

# HLA-DR antigens on human macrophages from bronchoalveolar lavage fluid

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**ABSTRACT** The expression of HLA-DR (Ia-like) antigens on human macrophages was investigated by analyses of cells from bronchoalveolar lavage fluid obtained from 12 patients with pulmonary sarcoidosis, six patients with extrinsic allergic alveolitis, nine patients with cryptogenic fibrosing alveolitis, 11 normal non-smokers, and 12 normal smokers. The HLA-DR antigen was demonstrated by the mouse monoclonal antibody OKIa by a peroxidase-antiperoxidase method performed on glass slides. No differences were found in the percentage of alveolar macrophages that expressed DR antigens between the five study groups. OKIa positivity was observed on more than 90% of macrophages in all cases. These observations suggest that the previously reported enhanced antigen presentation by alveolar macrophages in sarcoidosis is not linked with an increase in the percentage of DR<sup>+</sup> macrophages in the lung.

The expression of HLA-DR (Ia-like) antigens on cells of the monocyte/macrophage lineage is important for effective macrophage/T lymphocyte interactions,<sup>1</sup> especially in the presentation of antigen by macrophages to lymphocytes.<sup>1-4</sup> In patients with sarcoidosis the antigen presenting function of alveolar macrophages appears to be enhanced.<sup>5,6</sup> The question of whether this might be accompanied by increased expression of HLA-DR antigens on alveolar macrophages has been raised, and indeed a recent study reported that patients with sarcoidosis and those with fibrosing alveolitis had a higher percentage of alveolar macrophages expressing DR antigens than normal non-smokers.<sup>7</sup> These investigators found a rather low percentage of DR<sup>+</sup> macrophages in their normal study population (21%). Other studies have shown, however, that almost all alveolar macrophages obtained from normal volunteers express HLA-DR antigens.<sup>8,9</sup>

Because of these discordant reports, it was the aim of this study to compare the expression of HLA-DR antigens on macrophages obtained from a group of normal control patients with those obtained from groups of patients with sarcoidosis, extrinsic allergic alveolitis, and fibrosing alveolitis.

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## Methods

### PATIENTS

Twelve untreated patients with pulmonary sarcoidosis were investigated (mean age 39 (SD 14), range 19-62 years). They all had a clinical picture consistent with this disease along with biopsy evidence of non-caseating, epithelioid cell granulomas, and no evidence of mycobacterial, fungal, or parasitic infection, or exposure to organic or inorganic dusts. Chest radiographs were type I in six patients, type II in three patients, and type III in three patients. There was only one smoker in this group. Seven patients had an increased ratio of helper and inducer (OKT4<sup>+</sup>) to suppressor and cytotoxic (OKT8<sup>+</sup>) T cells in bronchoalveolar lavage fluid (above 3.5—that is, above the mean + 2 SD of our normal control values).

Six patients had untreated extrinsic allergic alveolitis (mean age 49 (16), range 21-67 years). All were non-smokers. The diagnosis was based on history and clinical and radiological features, and on respiratory function test results consistent with an interstitial lung disease. Serum from all six patients contained precipitins against the relevant antigens. Four patients were budgerigar fanciers, one patient had farmer's lung, and one patient had humidifier lung. Only one patient was studied during an acute episode.

Nine patients with cryptogenic fibrosing alveolitis (idiopathic pulmonary fibrosis) were studied (mean age 57 (19), range 18-81 years). Two patients were

receiving immunosuppressive treatment when they were investigated. All were non-smokers. All patients with known causes of pulmonary fibrosis were excluded from this group—that is, those with organic or inorganic dust disease, drug induced interstitial lung disease, histiocytosis X, or collagen disorders. Lung function tests showed restrictive ventilatory defects and reduced diffusing capacity in these patients.

As control subjects, 11 normal non-smokers (mean age 37 (15), range 16–61 years) and 12 normal, symptomless smokers (mean age 34 (16), range 16–69 years) were studied. The smokers had an average smoking history of 14 (SD 9) pack years. All subjects had normal chest radiographs and normal lung function with the exception of one smoker, who had mild central airways obstruction.

