Lymphocyte subsets in subjects exposed to asbestos: changes in circulating natural killer cells

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Several studies have shown alterations in lymphocyte subsets in the peripheral blood of asbestos workers.¹ During the past 10 years attention has focused on the role of natural killer (NK) cells, large granular lymphocytes believed to have a role in non-major histocompatibility (non-MHC) restricted cytotoxicity for tumour cells that may result in reduced growth and spread of lung cancer.²⁻⁴

Studies have shown inconsistent alterations in NK cell numbers, proportions, and activity in various groups of subjects exposed to asbestos.⁵⁻⁹ In all previous studies CD16 (Leu-11a) was the antibody used to identify the NK cells. Lanier *et al*¹⁰ suggested that at least two types of antigens are present on human natural killer cells; Leu-7 (CD57) (identifies human natural killer -1 (HNK-1) cells) and Leu-11a (CD16) (identifies natural killer cells -15 (NK 15)).

We have investigated lymphocyte subsets including NK cells using both CD16 and CD57 antibodies in patients with asbestosis, in asbestos workers without asbestosis, and in a control group.

Materials and methods

We studied 16 male patients with asbestosis (four

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smokers, one non-smoker, and 11 ex-smokers), 11 male asbestos workers with no evidence of asbestosis (two smokers and nine ex-smokers), and 24 control subjects (two women, 16 smokers, and eight non-smokers). None of the patients or normal subjects had any clinical evidence of malignancy, radiological evidence of lung cancer, or clinical evidence of connective tissue disorders. They had not received any corticosteroids during the past three months or experienced symptoms to suggest any viral disease during the six weeks before blood samples were taken.

Lymphocytes were separated, frozen gradually to -40°C and stored at that temperature in 2 ml fetal calf serum and 2 ml dimethyl sulphoxide (20%) until analysed later in batches. The viability of lymphocytes at the time of analysis was determined by trypan blue exclusion (viability ≥85%). The antibodies used were Leu 3a+ 3b (CD4) Leu 2a (CD8), Leu 11a (CD16), and Leu 7 (CD57) (Becton Dickinson). Antibody CD4 is present on helper inducer cells, CD8 identifies cytotoxic/suppressor cells, CD19 identifies B lymphocytes, CD16 identifies virtually all NK cells, and CD57 is expressed on a subset of NK cells and a subset of T cells.¹¹⁻¹³ Proportions of lymphocyte subsets were determined by flow cytometry (Coulter EPICS C system). A minimum of 10 000 cells were analysed for each antibody and the proportion of each lymphocyte subpopulation was determined. The number per litre of blood was calculated from absolute counts determined by impedance on a Coulter S-plus IV counter.

Table 1 Number of lymphocytes and lymphocyte subsets in subjects exposed to asbestos and in controls

	AS Group (n = 11)	AW Group (n = 16)	AS + AW Group (n = 27)	Control group (n = 24)
Lymphocytes	1.69 (0.76)	1.61 (0.60)	1.66 (0.76)	1.96 (0.74)
CD19	0.21 (0.16)	0.25 (0.26)	0.23 (0.20)	0.16 (0.39)
CD4	0.66 (0.44)	0.53 (0.20)	0.61 (0.38)	0.74 (0.34)
CD8	0.39 (0.28)	0.32 (0.14)	0.36 (0.26)	0.50 (0.29)
CD57	0.32 (0.24)	0.14 (0.17)	0.25 (0.22)	0.23 (0.15)
CD16	0.22 (0.15)	0.20 (0.13)*	0.21 (0.14)*	0.31 (0.15)
CD4:CD8	2.1 (1.2)	2.07 (1.2)	2.11 (1.68)	2.08 (1.13)

AS = Asbestos workers with asbestosis; AW = asbestos workers without asbestosis. Figures are mean × 10⁶ cells (SD).

*p = 0.014 compared with control group; †p = 0.034 compared with control group. There were no significant differences between AS and AW groups.

