaneous flares at 2 mg and 50 μg test sites; ++ = positive spontaneous flare at 2 mg test site only; + = positive delayed hypersensitivity reaction elicited only after 50 μg challenge dose applied; − = no cutaneous reactivity evident, even after 50 μg challenge dose applied.

Phytohaemagglutinin-induced lymphocyte proliferative assays. Approximately 30 ml of venous blood was collected into a moistened syringe containing 20 u of preservative-free heparin (Panheprin®, Abbott) per ml. Lymphomononuclear cells were obtained by dilution of the heparinized blood with an equal volume of saline and subsequent centrifugation on a Ficoll-sodium metrizoate (Lymphoprep®, Nyegaard & Co.) mixture (Harris & Ukaejiofo, 1969). The cells aspirated from the interface layer were then washed 3 times with M 150 medium and finally resuspended in M 150 medium at a concentration of 2×10^6 lymphocytes per ml. Quadruplicate cultures comprising 0·5 ml of lymphocytes (final concentration of 5×10^5 lymphocytes per ml) in 2 ml of M 150 medium (containing 20% autochthonous or allogeneic AB serum) were then set up. Phytohaemagglutinin (PHA) at a final concentration of 1 μg per ml (purified grade, Wellcome) was added to one set of lymphocyte cultures. Allogeneic AB serum was substituted for autochthonous serum in half the culture preparations, in order to exclude the presence of inhibitors of mitogen-induced lymphocyte proliferation (Kagan *et al.*, 1975).

All cultures were incubated at 37°C for 72 hr in stoppered test-tubes. Approximately 18 hr before termination of the cultures, methyl-^{3}H-labelled thymidine ([^{3}H]TdR) (specific activity 18·5 Ci/mMol) (Radiochemical Centre) was added to each culture tube (final concentration of 1 μCi per ml). At the completion of the test, the lymphocytes were lysed with

distilled water and the [^{3}H]TdR incorporation was assayed by a Millipore filter technique (Robbins *et al.*, 1972). The stimulation index was expressed as the ratio of the [^{3}H]TdR uptake (d/min) in cultures containing PHA to the [^{3}H]TdR uptake (d/min) in cultures without PHA. A particular serum was classed as having a serum inhibitor of PHA-induced lymphocyte proliferation if the stimulation index of cultures in allogeneic AB serum exceeded that of cultures in autochthonous serum by at least a factor of 2.

Phytohaemagglutinin-induced, lymphoid cell-mediated cytotoxicity. This assay of cytotoxic effector cell function involved a slight modification of a method previously described. Lymphomononuclear cells from approximately 30 ml of heparinized venous blood were obtained after centrifugation on Ficoll-sodium metrizoate. The lymphomononuclear cells and chicken erythrocyte target cells were prepared for culture as described (Sherwood & Blaese, 1973).

Triplicate cultures with and without PHA were incubated at 37°C in stoppered plastic tubes for 40 hr. Cultures (containing a total volume of 1·2 ml) comprised 1×10^6 lymphomononuclear cells in RPMI 1640 medium (containing 5% foetal calf serum) mixed with 5×10^4 ^{51}Cr-labelled chicken erythrocytes and $2{\cdot}5\times10^6$ washed human group O erythrocytes (originally collected into Alsever's solution). One set of cultures also contained 1 μg of PHA (purified grade). The human erythrocytes were added, in order to minimize the spontaneous release of ^{51}Cr into the supernatant medium. In this way, spontaneous release values were reduced from 20–25 to 8–14%.

At the end of the test, supernatant equivalent to half the culture volume was removed, after prior centrifugation. Both the supernatant and pellet were counted separately in a Packard Auto-Gamma Scintillation Spectrometer. The degree of cytotoxicity was calculated, by determining the percentage of total radioactivity released from the chicken erythrocytes into the supernatant. The following formula was employed:

$$\text{percentage } ^{51}\text{Cr release} = \frac{\text{supernatant ct/min}\times 2}{\text{supernatant ct/min}+\text{pellet ct/min}}\times 100.$$

RESULTS

Delayed hypersensitivity skin tests

The results of skin testing with a battery of recall antigens are illustrated in Table 1. Strongly positive cutaneous delayed hypersensitivity reactions to PPD were generally noted amongst all subjects tested and no significant differences were demonstrated by the χ^2 exact test between the patient and control groups. Only one subject in each group displayed cutaneous anergy to PPD.

