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Antiretroviral treatment reverses HIV-induced reduction in the expression of surface antigens on alveolar macrophages in AIDS patients

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SUMMARY

MoAbs and immunoperoxidase methods were used to identify antigen-presenting and phagocytic cells and to assess expression of HLA-DR molecules on cells obtained by bronchoalveolar lavage (BAL) from 33 AIDS patients and nine normal volunteers. In 17 patients, not receiving antiretroviral therapy, the expression of HLA-DR molecules (MoAb RFDR1) as well as the percentages of cells expressing RFD1 marker for antigen-presenting cells and RFD7 marker for mature phagocytes were significantly reduced. However, in BAL obtained after commencing treatment with zidovudine (AZT) in 21 patients or with 2′,3′-dideoxyinosine (DDI) in five patients, the expression of the markers studied was found to have returned to levels of expression seen in normal lavages. The changes observed were clearly associated with antiretroviral treatment and did not correlate with applications of other drugs, blood CD4 counts or presence of infectious organisms in BAL fluid. As the alterations in the expression of HLA-DR molecules and RFD1 marker on macrophages have been shown to be associated with functional capacities of these cells, the reversal of impaired expression of phenotypic markers on alveolar macrophages in AIDS patients by AZT and DDI signifies an important ability of these drugs to modify immune reactivity and emphasizes the need to monitor such functions in HIV disease.

Keywords alveolar macrophages antiretroviral therapy phenotypic macrophage markers HLA-DR

INTRODUCTION

Many studies of HIV focus on $CD4^+$ T cells as the principal target. There is increasing evidence, however, that the infection of cells belonging to the monocyte/macrophage lineage plays a crucial role in the pathogenesis and progression of HIV disease [1]. Of lung macrophages isolated from AIDS patients, 10–50% have been reported to be infected with HIV [2,3]. Macrophages are instrumental in inducing and sustaining local immunity via antigen presentation, phagocytosis and secretion of cytokines [4,5]. Antigen presentation is enhanced in activated macrophages and is associated with increased HLA-DR expression [5]. Previous studies have shown that the increase in the expression of HLA-DR molecules and phenotypic macrophage markers on alveolar macrophages is related to disease progression in sarcoidosis and can be abolished by treatment of the inhaled budesonide [6,7]. We have recently demonstrated dramatic reduction in the expression of these markers on alveolar macrophages in HIV-infected patients suffering from pneumonitis [8]. In a number of such patients, zidovudine (AZT) treatment reduces frequency of opportunistic infections [9,10], but it is not known whether antiretroviral treatment can affect functional capacities of macrophages. In order to establish the effect of such treatment, we studied the expression of cell markers, believed to be associated with function on alveolar macrophages in three groups of HIV-infected patients presenting with pneumonitis: (i) individuals not receiving antiretroviral drugs, and (ii) patients treated with AZT, or (iii) with 2′,3′-dideoxyinosine (DDI).

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MATERIALS AND METHODS

Bronchoalveolar lavage and study population

Bronchoalveolar lavage (BAL) of the appropriate lobe (as indicated by radiographic abnormality) or of the right middle lobe (in patients with normal radiographs or generalized radiographic shadowing) was carried out using a fibreoptic bronchoscope. A subsegmental bronchus was anaesthetized using 2% lignocaine and lavaged with 20-ml aliquots of 0·9% saline (buffered to pH 7·4 with $NaHCO_3$) to a total of 180 ml. The lavage fluid was gently aspirated after each aliquot and collected into a sterile, siliconized glass bottle maintained at 4°C.

BAL samples were obtained at 46 episodes of pneumonitis in 33 HIV-infected patients. Samples from nine normal volunteers acted as controls and were collected with the approval of the Royal Free Hospital Ethical Committee. At the time of BAL, 17 patients had never received antiretroviral therapy, while five patients were treated with DDI according to the MRC/INSERM alpha trial protocol involving an oral dose of 3 or 10 mg/kg daily (median duration of treatment 3 months) and 21 patients were taking AZT at the time of BAL (median duration of therapy 6 months). The AZT oral dosage regimen varied from 1200 mg to 500 mg daily in divided doses, reflecting the change in prescribing practice during the 2-year period of the study.