Table 2 Proportion of lymphocyte subsets in study groups

	AS (n = 16)	AW (n = 11)	AS and AW (n = 27)	Control (n = 24)
CD19	11 (5-36)	7 (4-23)	11 (4-36)	7 (1-17)
CD4	43 (12-62)	38 (10-47)	39 (10-62)	41 (10-79)
CD8	21 (5-44)	16 (8-32)	19 (5-44)	23 (9-50)
CD57	15 (2-40)	9 (3-22)	10 (2-40)	11 (2-24)
CD16	11 (5-23)*	14 (5-30)	11 (5-30)†	17 (1-27)

Figures are median (range).

*p = 0.041 compared with control group; †p = 0.048 compared with control group.

There were no significant differences between AS and AW groups.

Results

Tables 1 and 2 give the numbers and proportions of lymphocytes in different subsets for the total exposed group, the exposed groups with and without asbestos, and the control group. Numbers of total circulating lymphocytes were similar in all groups. Numbers

and proportions of CD16 positive cells were reduced in the total exposed group compared with the control group ($p = 0.014$ and $p = 0.048$ respectively; table 1, fig 1, table 2). There were no differences between numbers or proportions of CD57 positive cells in the total exposed group and the control group. The CD4:CD8 ratios were similar in all groups. There were no differences in numbers or proportions of lymphocytes in different subsets between exposed workers with asbestosis and those without.

Inverse correlations existed between numbers of CD16 positive cells and duration of exposure to asbestos ($r = -0.44$, $p = 0.026$; fig 2).

No differences were found in numbers or proportions of the different lymphocyte subsets in current smokers compared with non and ex-smokers combined in the total exposed group. Similarly there were no correlations between the numbers or proportions of lymphocytes in different subsets and age or time since first exposure to asbestos.

Figure 1 Numbers of circulating CD16 in the asbestos exposed subjects (AS + AW) compared with the control group (A) and in AS, AW, and control groups (B). Horizontal lines represent mean values.

Figure 2 Correlation between numbers of circulating CD16 and duration of exposure to asbestos.

Discussion

In this report we have shown a reduction in the number and proportion of circulating CD16 positive lymphocytes in subjects exposed to asbestos compared with a control group; CD57, however, did not differ between the two groups. Antibody CD16 defines a cluster of antibodies that recognise epitopes on the Fc receptor for human IgG (Fc γ RIII).¹³ Such receptors are present on a subpopulation of cells that exhibit NK activity. Antibody CD57 (also called human NK cell-1 (HNK-1)) exposed a subset of NK cells.^{10,13} Previous studies have suggested that Leu 11 (CD16) positive cells correlate with NK cell activity as measured by the radiolabelled chromium release method.^{14,15} The results shown here are therefore consistent with the hypothesis that exposure to asbestos suppresses NK cell activity. Support for this hypothesis has also come from *in vitro* studies. Robinson¹⁶ showed that direct exposure of NK cells from blood and lungs to asbestos fibres for 24 hours resulted in a dose related reduction in their activity. Activity could be restored by addition of recombinant interleukin-2.¹⁶ In the present report duration of exposure to asbestos correlated inversely with the number of circulating CD16 positive cells, consistent with a dose related effect.

Earlier studies have produced conflicting results. Kubota *et al*⁶ reported that the activity of NK cells

was reduced in peripheral blood of subjects with asbestosis. By contrast, Yoneda *et al*⁹ reported an increase in circulating NK cell activity in patients with asbestosis compared with normal controls. This increase tended to be greater in those patients with a less severe radiographic change. Other studies have reported reduced NK activity in smokers with asbestosis⁷ and smokers exposed to asbestos with and without asbestosis.⁸ De Shazo *et al*⁵ reported reduced numbers and activity of circulating NK cells in smokers with asbestosis compared with controls that smoked or smokers with pleural but no pulmonary asbestos related disease. Cigarette smoking alone has been associated with a reduction in the proportion of circulating NK cells as measured with the monoclonal antibody anti-Leu 11A (CD16).¹⁷ In the current report we found no differences in numbers or proportions of lymphocyte subsets between current smokers and non and ex-smokers in the group exposed to asbestos. The reasons for these conflicting results are not clear and further studies on larger numbers of subjects are needed.

From our data we conclude that circulating CD16 positive cells are reduced in subjects exposed to asbestos and that those with longer duration of exposure have lower numbers of circulating CD16 positive cells. This reduction may be at least partially responsible for the increased susceptibility to development of malignancies in asbestos workers.

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