Immune response in shipyard workers with x ray abnormalities consistent with asbestos exposure

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ABSTRACT The association of cellular and humoral immune responses with asbestos exposure was studied in two groups of 150 workers each from a shipyard population and compared with a population of Red Cross blood donors. One of the shipyard groups had both chest x ray evidence of asbestos exposure and work assignments that necessitated direct handling of asbestos. The other shipyard group had normal chest x ray films and no job assignments involving the direct handling of asbestos. Cellular immune responses studied were mitogen activation and measures of T helper and T suppressor cells. Humoral immune measures were IgG, IgA, and IgM. In shipyard workers with asbestos related x ray abnormalities there was a significant increase in pokeweed mitogen stimulation. In both shipyard groups compared with Red Cross blood donors were found (1) significantly higher IgG and IgA levels and (2) greater numbers of T helper and T suppressor cells; because of the disproportionately large number of T suppressor cells, Th/Ts ratios were lower than in the Red Cross controls. Observations of differences in humoral and cellular immune response may point the way to the early diagnosis of the clinical manifestation of asbestos related disease.

Both malignant and non-malignant diseases have been found to occur at a higher rate than expected in subjects exposed to asbestos.¹⁻⁷ These diseases include lung cancer, gastrointestinal cancer, mesothelioma, and fibrosis of the lung. Both malignant neoplasms and non-malignant lung disease have been associated with an impaired immunological response in the host.⁸⁻¹⁰ The host immunological response to asbestos exposure has been studied in both animal and man.⁸⁻¹⁶

An immune response in the form of immune stimulation or suppression should be considered an abnormal reaction and if it occurs in association with exposure to substances such as asbestos the question should be asked whether the response is predisposing to the clinical manifestation of disease. Thus we have measured immune response as a possible index of the toxic effect of asbestos exposure in the hope that such response may serve as a clue to the early detection of subjects at risk for developing asbestos related disease. It seems likely from studies using the animal model and human populations exposed to asbestos that exposure may modify immune responses before the appearance of any pathological lesion.¹¹⁻¹⁵

Our report is based on the study of several immunological markers tested in a well defined group of

shipyard workers exposed to asbestos and having chest x ray abnormalities consistent with exposure to asbestos including pleural plaques. The following hypotheses are tested:

(1) Subjects with chest x ray abnormalities consistent with asbestos exposure have altered levels of circulating immunoglobulins and their peripheral blood lymphocytes have an altered ability to respond in vitro to plant mitogens (pokeweed and phytohaemagglutinin) when compared with controls.

(2) Of subjects exposed to asbestos, those with an identified tissue response, predominately pleural plaque, have altered levels of circulating immunoglobulins and their peripheral blood lymphocytes have an altered ability to respond in vitro to plant mitogens (pokeweed and phytohaemagglutinin) when compared with controls.

Methods

SUBJECTS UNDER STUDY

Three groups were studied.

The experimental group consisted of 150 subjects of a West Coast shipyard randomly selected from those who were diagnosed as having chest x ray abnormalities consistent with asbestos exposure. These abnormalities were pleural plaques, present in all

members of the experimental group; a few members of this group also had pleural thickening and parenchymal fibrosis.

A control group consisted of 150 shipyard workers who had no chest x ray abnormalities but who worked in the same shipyard as the experimental group and matched the experimental group for age within five years. The control subjects had no job assignment at the shipyard necessitating direct handling of asbestos; however, some had incidental exposure to asbestos. It should be emphasised that the subjects in the control group were selected for the absence of chest x ray abnormalities rather than having been selected for the absence of asbestos exposure. The purpose of the study is to look for immune differences associated with the biological response of asbestos exposure.

A second control group consisted of 634 male Red Cross blood donors from Southern California. The age range in this group was similar to that of the shipyard worker groups. Blood samples were obtained at the same time and processed in the same laboratory as the samples from the shipyard groups.

DATA COLLECTION

Data were collected during interviews of each shipyard worker to obtain information about jobs necessitating exposure to asbestos held before joining the shipyard. Dates of employment were collected for each worker as were data about other exposures to chemicals. Additional questionnaire data included demographic information, habit patterns such as smoking and drinking, and medical history. An asbestos exposure index (AEI) was calculated using the following equation: $AEI = 3A + 2B + C$, where A = duration of employment in high intensity exposure shops, B = duration of employment in intermediate exposure shops, and C = duration of employment in low intensity exposure shops. Descriptive data on the shipyard groups are shown in table 1.