All patients were HIV$^+$ in a number of immunoassays [11] and presented with an acute respiratory episode. At the time of BAL, severity of respiratory episode and severity of HIV disease were assessed and detailed information was collected on the type of therapy each patient had received at the time of BAL. BAL was also carried out on normal volunteers who had no past history of lung disease and no symptoms suggesting viral infections in the 2 weeks before lavage.

Infectious agents were identified in 20 ml of BAL fluid using a combination of Papanicolaou and Grocott stain for detection of *Pneumocystis carinii*, a combination of cell culture and Detection of Early Antigen Fluorescent Foci (DEAFF) testing for identification of cytomegalovirus (CMV) [12] and standard microbiological culture techniques for detection of bacteria and mycobacteria.

Immunocytochemistry

Cells obtained from 60–80 ml of BAL fluid were washed and cytospins prepared and stored, as described before [13]. One slide from each BAL was prepared with a modified May–Grünwald–Giemsa stain and used to assess the percentages of macrophages, lymphocytes and granulocytes.

An indirect immunoperoxidase method [14] and MoAbs were used to identify antigen-presenting (dendritic) cells (MoAb RFD1, HLA-class II) [15], phagocytic cells (MoAb RFD7) [15] and to assess expression of HLA-DR molecules (MoAb RFDR1, HLA-class II) [16]. Staining with MoAb RFDR1 was quantified via measurement of optical density using a Seescan Image Analyser (Seescan Ltd) and HLA-DR density was expressed as optical density per unit area. For all remaining MoAbs, percentages of positive macrophages were calculated as a proportion of total macrophages identified on morphological grounds. All assessments were carried out by two investigators blind to the identity of samples.

Statistical analysis

The expression of surface antigens on alveolar macrophages between the different groups of subjects was compared using the Mann–Whitney test and 95% confidence interval analysis. Spearman's rank correlation was used to assess the correlation between the expression of macrophage markers and severity of pneumonitis within each group of patients.

Table 1. Characteristics of respiratory episodes in HIV-infected patients included in the study

Sample no.	CD4 count ($\times 10^9$/l)	Severity of HIV disease	Severity of respiratory episode*	Organisms detected in BAL		
				CMV	PC	Other
a. Patients without antiretroviral therapy						
183	0·003	AIDS	6	+	+	–
123	0·270	AIDS	8	+	+	–
182	0·208	AIDS	10	+	+	–
181	0·172	AIDS	1	+	+	–
116	0·072	AIDS	1	–	+	–
179	0·052	AIDS	6	–	+	–
189	0·040	AIDS	5	–	+	–
206	0·045	CDC III	2	–	–	aafb.pos.
131	0·042	AIDS	4	–	–	HSV
162	0·002	AIDS	4	+	–	St.pn.
119	ND	AIDS	1	+	+	–
171	0·288	CDC III	1	–	–	–
281	0·216	AIDS	5	–	+	–
322	0·120	AIDS	3	–	–	–
303	0·360	AIDS	4	–	+	–
307	0·195	AIDS	4	–	+	–
63	0·079	AIDS	1	–	+	–
261	0·040	AIDS	4	–	–	ADN
b. Patients treated with AZT at time of BAL						
138	0·042	AIDS	4	–	–	–
288	0·216	AIDS	9	–	–	–
192	0·050	AIDS	10	+	+	St.pn.
191	0·033	AIDS	6	+	+	–
236	0·032	AIDS	2	–	–	–
264	0·007	AIDS	1	–	–	–
137	0·130	AIDS	2	–	–	–
267	0·028	AIDS	2	+	–	–
238	0·056	AIDS	1	–	–	–
68	0·001	AIDS	3	+	+	S.a.
193	0·048	AIDS	6	–	–	–
163	0·002	AIDS	1	–	–	H.i.
164	0·002	AIDS	1	–	–	–
254	0·011	AIDS	3	+	+	–
200	0·007	AIDS	5	–	+	–
202	0·001	AIDS	4	–	+	–
320	0·090	AIDS	7	–	–	–
324	0·072	AIDS	2	–	–	–
306	0·308	AIDS	4	–	–	–
325	0·009	CDC III	1	–	–	–
319	0·050	AIDS	1	–	–	–
310	0·110	AIDS	1	–	–	–
c. Patients treated with DDI at time of BAL						
284	0·010	AIDS	5	–	–	–
293	0·010	AIDS	0	–	–	–
260	0·003	AIDS	7	–	+	–
318	0·003	AIDS	2	–	–	Tox
234	0·002	AIDS	5	+	–	–
283	0·003	AIDS	1	–	–	–

