

Changes in lung lymphocyte populations reflect those seen in peripheral blood in HIV-1 positive individuals

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Changes in lung lymphocyte populations reflect those seen in peripheral blood in HIV-1 positive individuals. M. Bofill, M. Lipman, J.E. McLaughlin, M.A. Johnson, L.W. Poulter. ©ERS Journals Ltd 1998.

ABSTRACT: We have investigated the level of lymphocytosis present in the lung of human immunodeficiency virus (HIV)-1+ infected patients with and without pulmonary disease and how changes in natural killer (NK), B and T-cells seen in peripheral blood (PB) compare with those seen in bronchoalveolar lavage fluid (BALF).

Lymphocyte subpopulations and their expression of activation, cytotoxic markers and memory status were characterized by triple immunofluorescence.

Macrophages accounted for over 80% of the BAL cells. Only three out of 72 patients had a lymphocyte percentage >30%. No statistically significant differences in the relative proportions of NK, CD4 and CD8 populations were seen in BALF when compared to PB, except for a twofold increase in the percentage of activated CD8 cells in BALF. The only differences in BALF populations between the HIV-1+ groups were a lower percentage of CD4+ cells, and a higher percentage of activated CD8+ cells in the patients with pneumonitis.

In the present cohort of patients there was little evidence for an overall lymphocytosis in bronchoalveolar lavage fluid of HIV-1+ subjects. Changes observed in lymphocyte subsets of bronchoalveolar lavage fluid populations reflected those in peripheral blood, and were similar for patients with and without pneumonitis. Evidence of increased CD8 subset activity in bronchoalveolar lavage fluid did, however, emerge.

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The effects of human immunodeficiency virus (HIV-1) infection in peripheral blood (PB) of HIV-infected patients have been described in detail by several groups, including this laboratory [1–4]. The most striking effect is the fall in absolute CD4 T-lymphocytes [1, 2], paralleled in many instances by an increase in the numbers of short-lived terminal CD8 T-lymphocytes with high expression of activation markers [3].

Several reports have shown that HIV-1 affects the lymphocyte population in the lung [5–11]. Up to 75% of patients have increased percentages of CD8 lymphocytes in bronchoalveolar lavage fluid (BALF) [6, 8, 10], mainly due to an expansion of CD8+ CD57+ T-cells [6]. It has also been documented that a proportion of these cells express activation markers such as major histocompatibility complex (MHC) Class II and CD25 [11]. A reduction in CD8+ CD28+ cells has also been noted [12]. Nevertheless, in most studies, the patients were selected either because they had respiratory disease or had a documented lymphocytosis in their BALF. Furthermore, PB and BALF populations obtained at the same time were rarely compared.

We have expanded these studies and compared the effects of HIV-1 infection on the lymphoid populations in PB and BALF, excluding drug users as they have reduced pulmonary function tests when compared to other HIV-1

seropositive (HIV-1+) groups [13]. We have focused on three points. Firstly, we considered whether our cohort of patients had alterations in the relative proportions of macrophages, lymphocytes and granulocytes in BALF when compared to controls. In seven cases we confirmed our data by looking at post-mortem tissues from patients who had previously been lavaged, to exclude the possibility that the results obtained were due to sampling anomalies at bronchoscopy. Secondly, we compared the results obtained from HIV-1+ individuals with and without pneumonitis to determine whether clinical respiratory disease altered the lymphocyte profile of the BALF. Thirdly, we investigated how the changes in T, B and natural killer (NK) cells seen in the BALF compared with those seen in the PB.

Materials and methods

Patient and control samples

Heparinized peripheral blood was donated by 103 individuals who underwent bronchoscopy with bronchoalveolar lavage. The individuals were classified into three groups: A) healthy laboratory workers; B) asymptomatic HIV-1+ patients with no respiratory disease (HIV_{nr}); and C) HIV-1+ patients with symptoms and signs of pneumonitis (HIV_p). The study was approved by the ethics committee of the Royal Free Hospital.

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Group A comprised 31 healthy adult volunteers with no past history of lung disease or symptoms suggesting viral infection in the last 3 months.

Group B comprised 39 HIV-1 antibody positive individuals with no history of acute or chronic respiratory disease determined by respiratory questionnaire, normal spirometry and chest radiograph and who volunteered for the study. Within this population 31 of 39 (79%) subjects were homosexual males and eight of 39 (21%) were heterosexual (five males and three females). According to the Center for Disease Control (CDC) classification, 18 were HIV group II (asymptomatic), 12 were group III (persistent generalized lymphadenopathy) and nine were group IV (seven with minor opportunistic disease and two with clinical nonpulmonary acquired immune deficiency syndrome (AIDS)). This population had blood CD4 counts ranging 0–1,450 cells· μL^{-1} , median 480 cells· μL^{-1} . None of this group were taking antiretroviral therapy or had a history of injecting drug use.