#### BRONCHOALVEOLAR LAVAGE

Bronchoalveolar lavage was performed as previously described.<sup>10–12</sup> After informed consent had been obtained from all patients and control subjects, a total of 100 ml of 0.9% saline in five 20 ml aliquots was instilled into the middle lobe or the lingula with a flexible bronchoscope. The fluid was immediately aspirated and filtered through gauze. The recovered cells were counted, washed three times in Eagle's minimum essential medium containing 0.2% bovine serum albumin and 0.1% EDTA, and resuspended in Eagle's medium. A trypan blue exclusion test for cell viability was performed. The cell viability was between 80–95%. Before the first washing differential counts were made from smears stained with May-Grünwald-Giemsa by counting 600–1000 cells.

#### IDENTIFICATION OF CELL SURFACE ANTIGENS

The cell suspensions were transferred on to glass slides coated with poly-L-lysine for staining of cell surface antigens by the peroxidase-antiperoxidase method,<sup>13</sup> a glass slide assay being used.<sup>11 14 15</sup> Briefly, 10  $\mu$ l cell suspension ( $2 \times 10^6$  cells/ml) was added to poly-L-lysine coated reaction areas of siliconised glass slides. After 10 minutes the cells were settled and firmly attached to the glass surface. After fixation with 0.05% glutaraldehyde for 20 minutes immunocytochemical staining was carried out. For this study the following

murine monoclonal antibodies (Ortho Pharmaceutical Corporation) were used: OKIa for recognition of HLA-DR antigens on cells of the mononuclear phagocyte system,<sup>16</sup> OKT4 for helper-inducer T cells,<sup>17</sup> and OKT8 for suppressor-cytotoxic T cells.<sup>18</sup> To evaluate the reaction, the slides were viewed under a light microscope with a magnification of 400–1000. A positive reaction was denoted by the presence of a dark brown, granular staining of the cell membrane. At least 200 cells were counted in each reaction area.

#### STATISTICS

All data are expressed as mean values with standard deviations in parentheses. Comparisons between groups were made with Student's two tailed *t* test.

#### Results

##### GENERAL CHARACTERISTICS OF LAVAGE FLUID

The total numbers of cells recovered from all disease groups and from the smoking controls were significantly greater than those from normal non-smokers (table). The cell differential counts showed a predominance of lymphocytes in lavage fluid from patients with sarcoidosis and from those with extrinsic allergic alveolitis, and a predominance of polymorphonuclear leucocytes from the group with cryptogenic fibrosing alveolitis (table). The ratio of OKT4<sup>+</sup> to OKT8<sup>+</sup> T cells was appreciably increased in lavage fluid obtained from the patients with sarcoidosis (mean ratio 8.7 (8.2)) and this differed significantly ( $2p < 0.001$ ) from the ratio for the patients with extrinsic allergic alveolitis, which was reduced to 1.0 (0.6).

##### HLA-DR ANTIGENS ON LAVAGE FLUID MACROPHAGES

There was no significant difference in the percentage of macrophages that expressed DR antigens between the different study groups. OKIa positivity was observed on virtually all macrophages (>90% in all cases; figs 1 and 2). The mean (SD) values for the different groups were: normal non-smokers 98.6% (2.3%), normal smokers 97.9% (2.2%), sarcoidosis

General characteristics of lavage (BAL) fluid (mean values with standard deviations in parentheses)

Group	BAL fluid recovered (%)	Total cells ( $\times 10^6$ )	Macrophages Lymphocytes Polymorphonuclear cells		
			(% of total cells)		
Normal non-smokers (n = 11)	54 (15)	7 (3)	92 (4)	7 (3)	1 (1)
Normal smokers (n = 12)	52 (13)	23 (13)	96 (3)	3 (2)	1 (1)
Sarcoidosis (n = 12)	61 (14)	18 (9)	61 (18)	37 (18)	2 (2)
Extrinsic allergic alveolitis (n = 6)	53 (9)	29 (17)	27 (11)	60 (14)	13 (10)
Fibrosing alveolitis (n = 9)	51 (17)	19 (15)	68 (20)	13 (11)	19 (19)