* Criteria for ranking severity of respiratory episode:

Feature	*Score*
Fever >38 C before lavage	1
O_2 saturation >95% before lavage and desaturation on exertion	1
O_2 saturation <95% >90%	2
O_2 saturation <90% >85%	3
O_2 saturation >85% or requiring O_2 therapy	4
Chest X-ray shadowing one zone	1
Chest X-ray shadowing two zones	2
Chest X-ray shadowing three zones/confluent/generalized	3
Requiring ventilatory support	2
Death from respiratory episode (max. possible)	12

ND, not done; CMV, cytomegalovirus; PC, *Pneumocystis carinii*; S.a., *Staphylococcus aureus*; HSV, *Herpes simplex* virus; St. pn., *Streptococcus pneumoniae*; ADN, adenovirus; H.i., *Haemophilus influenzae*; Tox, *Toxoplasma gondii*; aafb.pos., acid-fast bacteria positive.

RESULTS

Results were analysed according to whether or not patients were receiving antiretroviral treatment at the time of the lavage. Data on the severity of each episode, the severity of HIV disease, and the nature of the infectious agents isolated are shown in Table 1.

Infectious agents, including *P. carinii* and CMV, were identified at 15/18 episodes (83%) in patients who had never received antiretroviral therapy compared with 8/22 (36%) in AZT-treated group and with 3/6 (50%) in DDI group. No infectious agents were identified from BAL fluid of normal volunteers.

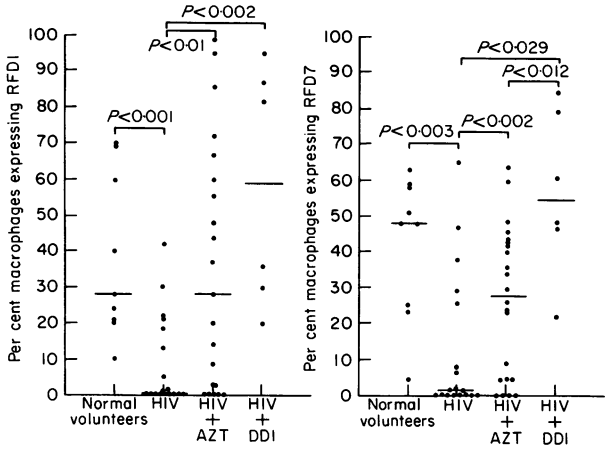

Fig. 1. Expression of dendritic and phagocytic cell markers on alveolar macrophages. AZT, Zidovudine; DDI, 2′,3′-dideoxyinosine.