Group C comprised 33 HIV-1 infected subjects with clinical features of respiratory infection. Within this group 28 of 33 (85%) were homosexual males, in the remaining five the risk was heterosexual sex in two males and one female and contaminated blood transfusion in two females. Of these, 18 had a previous clinical AIDS diagnosis (CDC group IV), 11 minor opportunistic infections (CDC IV) and four had no history of HIV-related illnesses (CDC II, CDC III). Blood CD4 counts within this population ranged 0–190 cells· μL^{-1} , with a median of 30 cells· μL^{-1} . Nine of 33 subjects were taking antiretroviral therapy at the time of their respiratory episode (five zidovudine (AZT) alone, two dideoxyinosine (ddI) alone, one dideoxycytosine (ddC) alone and one combination AZT and ddI).

Infectious agents recovered from the BAL

The presence of infectious agents was determined by cytology (looking for fungi, acid fast bacilli, protozoa and viral inclusions, as well as cytological abnormalities), bacteriology (including aerobic and anaerobic cultures as well as fungal cultures, mycobacteriology (both smear and prolonged cultures) and virology (using both early antigen detection methods and cultures). Infectious agents recovered from the BALF of the HIV pneumonitis group were: *Pneumocystis carinii* in 39% of the cases; bacteria in 15%; respiratory syncytial virus in 6%; *Mycobacterium avium* complex in 6%, and Cytomegalovirus in 24%. No pathogen was isolated in 30% of cases, while in some cases more than one pathogen was detected. In subsequent analyses the HIV pneumonitis patients were dealt with as one group as no difference was found in measured immune parameters between subjects with and without *P. carinii* in their BALF.

P. carinii pneumonia prophylaxis (PCP) was used in eight of 39 (21%) asymptomatics and 27 of 33 (82%) HIV-1 individuals with pneumonitis

Bronchoscopy

BALF samples were obtained as described previously: fibreoptic bronchoscopy was performed using three 60 mL

aliquots of warm sterile saline. Each aliquot was aspirated immediately after instillation and collected in silicone coated glass bottles [14]. Differential counts were performed using PB with a routine cytometer and from BALF samples on May Grünwald-Giemsa stained cytopspins by counting 500 cells. Special care was taken to exclude any samples that had more than 5% red cells in their BALF as this indicated haemorrhage during the procedure and that the BALF was contaminated by blood. Patients with pneumonitis tended to bleed more easily than asymptomatic patients and care with the technique was essential to obtain reliable data.

Histopathology

Lung tissue was obtained during post-mortem examination of seven patients who during their last illness had undergone bronchoscopy and lavage to investigate an episode of pneumonitis. These tissues were embedded in paraffin wax and microtome sections prepared.

Cell staining

Peripheral blood mononuclear cells and BALF lymphoid suspensions were stained with double and triple immunofluorescent antibody mixtures using fluorochrome conjugated monoclonal antibody reagents (summarized in table 1) [15]. This was followed by analysis on the fluorescence-activated cell sorter scan (FACScan; Becton Dickinson UK Ltd., Oxford, UK). In BALF samples some small macrophages and debris fall inside the lymphocyte gate. To overcome this problem we have given either the relative proportion of two or more popula-

Table 1. – Reagents

CD	Isotype	Molecular structure	Reactivity
CD16	IgG1	50–60 kD	NK cells, granulocytes
CD3	IgG1	16, 20, 25–28 kD (5 chains)	T-cells
CD4	IgG1	50 kD	T-helper cells
CD8	IgG1	34 kD (2 chains)	T-suppressor/cytotoxic T-cells
CD57	IgG1	110 kD	NK cells, T-cell subset
CD5	IgG1	67 kD	T-cells and a subset of B-cells
Class II	IgG1	34 kD	B-cells, interdigitating cells, activated macrophages and T-cells
CD38	IgG1	45 kD	Activate T and B-cells, plasma cells
CD7	IgG1	24 kD	Activated T-cells
CD45RO	IgG2a	180 kD	Primed T-cells
CD28	IgG1	44 kD	CD4 cells and a subset of CD8+ cells
CD68	IgG1	110 kD	Macrophages
TIA-1	IgG1	15 kD	Cytotoxic granules of T and NK cells
Bcl-2	IgG1	25 kD	Majority of resting B and T-cells