LABORATORY METHODS

Specimen preparation

Blood samples were collected from each worker on Mondays at 0700 before breakfast and then transferred for processing to the central laboratory. Serum samples were separated from clot within three to five hours of collection and frozen at -70°C . A heparinised blood sample was used to perform a white blood cell count (WBC), differential count, and T cell subset analysis. These measurements were also made within three to five hours. An additional sample of heparinised blood was drawn for Ficoll-Hypaque separation to permit analysis of lymphocyte subsets by dual fluorescence and to permit mitogen activation assays.

Lymphocyte subset determinations

Total lymphocyte count—Total lymphocyte count

was determined from a white blood cell count using a Coulter counter and a differential count on 100 cells.

Immunofluorescent measurement of lymphocyte subsets—Levels of T cells, T helper (Th) cells, and T suppressor (Ts) cells were determined using the monoclonal antibodies listed below.

Antibody	Reactive cell
OKT3	Total T cells
OKT4	T helper/inducer cells
OKT4A	T helper/inducer cells
OKT8	T suppressor/cytotoxic cells

Both indirect and direct immunofluorescence were routinely performed. Indirect immunofluorescence uses a monoclonal antibody as the primary antibody and a FITC conjugated goat antimouse immunoglobulin antibody as the secondary antibody. Background immunofluorescence was determined using murine gamma-2a myeloma protein as primary antibody. Direct immunofluorescence used FITC conjugated monoclonal antibody. Whole blood collected in EDTA vacutainer tubes was treated with monoclonal antibodies and fluorescinated cells were analysed using an automated flow cytometer (Spectrum III Ortho Diagnostics). Lymphocyte populations were distinguished from monocytes and granulocytes by correlated analysis of forward and right angle scatter. The percentage of cells positive for immunofluorescence was used in conjunction with the absolute lymphocyte count to determine the absolute number of cells bearing a given marker and the Th/Ts ratio.¹⁷ The above assays were performed on a subsample of our experimental and control groups.

Lymphocyte mitogen activation

Lymphocyte blastogenic response assays were performed on all subjects. Lymphocytes were cultured in RPMI 1640 with 20% fetal calf serum (Reheis), penicillin, and streptomycin. One hundred thousand cells per well were distributed in microtitre plates and phytohaemagglutinin (PHA) and pokeweed mitogen (PWM) were added in different doses (PHA in three doses 0.5, 1.0, and 2.0 mg% and PWM in two doses 0.25 and 0.5 mg%). Cultures were incubated at 37°C for 72 hours in a humidified 5% CO_2 atmosphere. Labelled ^3H thymidine was added and the cells were harvested and counted using a liquid scintillation counter.¹⁸

Quantitative immunoglobulins

IgG, IgA, and IgM levels were determined using a rate nephelometric assay system developed by Beckman.

DATA ANALYSIS

Analyses of the data consisted mainly of characterising the distributions of the various blood assays. We evaluated the mean, median, mode, standard deviation,

tion, and minimum and maximum values of the blood measures periodically as a check on coding, programming, and laboratory procedures.

Statistical analysis was performed using the BMDP statistical software. Standard statistical methods (chi-squared tests for contingency tables, *t* tests, and analysis of variance) were used. Two way analysis of variance was performed on each of the immunological markers with classification of subjects by test groups, smoking status, and asbestos exposure measurements.

Results

Table 1 shows the descriptive measures for the experimental and control groups from the shipyard. The mean age and the age at first exposure to asbestos were similar for the experimental and control groups.

There was, however, a significant difference between the experimental and control groups in the duration of exposure to asbestos, 25.1 and 21.4 years respectively and in the duration of employment, with a shorter duration of employment in the experimental group than the control group, 14.3 and 17.0 years respectively. The asbestos exposure index was significantly higher in the experimental group than in the control group, 24.5 and 9.5 respectively. A summary of the results of smoking history shows that the frequency of current and former smokers in both the experimental and control groups was similar. The mean pack years smoked in the experimental group was also similar to that in the control group, 20.9 versus 19.4.