TABLE 1. Comparative frequencies of delayed hypersensitivity cutaneous reactions to recall antigens amongst patients with asbestosis and control subjects

Recall antigen	Reaction score	Frequency of reaction elicited: Patient group (Total = 26)	Control group (Total = 30)
Streptokinase–streptodornase	++	11	8
	+	1	16
	−	14	6
		←$P<0{\cdot}05$→	
C. albicans allergenic extract	++	19	25
	+	2	5
	−	5	0
		←$P<0{\cdot}05$→	
Tuberculin purified protein derivative	++	25	26
	+	0	3
	−	1	1

* For interpretation of symbols see text.

Although all control subjects manifested positive skin reactions to *C. albicans* extract, negative reactions were recorded in five of twenty-six patients with asbestosis. This difference was significant ($P<0{\cdot}05$). Negative cutaneous reactions to streptokinase–streptodornase (SK–SD) extract occurred significantly

more frequently ($P<0{\cdot}05$) amongst the patients (fourteen of twenty-six) than amongst the controls (six of thirty).

The results of testing for cutaneous reactivity to DNCB are shown in Table 2. All the control subjects manifested strong (++ and +++) reactions to this antigen, which contrasted strikingly with the reactions noted in the patient group. Cutaneous anergy to DNCB was shown in seventeen of twenty-six subjects in this group, and a further two individuals manifested only weak (+) reactions. These findings were significantly different from those noted amongst the controls ($P<0{\cdot}01$).

TABLE 2. Comparative frequencies of delayed hypersensitivity cutaneous reactions to 2,4-dinitrochlorobenzene amongst patients with asbestosis and control subjects

Reaction score*	Frequency of reaction elicited: Patient group (Total = 26)	Control group (Total = 30)
+++	2	4
++	5	26
+	2	0
−	17	0
	←$P<0{\cdot}01$→	

* For interpretation of symbols see text.

Phytohaemagglutinin-induced lymphocyte proliferative assays

The results of the PHA-induced lymphocyte proliferation studies are illustrated in Fig. 1, and expressed as stimulation index values. The latter showed considerable variation within both groups of subjects studied. Nevertheless, group differences were apparent. The Wilcoxon–Mann–Whitney test revealed highly significant differences between the stimulation indices of cultures in autochthonous

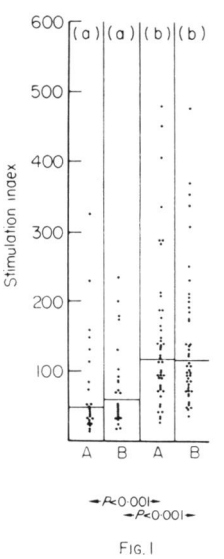

FIG. 1

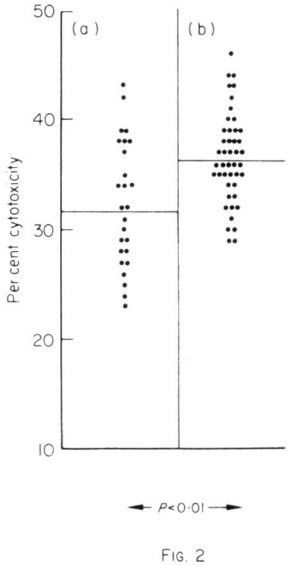

FIG. 2

FIG. 1. Phytohaemagglutinin-induced lymphocyte proliferative assays. Horizontal lines indicate geometric means. A = Autochthonous serum cultures; B = AB serum cultures. (a) Patient group; (b) control group.

FIG. 2. Phytohaemagglutinin-induced, lymphoid cell-mediated cytotoxicity. Horizontal lines indicate geometric means. (a) Patient group; (b) control group.

serum amongst the group of patients with asbestosis, when compared with the control group ($P<0{\cdot}001$).

Overt group differences were also evident for cultures in allogeneic AB serum. Significantly lower stimulation indices were again noted amongst patients, when compared with controls ($P<0{\cdot}001$). It is of interest that serum inhibitors of PHA-induced lymphocyte transformation were detected in five of twenty-six patients, but in only one of forty-five control subjects; however, the numbers involved were below the level of statistical significance.

Phytohaemagglutinin-induced, lymphoid-cell-mediated cytotoxicity

The values obtained for the PHA-induced cytotoxicity assays are shown in Fig. 2. The results showed considerably less scatter than was observed for the PHA-induced proliferative assays. Distinct group differences were shown once again, since the values recorded in the patient group were significantly lower than those obtained in the control group ($P<0{\cdot}01$).