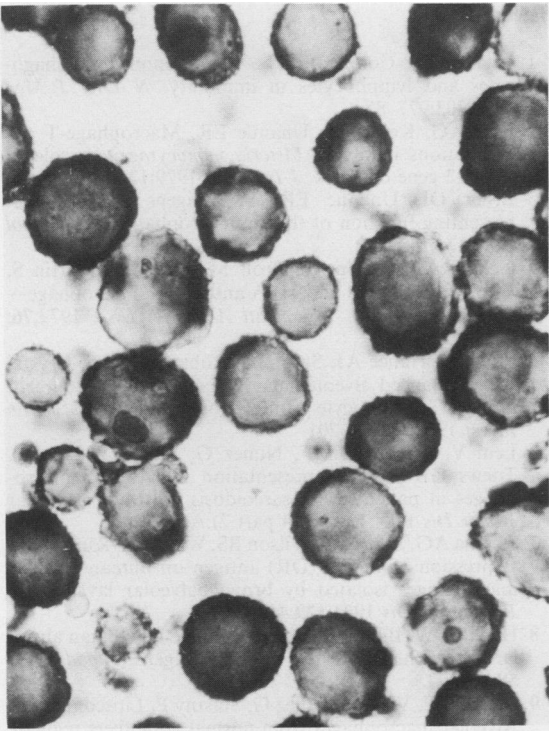


Fig 1 Bronchoalveolar fluid cells from a normal non-smoker. The large cells are alveolar macrophages, virtually all of them positive for OKIa—that is, they have intense, dark brown staining on their surface. (Peroxidase-antiperoxidase method performed on glass slides, staining with the monoclonal antibody OKIa;  $\times 565$ .)

98.6% (2.3%), extrinsic allergic alveolitis 97.5% (2.9%), cryptogenic fibrosing alveolitis 99.6% (1.3%). Moreover, there was no difference in the percentage of OKIa<sup>+</sup> macrophages when the patients with sarcoidosis were divided into those with a raised OKT4/OKT8 ratio ( $n = 7$ ) and those with a normal ratio ( $n = 5$ ); the percentages of OKIa<sup>+</sup> macrophages in these two groups were 98.0 (2.2) and 99.4 (0.5).

## Discussion

Immunoregulatory activities are mediated, in part, by intercellular signals based on products of the I region (Ia antigen) of the major histocompatibility complex (MHC) in the mouse. The corresponding human Ia like antigens are HLA-DR gene products belonging to MHC class II antigens. Such HLA-DR antigens are expressed on monocytes and macrophages, B cells, and activated T cells after allogenic or mitogenic stimulation, but not on resting T cells.<sup>1-4 16 19</sup>

Local replication of T lymphocytes in the alveolar structures is widely held to lead to an expansion in lung T cell numbers in patients with sarcoidosis.<sup>5 20</sup> Most of these T cells are OKT4<sup>+</sup> helper-inducer cells.<sup>10 11 21</sup> They spontaneously secrete interleukin 2.<sup>22</sup> Since OKT4<sup>+</sup> cells are capable of proliferating in response to HLA-DR antigens, the process of antigen presentation by which HLA-DR positive mononuclear phagocytes interact with antigen and induce T lymphocytes to proliferate might be increased in the lungs of patients with sarcoidosis. Indeed, enhanced antigen presentation by alveolar macrophages obtained from patients with sarcoidosis has recently been found.<sup>5 6</sup> We do not know whether this process is accompanied by increased expression of HLA-DR antigens on alveolar macrophages.