Table 2 gives a summary of the immunological and other blood test results in the experimental and control groups from the shipyard and the tests of comparison

Table 1 Descriptive measures of asbestos exposure and cigarette smoking in shipyard experimental and control groups

	Experimental group			Control group			Statistical test p value
	Mean	SD	No	Mean	SD	No	
Age at time of study (y)	53.1	7.8	151	51.6	7.4	150	0.07*
Age at first asbestos exposure (y)	25.4	9.1	150	26.5	8.6	141	0.29*
Duration of asbestos exposure (y)	25.1	10.2	150	21.4	10.0	141	0.002*
Duration of employment at shipyard (y)	14.3	6.7	151	17.0	6.2	150	0.001*
Asbestos exposure index	24.5	19.7	151	9.5	10.5	150	0.001†
Cigarette smoking (pack-years)	20.9	22.9	145	19.4	22.8	146	0.35†
Smoking status:	No	%		No	%		
Current	51	34.0		48	32.0		
Former	63	42.0		55	36.7		
Never	36	24.0		47	31.3		

**t* Test comparison of data in original units.

†Wilcoxon rank sum test.

Table 2 Immunological and other blood test results in shipyard experimental and control groups

Laboratory test	Experimental group			Control group			Statistical test p value
	No	$\bar{X}$	SD	No	$\bar{X}$	SD	
WBC cells/m ³	147	7361	2478	149	7046	1961	0.23*
Total lymphocytes	146	2625	917	142	2591	965	0.76*
Eosinophils	142	255	251	140	184	198	0.01†
Monocytes	145	306	226	141	295	197	0.89†
Bands	146	641	419	142	614	388	0.89†
Segmented cells	146	3464	1599	142	3305	1155	0.33*
T cells	141	1842	718	142	1706	712	0.11*
B cells	68	208	145	85	203	116	0.81*
T helper cells	47	1138	395	37	1118	502	0.84*
T suppressor cells	47	813	544	37	768	365	0.78†
Th/Ts	47	1.72	0.89	37	1.58	0.59	0.39*
Mitogen activation (DPM):							
PWM 0.25 mg/100 ml	147	7037	6329	147	5123	4502	0.001†
PWM 0.5 mg/100 ml	146	7366	6417	146	5542	4550	0.003†
PHA 0.5 mg/100 ml	147	24399	13989	147	21341	7358	0.26†
PHA 1.0 mg/100 ml	147	28217	15813	147	24024	8291	0.09†
PHA 2.0 mg/100 ml	147	30277	17426	147	26404	8836	0.19†
Immunoglobulins (mg/100 ml):							
IgA	136	271	109	142	258	112	0.34*
IgG	135	1210	317	143	1187	300	0.54*
IgM	136	143	70	143	139	83	0.62†

**t* Test comparison of data in original units.

†Wilcoxon rank sum test.

Table 3 Comparison of the T helper and T suppressor cells between shipyard groups and Red Cross normal controls

No	Th	Ts	Th/Ts ratio
(1) Red Cross normal controls: 617	876.2 + 336.0	468.6 + 211.9	2.08 + 0.86
(2) Shipyard experimental group: 47	1137.9 + 395.2	812.8 + 544.5	1.72 + 0.89
(3) Shipyard control group: 37	1118.2 + 501.9	768.2 + 365.2	1.58 + 0.59
Comp of 1 v 2: t Value p <	16.60 0.001	9.11 0.001	2.76 0.005
Comp of 1 v 3: t Value p <	12.12 0.001	7.93 0.001	3.49 0.001
Comp of 2 v 3: t Value p <	0.20 0.42	0.43 0.33	0.82 0.21

between them. There was no significant difference between the mean white cell counts, total lymphocytes, lymphocyte subpopulations, monocytes, band cells, and segmented cells between the experimental and control groups. The difference in number of eosinophils, however, was statistically significant.

Humoral immune measurements, IgA, IgM, and IgG, are also presented in table 2. No difference was observed between the means of the experimental and control groups.

The results for mitogen activation show a significantly higher mean pokeweed mitogen stimulation at both concentrations of mitogen in the experimental group compared with the control group ($p < 0.001$ and < 0.003). Although the experimental group had a higher mean stimulation, the differences in mean PHA mitogen stimulation were not significant at the three doses of PHA.