DISCUSSION

The present investigation was undertaken in accordance with a recommendation of the Advisory Committee on Asbestos Cancers (Report, 1973). The purpose of the study was to evaluate the degree of immunocompetence of a group of patients with radiographic evidence of parenchymal asbestosis, who might be at risk of developing asbestos-related neoplasms.

Significant cutaneous anergy to extracts of *C. albicans* (19% of cases) and SK–SD (54% of cases) was a notable feature of the patient group in this study. Even more striking was the failure of contact sensitization with DNCB, which was apparent in two-thirds of the patients with asbestosis, but in none of the controls.

It is conceivable that the manifestations of cutaneous anergy to recall and new antigens exhibited by the patients with asbestosis might be due to impaired function of one or more components of the cellular immune response. The expression of cutaneous delayed hypersensitivity requires a non-specific inflammatory component, together with an integrated series of immunological events, which include antigen processing, immunological memory, lymphocyte–macrophage interaction, lymphocyte proliferation and lymphokine production. A defective inflammatory response seems unlikely in these subjects, since all but one displayed non-specific, irritant, inflammatory reactions within 24 hr of application of 2% DNCB (results not shown).

A curious discrepancy was noted in the patient group between the delayed hypersensitivity cutaneous reactions elicited with DNCB (and to a lesser extent with SK–SD) and with PPD and *C. albicans* extract. The predominence of strong (++) reactions to PPD may relate to contact with *Mycobacterium tuberculosis* during childhood, when no occupational exposure to asbestos would yet have occurred. There is a high prevalence of active tuberculosis amongst young schoolchildren in certain parts of South Africa, where up to 10% of school entrants may be infected (Taute, 1975). It is thus conceivable that most of the patients (and controls) in this study may have been sensitized to mycobacterial products at an early age. A similar situation may pertain with respect to previous sensitization to *C. albicans*. These findings suggest that immunological recall in relation to both these ubiquitous 'early age sensitizers' was essentially intact amongst most patients with asbestosis. However, in the case of newly introduced antigens such as DNCB, some impairment of immunological memory seems apparent at a time when the radiographic stigmata of parenchymal asbestosis are already manifest. SK–SD extract was found to be a 'weak' elicitor of cutaneous delayed hypersensitivity amongst both the patient and control groups. The higher frequency of cutaneous anergy to this antigen amongst the patients may also relate to some disturbance of memory function for 'weak' elicitors in association with asbestosis.

Proliferative assays on lymphocytes from the patient group showed, both in autochthonous and allogeneic serum, a significant reduction in mitogen-induced lymphocyte transformation, when compared with similar studies on lymphocytes from controls. Since PHA stimulates blastogenesis of T lymphocytes predominantly and, to a lesser extent, of B lymphocytes (Chess, Macdermott & Schlossman, 1974; Kreeftenberg, Leerling & Loggen, 1975), it is possible that a qualitative T-cell defect is present in patients with asbestosis.

The discovery of serum inhibitors of PHA-induced lymphocyte proliferation in 19% of patients with asbestosis (but in only one control subject) is of interest. Although the significance of these inhibitors is unknown, similar inhibitors of lymphocyte blastogenesis have been described in patients with cancer (Catalona, Sample & Chretien, 1973), sarcoidosis and in a variety of diseases characterized by derangement of immunity (Mangi, Dwyer & Kantor, 1974).

Another parameter of cell-mediated immunity, PHA-induced cytotoxic effector cell function, was also found to be significantly reduced amongst asbestosis patients, when compared with controls. This assay of cellular effector mechanisms appears to involve the interaction of T and B lymphocytes, 'null' cells and monocytes (Wisløff, Frøland & Michaelsen, 1974; Muchmore *et al.*, 1975). No significant correlation between PHA-induced blastogenesis and cytotoxicity was noted in any of the groups tested ($P>0{\cdot}05$).

We have shown elsewhere that both the proportion and absolute amount of circulating T lymphocytes were reduced in a number of the patients with asbestosis (Kagan *et al.*, submitted for publication). A quantitative T-cell deficit might account for some of the defective expressions of cell-mediated immunity noted in this group of subjects. Indeed, in several patients, reduced percentages and absolute numbers of T cells in the blood were found in association with cutaneous anergy to DNCB and with low PHA-induced proliferative assay values (both in autochthonous and allogeneic AB serum). Consideration of the patient group as a whole, however, showed no significant correlation between the relative or absolute numbers of circulating T cells and any of the parameters of cellular immunity investigated ($P>0{\cdot}05$).