In the present study no differences in the percentage of alveolar macrophages expressing HLA-DR antigens were found in the five study groups. OKIa positivity was observed on almost all macrophages from patients with sarcoidosis, extrinsic allergic alveolitis, and cryptogenic fibrosing alveolitis and from normal subjects. In our study smoking did not appear to influence the expression of HLA-DR antigens on macrophages. This observation differs from that of Lawrence *et al*<sup>23</sup> who reported that smoking decreases DR antigen expression on human alveolar macrophages. These authors used an immunofluorescent technique, which might explain the discrepancy because smokers' macrophages are known to show autofluorescence.<sup>24 25</sup> This might interfere with the interpretation of the specific fluorescence of surface markers.

The finding of a very high percentage of DR<sup>+</sup> macrophages in lavage fluid from normal subjects confirms results from other centres.<sup>8 9</sup> The absence of

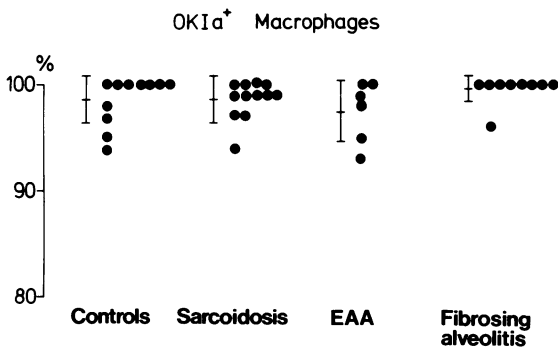


Fig 2 Percentages of macrophages expressing HLA-DR surface antigen assessed by reaction with OKIa monoclonal antibody in non-smoking control subjects and in patients with sarcoidosis, extrinsic allergic alveolitis (EAA), and fibrosing alveolitis.

a difference in DR expression between alveolar macrophages from normal subjects and from patients with sarcoidosis is in agreement with a recent report by Venet *et al.*<sup>5</sup> Razma *et al.*<sup>7</sup> however, detected a higher percentage of DR<sup>+</sup> macrophages in the lavage fluid from patients with sarcoidosis and from patients with diffuse interstitial pulmonary fibrosis than in lavage fluid from normal non-smokers. This discordance may be due to different methods. Firstly, we applied an immunoperoxidase assay based on the unlabelled antibody enzyme method, and thus is more sensitive than the direct immunofluorescence used by Razma *et al.*<sup>7</sup> Our technique may have identified cells that expressed small amounts of HLA-DR antigens on their surface. Secondly, Razma *et al.* studied adherent macrophages after a 24 hour culture period, rather than macrophage preparations made immediately after lavage. Cells in culture may alter their surface marker characteristics. On the other hand, Lipscomb *et al.*<sup>26</sup> reported that extended culture for 13 days did not change the percentage of DR<sup>+</sup> alveolar macrophages. Thus we must consider other factors to explain the disparate results in surface expression of DR antigens on human alveolar macrophages. Differences in the reactivity and specificity of the different anti-DR-antibodies used by different groups as well as the fact that interpretation of positivity may be very subjective may also account for the differences between the results of these studies.

Our method offers advantages other than increased sensitivity by comparison with immunofluorescence techniques; these include the production of permanent preparations and the capacity to investigate several antibodies in parallel as small numbers of cells are needed for each test.<sup>14 15</sup>

We conclude from this study that the enhanced antigen presentation by alveolar macrophages in sarcoidosis<sup>5 6</sup> is not linked with an increase in the percentage of HLA-DR<sup>+</sup> macrophages in the lung. We cannot, however, exclude the possibility that the total amount of DR antigen expressed by a single macrophage might be increased. This question should be investigated by quantitative methods such as radioimmunoassay or ELISA techniques. Currently, no established assays are available, but the development of an ELISA technique for quantitating DR antigens and other cell surface antigens is in preparation (G Dölken, personal communication). Such quantitative measurements of cell surface markers and their correlation with the functional properties of cells concerned in local immunoregulation should prove helpful in expanding our understanding of hitherto unresolved questions in immune diseases of the lung.

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