There were no significant differences between the experimental and control groups in the mean number of Th and Ts cells or the Th/Ts cell ratio. As shown in table 3, however, shipyard experimental and control subjects had 1.3 times more Th cells than the Red Cross controls. This excess was significant at $p <$

0.001. Similar results were obtained for Ts cells ($p < 0.001$) when shipyard workers had about 1.7 times as many suppressor cells as the Red Cross controls. On the other hand, Th/Ts ratio was significantly lower in shipyard workers compared with Red Cross controls ($p < 0.005$). There were no significant differences in the levels of Th cells, Ts cells, or Th/Ts between the experimental and control shipyard workers. The sub-samples of experimental and control subjects on which immunofluorescent measurement of lymphocyte subsets were performed had similar levels of asbestos exposure as measured by the asbestos index when compared with the experimental and control samples.

Discussion

Our findings indicate that changes in both cellular and humoral immune responses are associated with asbestos exposure and with the tissue changes diagnosed by chest x ray films as consistent with asbestos exposure. Cellular immune responses, as measured by T and B cell mitogen stimulation are affected, confirming the findings of Hartmann¹⁵ and Wagner.¹⁹

Pokeweed mitogen stimulation was significantly raised in the experimental group. This indicates that B-cells are responsive to asbestos exposure in agreement with previous results reported for in vitro experiments,²⁰ the animal model,²¹ and in man.^{14 19 22 23} Two way analyses of variance were performed to assess the effects of chest x ray findings and the effects of age, smoking, and the asbestos exposure index as well as their possible interaction on mitogen activation. Differences in pokeweed induced mitogen stimulation (table 2) were confirmed for chest x ray abnormality after correction for the effects of age, smoking, and exposure to asbestos. Because of the skewed distributions in the levels of mitogen stimulation, data were transformed by logarithms; similar results were obtained when analyses of variance were performed on the transformed data. We are unaware of these findings having been reported previously.

With regard to humoral immune response, we found no difference in IgM levels between shipyard workers (with or without asbestos stigmata) and Red Cross normal blood donors (table 4). This observation

Table 4 Comparison of shipyard worker group immunoglobulins with those of Red Cross normal controls

	IgA			IgM			IgG		
	No	Mean	SD	No	Mean	SD	No	Mean	SD
(1) Population controls	573	228	105	574	143	112	572	1081	268
(2) Shipyard experimentals	136	271	109	136	143	70	135	1210	317
(3) Shipyard controls	142	258	112	143	139	83	143	1187	300
(1) v (2)	t = 4.26 p < 0.001			t = 0.00 p < 1.00			t = 4.85 p < 0.001		
(1) v (3)	t = 3.01 p < 0.01			t = 0.40 p < 0.70			t = 4.13 p < 0.001		
(2) v (3)	t = 0.98 p < 0.17			t = 0.43 p < 0.34			t = 0.62 p < 0.27		

is consistent with the fact that the IgM mediated primary humoral response subsides with continuing exposure to an antigen. Our finding that both shipyard groups had significantly higher levels of IgG than the Red Cross controls (table 4) is, however, consistent with the idea that asbestos behaves in the body as a persisting antigen. Immunoglobulin A was also found to be higher in shipyard workers than in the Red Cross controls. The immunoglobulin levels in shipyard experimental subjects were similar to those in shipyard control subjects. Of interest here is the observation of Huuskonen *et al* that patients with asbestosis have higher IgA values than controls.¹⁴ They concluded that the increase in humoral immune response could be used for the evaluation of patients with pulmonary fibrosis.

When we looked at Th and Ts cells, both types were present in significantly greater numbers in the two shipyard groups compared with the Red Cross blood donors, the Ts excess being considerably greater than the Th excess (table 3). The greater excess of Ts cells results in Th/Ts mean ratios that are smaller than those found in the Red Cross controls, a condition also found in immunodeficient people. Our findings are similar to those of Lew *et al* who found raised Ts but not raised Th cells in workers exposed to asbestos.²²

Although statistically significant differences were found when comparisons were made in several of the laboratory tests, their biological importance remain to be determined. It is also recognised that many comparisons were made and that differences can occur as random events and not the result of real effects. Study hypotheses were selected before inspection of the data, and thus results are not selectively reported.

In conclusion, our observations of the humoral immune response as measured by changes in IgG and IgA, the cellular immune response as measured by mitogen stimulation and the disproportionate increase in Ts cells and decrease in Th/Ts cell ratios may, one day, serve as clues to the early diagnosis of susceptibility for asbestos caused malignancy. The test to these clues will depend on a prospective study.

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