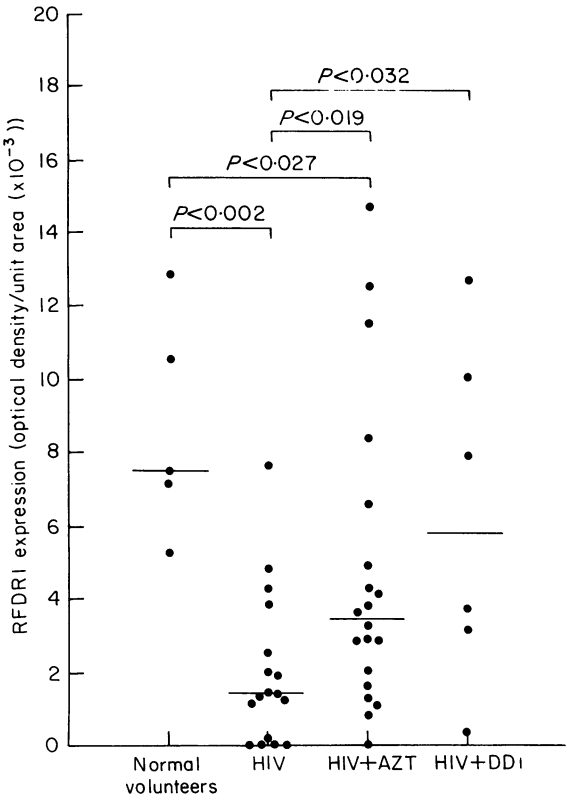

Fig. 2. Expression of HLA-DR molecules on bronchoalveolar lavage (BAL) cells. AZT, Zidovudine; DDI, 2′,3′-dideoxyinosine.

Differential cell counts revealed that HIV-infected patients who were not treated with antiretroviral drugs had significantly higher percentages of lymphocytes in BAL fluid when compared with normal controls, leading to lymphocytosis in 70·6% of cases. Treatment with AZT or DDI reduced the incidence of lymphocytosis (40·9% in AZT group, 0% in DDI group), bringing the median percentage of lymphocytes in BAL to normal levels.

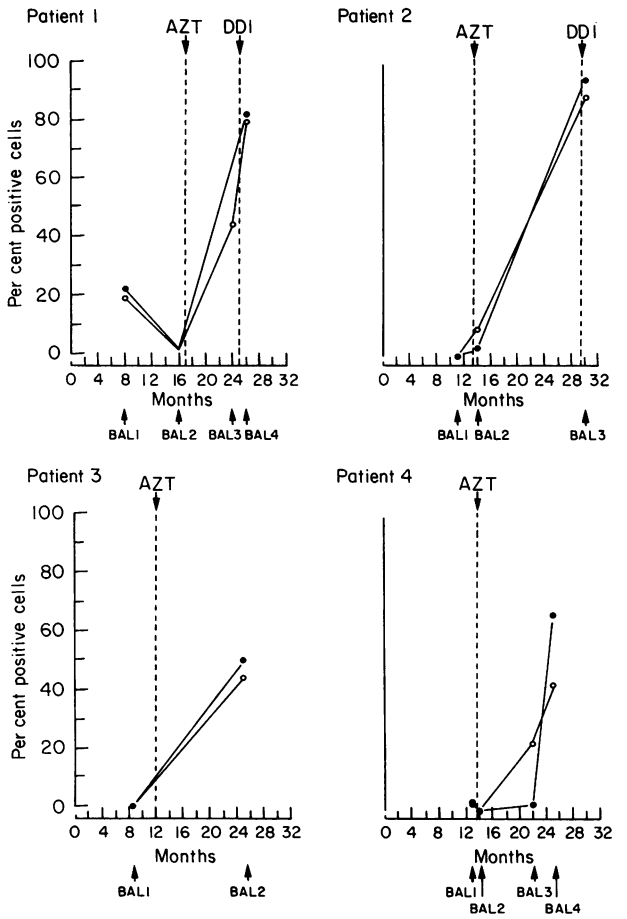

Fig. 3. Changes in the expression of antigen-presenting and phagocytic cell markers on alveolar macrophages in sequential bronchoalveolar lavage (BAL) samples. ○, RFD7; ●, RFD1. AZT, Zidovudine; DDI, 2′,3′-dideoxyinosine.

Patients who received no antiretroviral therapy had markedly reduced proportions of alveolar macrophages expressing the antigen-presenting (MoAb RFD1) and phagocytic (MoAb RFD7) cell markers when compared with the normal volunteers (medians 0·5% and 1·35% respectively *versus* 28% and 48% in volunteers, Fig. 1). Expression of HLA-DR molecules on BAL cells isolated from the same patients was also dramatically impaired (median of 1·44 (optical density/unit area) *versus* 7·50 (optical density/unit area) in volunteers, Fig. 2).