It therefore seems likely that multiple factors, including possibly altered macrophage or monocyte function, may be responsible for the disturbances of cell-mediated immunity which were observed amongst the patients with asbestosis. Although macrophage function was not specifically investigated, functional derangements of monocytes or macrophages could have relevance to the findings in this study, since these cells are important in delayed hypersensitivity responses and are known to be injured by asbestos. Thus certain varieties of asbestos (especially chrysotile) have been shown to exert pronounced cytotoxic effects on macrophage cultures *in vitro* (Allison, 1974). Moreover, crocidolite asbestos is capable of producing striking *in vivo* alterations of alveolar macrophage surface topography (Miller & Kagan, 1976).

The results of this study may have relevance to the possible development of cancer subsequent to asbestos exposure. Although the classical concept of immunological surveillance (Burnet, 1970) has recently been subjected to critical reappraisal (Schwartz, 1975; Möller & Möller, 1976), some of the principles on which the theory is based may apply in certain select situations. The manifestations of defective cellular immunity which were found in the group of patients with asbestosis may have relevance in this regard.

These observations are also compatible with the alternative theory of immunostimulation of tumour development (Prehn & Lappé, 1971). It is therefore conceivable that presumed carcinogenic properties of asbestos dust might act in concert with a defective immune apparatus in patients with asbestosis, the subsequent development of malignancy occurring as a result of these phenomena. Whatever the possible significance of these findings, the present study has shown that immunological procedures may prove useful for screening selected populations at risk of developing cancer in certain situations.

We wish to thank Dr B. Goldstein and Dr G. K. Sluis-Cremer for helpful advice and criticism, and Mr R. I. M. Handfield for preparing the illustrations. Chest roentgenograms were taken in the Radiology Department of the Medical Bureau for Occupational Diseases.

REFERENCES

ALLISON, A.C. (1974) Pathogenic effects of inhaled particles and antigens. *Ann. N.Y. Acad. Sci.* **221**, 299.

BERRY, G., NEWHOUSE, M.L. & TUROK, M. (1972) Combined effect of asbestos exposure and smoking on mortality from lung cancer in factory workers. *Lancet*, ii, 476.

BURNET, F.M. (1970) The concept of immunological surveillance. *Prog. exp. Tumor Res.* **13**, 1.

CATALONA, W.J., TAYLOR, P.T., RABSON, A.S. & CHRETIEN, P.B. (1972) A method for dinitrochlorobenzene contact sensitization: a clinicopathological study. *New Engl. J. Med.* **286**, 399.

CATALONA, W.J., SAMPLE, W.F. & CHRETIEN, P.B. (1973) Lymphocyte reactivity in cancer patients: correlation with tumor histology and clinical stage. *Cancer*, **31**, 65.

CHESS, L., MACDERMOTT, R.P. & SCHLOSSMAN, S.F. (1974) Immunologic functions of isolated human lymphocyte subpopulations. I. Quantitative isolation of human T and B cells and response to mitogens. *J. Immunol.* **113**, 1113.

ENTERLINE, P.E. & KENDRICK, M.A. (1967) Asbestos-dust exposures at various levels and mortality. *Archs envir. Hlth.* **15**, 181.

GERBER, M.A. (1970) Asbestosis and neoplastic disorders of the hematopoietic system. *Amer. J. clin. Path.* **53**, 204.

HARRIS, R. & UKAEJIOFO, E.O. (1969) Rapid preparation of lymphocytes for tissue-typing. *Lancet*, **ii**, 327.

INTERNATIONAL LABOUR OFFICE. (1972) *ILO U/C International Classification of Radiographs of Pneumoconioses*, 1971. International Labour Office, Geneva.

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.

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

KREEFTENBERG, J.G., LEERLING, M.F. & LOGGEN, H.G. (1975) B- and T-cell markers on human lymphoblasts after stimulation with mitogen or antigens. *Clin. exp. Immunol.* **22**, 121.

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

LIBSHITZ, H.I., WERSHBA, M.S., ATKINSON, G.W. & SOUTHARD, M.E. (1974) Asbestosis and carcinoma of the larynx: a possible association. *J. Amer. med. Ass.* **228**, 1571.