IgG: immunoglobulin G. NK: natural killer.

tions, or the percentages of positive cells in a positively gated population. For example, the triple combination CD3, CD4, CD8 allowed us to gate on CD3+ T-cells and find the relative proportions of CD4+ and CD8+ T-cells without including CD4+ monocytes or CD8+ natural killer (NK) cells. Intracellular antigens were stained after permeabilization of the cell membrane using ORTHO Permeafix™ (OPF, Ortho Diagnostic Systems Inc., Raritan, NJ, USA; 800 µL-tube⁻¹; 30 min at room temperature) to effect simultaneous fixation and permeabilization of the peripheral blood mononuclear cells (PBMC) in whole blood.

Cytospin preparation and immunocytochemistry

BALF suspensions were adjusted at 10⁶ cells·µL⁻¹ and cytocentrifuged. The proportions of immunocompetent cells were determined in these cytopsins and the paraffin sections of lung tissues by indirect immunoperoxidase staining using anti-CD68 to stain macrophages and anti-CD3 to label T-cells. Staining was followed by analysis with an optical microscope or an image analysis system (Seescan Imaging Ltd., Cambridge, UK) [16, 17].

Statistical analysis

The data are presented as arithmetic mean±SEM with the range in parenthesis. The mean values were compared using the Student's t-test for independent means; p-values of less than 0.01 were considered significant.

Results

Macrophages are the predominant population in BALF in HIV infection

Macrophages were the most prominent cell type in all groups representing more than 80% of all white cells in the BALF (table 2). Nevertheless, a proportion of individuals, especially patients with pneumonitis, had an increased percentage of lymphocytes, although this remained below 15% in 90% of HIVnrd and 73% of HIVp compared to 97% in the controls. Only 4% of the HIV-1+ patients had a percentage of lymphocytes equal to or greater than 30%. The granulocytic population was the least represented in all groups although significantly greater proportions were seen in the HIVp group. The percentage of red cells was always below 5%.

As shown in table 2, the absolute numbers of different populations of cells recovered from BALF, expressed as 10⁶ cells·total lavage⁻¹ were not significantly increased in the HIVnrd and HIVp groups when compared to controls.

Proportion of macrophages, lymphocytes and granulocytes in lung tissue

As we found lower levels of lymphocytosis than reported by other authors, we wanted to confirm our data by looking at lung tissue obtained at post-mortem from patients who had previously been lavaged. Sections were stained with panmacrophage and T-cell monoclonal antibodies (CD68, CD3) on consecutive sections. The same areas were identified and macrophages and T-cells from interstitial and perialveolar areas were counted using an image analyser. The ratio of macrophages to lymphocytes was 31.0±9.6 with a range of 4.7–67. The lymphocytic population, consisting mainly of T-cells, was very scarce and scattered throughout the interstitium. In some cases, clusters of granulocytes were seen in the interstitium.

Differences in lymphocytic subpopulations from PB and BALF in HIV-1+ subjects

Lymphocyte subpopulations were studied in detail in PB and BALF samples obtained at the same time from a small number of patients with BALF lymphocytosis. This group comprised six controls, seven HIV-1+ patients without and seven HIV with respiratory disease. The patient samples were chosen because they had a high proportion of lymphocytes (>10%) of which more than 95% were viable. Within the patients with no respiratory disease, four were CDC group II and three were CDC group III. All seven patients with respiratory disease were CDC group IV. The differential counts of these patients are given in table 3. The results fell into two distinct groups: all the patients had very similar results between PB and BALF except one asymptomatic HIV-1+ patient who had major differences between some of the populations from the two sites. This patient was excluded from the main set of results and will be described separately at the end of the section.

When we compared the lymphocytic populations from PB and BALF obtained at the same time we found that the results were very similar in the HIVnrd and HIVp groups and, thus, we expressed the results as a single group. No statistically significant differences were seen in the percentages of NK (CD16) cells, CD4 and CD8 T-lymphocytes from PB and BAL (table 4). In BALF, twice the number of CD8+ cells expressed the memory phenotype CD45RO and the activation marker CD38. A significantly greater percentage of CD8+ cells expressing the activation marker CD7 were also present in BALF. CD8+ T-cells expressing Class II were also high, but were already elevated in the blood when compared to HIV negative controls (controls: 8.0±5.0%; HIV-1+ 36.3±6.5%).