However, in patients who at the time of BAL were receiving AZT or DDI, median percentage of alveolar macrophages expressing RFD1 and RFD7 molecules was found to be similar or higher than in normal volunteers (Fig. 1). A similar pattern was observed in the expression of HLA-DR molecules: reduction in patients who were not treated with antiretrovirals and the reversal of such loss in individuals treated with AZT or DDI (Fig. 2).

We also analysed changes in the expression of dendritic and phagocytic cell markers in four patients who presented with two or more consecutive episodes of pneumonitis (Fig. 3). Expression of both RFD1 and RFD7 markers in alveolar macrophages in all four patients was reduced before antiretroviral therapy but was found to have returned to normal or higher levels after commencing such treatment.

When the data for all patients were analysed together, the changes in the expression of HLA-DR molecules and phenotypic macrophage markers did not correlate with peripheral blood CD4 count, lung lymphocytosis, severity of respiratory episodes, presence of infectious organisms in BAL fluid or other drugs these patients were taking, but were clearly associated with antiretroviral therapy.

DISCUSSION

This study has demonstrated that the expression of HLA-DR molecules as well as markers for antigen-presenting and phagocytic cells is clearly decreased on alveolar macrophages obtained from AIDS patients who had presented with an acute respiratory episode. Moreover, our investigation has also shown that treatment with antiretroviral drugs enhances the expression of such markers and therefore restores the values of HLA-class II expression to levels observed in normal volunteers. As the reversal of these markers is dependent on antiretroviral therapy, we conclude that the alterations in the phenotypic characteristics of alveolar macrophages are not necessarily directly associated with the emergence of a respiratory episode but they may be related to HIV disease.

These observations confirm the findings on three HIV-infected patients where reduced levels of HLA-DR expression on alveolar macrophages were recorded [17]. Diminished expression of class II molecules has also been found in other tissues. In AIDS patients, substantial loss of HLA-DR-positive epidermal Langerhans cells has been observed [18] as well as a decrease in HLA-DR expression on peripheral blood monocytes [19]. HIV can alter phenotype of macrophage-like cells cultured *in vitro*. Studies on human promonocytic U937 cell line using six anti-HLA class II MoAbs have provided strong evidence that HIV infection down-regulates expression of HLA class II antigens [20].

It is likely that the reduction in the expression of RFD1, RFD7 and HLA-DR molecules on alveolar macrophages in AIDS patients reported here is manifested in the functional impairment of these cells. We have previously demonstrated that the antigen-presenting function of alveolar macrophages is related to their ability to express RFD1 molecules, and that anti-RFD1 MoAb can block antigen presentation when added to cell culture [21,22]. HLA-DR antigens on alveolar macrophages also play an important role in the antigen-induced T lymphocyte proliferation and MoAb against HLA-DR molecule is capable of reducing the stimulatory function of these macrophages [23]. Recently, it has been documented that in the course of HIV disease peripheral blood dendritic cells and monocytes obtained from AIDS patients lose their ability to stimulate T cell proliferation [24,25]. Similar results were obtained when these cells were exposed to HIV *in vitro*.

The re-emergence of phenotypic macrophage markers after treatment with antiretroviral drugs signifies the ability of these drugs to induce immune changes within the lung. In this study the incidence of lung lymphocytosis and isolation of opportunistic organisms were reduced in AZT- and DDI-treated patients, matching results of previous controlled trials [7,8]. Lately, increased percentage of HLA-DR-positive (activated) T cells has been correlated with progression of HIV disease [26]. It is not known whether AZT treatment can influence HLA-DR expression on T cells. $CD4^+$ T cells from AIDS patients have impaired responses to antigen receptor stimulation [27], and this impairment can be corrected by AZT therapy [28].

Further studies are necessary to elucidate the clinical significance of altered expression of HLA class II molecules in the pathology of AIDS. Monitoring such changes may prove to be a useful tool for assessment of disease progression and efficacy of novel therapies in AIDS.

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