LIEBEN, J. (1966) Malignancies in asbestos workers. *Archs envir. Hlth.* **13**, 619.

MAGNER, D. & MCDONALD, A.D. (1972) Malignant mesothelial tumors—histologic type and asbestos exposure. *New Engl. J. Med.* **287**, 570.

MANGI, R.J., DWYER, J.M. & KANTOR, F.S. (1974) The effect of plasma upon lymphocyte response *in vitro*. Demonstration of a humoral inhibitor in patients with sarcoidosis. *Clin. exp. Immunol.* **18**, 519.

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.

MÖLLER, G. & MÖLLER, E. (1976) The concept of immunological surveillance against neoplasia. *Transplant. Rev.* **28**, 3.

MUCHMORE, A.V., NELSON, D.L., KIRCHNER, H. & BLAESE, R.M. (1975) A reappraisal of the effector cells mediating mitogen induced cellular cytotoxicity. *Cell Immunol.* **19**, 78.

PREHN, R.T. & LAPPÉ, M.A. (1971) An immunostimulation theory of tumor development. *Transplant Rev.* **7**, 26.

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.

ROBBINS, J.H., GART, J.J., LEVIS, W.R. & BURK, P.G. (1972) The millipore filter assay technique for measuring tritiated thymidine incorporation into DNA in leucocyte cultures. *Clin. exp. Immunol.* **11**, 629.

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

SELIKOFF, I.J., HAMMOND, E.C. & CHURG, J. (1972) Carcinogenicity of amosite asbestos. *Archs envir. Hlth.* **25**, 183.

SELIKOFF, I.J., HAMMOND, E.C. & SEIDMAN, H. (1973) Cancer risk of insulation workers in the United States. *Biological Effects of Asbestos—Proceedings of a Working Conference held at the International Agency for Research on Cancer, Lyon, France, 2–6 October*, 1972 (ed. by P. Bogovski, J. C. Gilson, V. Timbrell and J. C. Wagner), pp. 209–216. World Health Organization International Agency for Research on Cancer, Lyon.

SHERWOOD, G. & BALESE, R.M. (1973) Phytohaemagglutinin-induced cytotoxic effector lymphocyte function in patients with the Wiskott–Aldrich syndrome (WAS). *Clin. exp. Immunol.* **13**, 515.

STANSFIELD, D. & EDGE, J.R. (1974) Circulating rheumatoid factor and anti-nuclear antibodies in shipyard asbestos workers with pleural plaques. *Brit. J. Dis. Chest*, **68**, 166.

STANTON, M.F. (1973) Some etiological considerations of fibre carcinogenesis. *Biological Effects of Asbestos—Proceedings of a Working Conference held at the International Agency for Research on Cancer, Lyon, France, 2–6 October*, 1972 (ed. by P. Bogovski, J. C. Gilson, V. Timbrell and J. C. Wagner), pp. 289–294. World Health Organization International Agency for Research on Cancer, Lyon.

STELL, P.M. & MCGILL, T. (1973) Asbestos and laryngeal carcinoma. *Lancet*, **ii**, 416.

TAUTE, J.F. (1975) Tuberculosis control in the farming situation. *Proceedings of the Symposium on Tuberculosis*, p. 46. South African National Tuberculosis Association, Johannesburg.

TIMBRELL, V. (1973) Physical factors as etiological mechanisms. *Biological Effects of Asbestos—Proceedings of a Working Conference held at the International Agency for Research on Cancer, Lyon, France, 2–6 October* 1972 (ed. by P. Bogovski, J. C. Gilson, V. Timbrell and J. C. Wagner), pp. 295–303. World Health Organization International Agency for Research on Cancer, Lyon.

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

WAGNER, J.C., SLEGGS, C.A. & MARCHAND, P. (1960) Diffuse pleural mesothelioma and asbestos exposure in the North Western Cape Province. *Brit. J. ind. Med.* **17**, 260.

WEBSTER, I. (1973) Asbestos and malignancy. *S. Afr. med. J.* **47**, 165.

WISLØFF, F., FRØLAND, S.S. & MICHAELSEN, T.E. (1974) Characterization of subpopulations of human lymphoid cell participating in phytohemagglutinin and concanavalin A-induced cytotoxicity. *Int. Arch. Allergy*, **47**, 488.