Table 2. – Distribution of white cells in bronchoalveolar lavage fluid (BALF) from human immunodeficiency virus-1 seronegative (HIV-1-) and human immunodeficiency virus-1 seropositive (HIV-1+) individuals

	Macrophages		Lymphocytes		Granulocytes	
	%	n×10 ⁶	%	n×10 ⁶	%	n×10 ⁶
HIV-1- (n=31)	92.6±1.0	17.1±1.9	6.6±1.1	0.9±0.1	1.0±0.2	0.8±0.1
HIV-1+ (n=72)	86.3±1.67	16.9±1.8	10.1±1.4	1.3±0.1	3.9±1.0	1.0±0.3
With no respiratory disease (n=39)	89.6±1.8	17.8±2.0	8.9±1.8	1.4±0.2	1.7±0.4	0.5±0.2
With pneumonitis (n=33)	81.9±2.9	15.8±3.3	11.6±2.3	1.19±0.2	6.6±2.2	1.8±0.6

Values are presented as percentages of white cells or number (×10⁶) of cells recovered from the total volume of BALF.

Table 3. – Distribution of white cells in bronchoalveolar lavage fluid (BALF) from individuals chosen for the lymphocyte study

	Macrophages		Lymphocytes		Granulocytes	
	%	n×10 ⁶	%	n×10 ⁶	%	n×10 ⁶
HIV-1- (n=6)	90.5±0.4	15.1±4.1	8.5±0.4	1.4±0.4	1.0±0.2	2.1±0.1
HIV-1+ (n=14)	78.5±2.6	8.1±1.4	18.2±2.3	2.1±0.5	4.0±1.5	0.4±0.1
With no respiratory disease (n=7)	78.3±3.9	10.3±2.1	20.1±4.1	2.9±0.8	2.5±0.9	0.3±0.1
With pneumonitis (n=7)	78.7±3.6	5.7±1.7	15.8±2.1	1.2±0.4	5.5±2.0	0.6±0.2

Values are presented as percentages of white cells or number (×10⁶) of cells recovered from the total volume of BALF. HIV-1-, HIV-1+: human immunodeficiency virus-1 seronegative and seropositive, respectively.

Table 4. – Lymphocyte populations from peripheral blood (PB) and bronchoalveolar lavage fluid (BALF)

	PB %	BALF %	p-value
T-lymphocytes*	84.7±3.3	92.1±1.8	NS
Natural killer cells*	13.9±2.5	7.6±1.9	NS
CD4+ T-lymphocytes†	21.7±4.9	23.6±4.4	NS
CD8+ T-lymphocytes†	75.8±5.4	80.6±4.5	NS
Percentages within the CD8+ population			
Class II	45.2±3.7	48.3±5.1	NS
CD57+	25.1±2.8	27.9±4.9	NS
CD45RO+	23.0±4.1	48.8±6.5	p<0.01
CD38+	50.0±7.7	42.8±7.2	NS
CD45RO+ CD38+	12.5±3.2	20.3±5.0	p<0.01
CD7+	52.7±3.7	70.3±5.9	p<0.01
CD5+	>95	>95	
TIA-1+	62.1±8.7	62.1±8.7	NS
CD28+	36.6±3.8	41.6±3.8	NS
Bcl-2+	79.6±3.9	84.0±6.1	NS

Samples were taken from 13 human immunodeficiency seronegative patients. *: percentage of total lymphocytes; †: percentage of CD3+ cells. NS: nonsignificant.

There was no differences in the proportion of CD8+ cells that expressed TIA-1, CD28, CD57 or Class II MHC molecules (table 4). In both PB and BALF all CD3+ T-cells expressed CD5 on their surface suggesting no significant presence of intraepithelial lymphocytes, which always had a very weak expression [18]. In both PB and BALF over 80% of cells expressed Bcl-2.

As mentioned earlier, one patient without respiratory disease had a different set of results from the others. In

this case a much greater proportion of CD8 cells infiltrated the BALF than the blood (BALF: 58%; PB: 41%). There was a huge increase in the percentage of CD8+ expressing CD57 (PB: 27%; BALF: 96%). The BALF data was corroborated by the findings of very low levels of CD28 positive cells in the CD8 population (28%); more than 98% contained TIA-1+ granules. These cells expressed very high levels of Bcl-2, indicating that they are probably a long-lived population (PB: 65%, BALF: 98%).

Lymphoid population in HIV-1 infected bronchoalveolar lavage fluid

The changes in the lymphoid population in the BALF are summarized in table 5. As expected, there was a decrease in percentage of CD4 in the HIV-1+ group when compared to controls. The decrease in CD4 was more pronounced in patients with pneumonitis, where the percentage of CD4 was less than 7% of total compared to 62% in the controls. This loss was reflected in an increase in the percentage of CD8 cells. Relative proportions of NK cells and T-cells were the same in all groups.

When we examined the phenotype of CD8 cells, the changes in the BAL reflected those previously described in blood for the different study groups. The percentage of CD8+ cells expressing the NK marker CD57 tripled and the CD8+ cells expressing cytotoxic granules doubled in the HIV-1+ groups when compared to controls. There was also a fourfold increase in the percentages of CD8+ cells expressing the memory phenotype CD45RO or the activation marker CD38. Coexpression of both markers was

Table 5. – Lymphocyte populations recovered from bronchoalveolar lavage fluid

	Controls (n=6)	HIV-1+		p-values	
		Symptom free (n=6)	Pneumonitis (n=7)	HIV-1- vs HIV-1+	Symptom free vs pneumonitis
Natural killer cells*	8.5±4.5	4.4±2.7	10.0±2.9	NS	NS
T-lymphocytes*	90.5±4.5	95.2±1.4	90.0±2.9	NS	NS
CD4+ T-lymphocytes†	62.1±1.0	32.3±3.7	7.1±5.2	p<0.01	p<0.01
CD8+ T-lymphocytes†	38.2±5.0	67.8±3.6	93.5±3.5	p<0.01	p<0.01
Class II‡	36.3±6.5	48.2±2.7	48.5±7.1	NS	NS
CD57‡	7.7±0.7	37.4±7.1	17.5±3.3	p<0.01	p<0.01
CD45RO‡	14.4±4.0	37.6±10.5	56.7±7.0	p<0.01	p<0.01
CD38‡	10.5±3.5	26.8±0.9	56.6±9.1	p<0.01	p<0.01
CD45RO+ CD38‡	1.0±0.1	9.4±4.5	29.3±6.2	p<0.01	p<0.01
CD7‡	60.9±5.1	68.7±7.4	72.6±7.6	NS	NS
CD5‡	>95	>95	>95		
TIA-1‡	32.0±10.0	73.2±7.3	66.5±10.5	p<0.01	NS
CD28‡	66.0±14.0	41.2±6.4	41.7±4.9	p<0.01	NS
Bcl-2‡	70.5±7.7	84.0±6.0	85.7±4.9	NS	NS

*: percentage of lymphocytes; †: percentage of positive cells within the CD3+ population ‡: percentage of positive cells within the CD8+ population. NS: nonsignificant. HIV-1-, HIV-1+: human immunodeficiency virus-1 seronegative and seropositive, respectively.

seen in 20% of the CD8+ cells in the HIV-1+ group although it was almost absent from the HIV-1 negative group. A lower expression of the co-stimulatory molecule CD28 (HIV-1: 66.0±14.0%; HIV-1+: 41.6±3.8%) was also seen.

When we compared the two positive groups we found that the percentage of CD8+ cells expressing CD45RO or CD38 was twofold higher in the HIVp group, and the percentage of cells expressing both markers tripled. Furthermore, there was a twofold decrease in the percentages of cells with CD57 in patients with pneumonitis, although the percentages were still three times higher than in the control group. There was no statistical significance in the percentages of any other markers including Bcl-2, which was high in all groups.

Discussion

In the present cohort of HIV-1+ patients CD8 lymphocytosis in BALF was a much rarer event than reported by other groups [6, 8, 10, 11]. Only 7% of asymptomatics and 22% of patients with pneumonitis had >15% of lymphocytes in the BALF, and only one patient had >45%. This compares to other reports which state that the percentages of lymphocytes range 15–75% [6, 8, 10, 11]. Our data, nevertheless, confirm that a lymphocytosis was seen in a higher proportion of patients with pneumonitis compared to asymptomatic patients [11]. Analysis of post-mortem lung from seven previously lavaged patients showed a lymphocyte distribution consistent with the BALF result. Thus, it is unlikely that the lavage data reflected poor sampling. An explanation for the apparent discrepancy in the frequency of BALF lymphocytosis reported here might be that this study did not exclusively target patients with pneumonitis or lymphocytic alveolitis, and also included patients at all stages of disease. The present study population also specifically excluded injecting drug users, a population reported to have inherent abnormalities of lung function [13]. Furthermore, the rejection of any BALF sample with a red cell blood count above 5%, excluded the possibility of PB leucocytes contaminating the BALF.

As the absolute numbers of macrophages recovered from total BALF remained constant in all group of patients, it is puzzling why these cells (the effector cells of natural immunity [19]) failed to prevent the establishment of opportunistic infections. Several explanations can be postulated: 1) The gradual disappearance of the CD4 population might affect macrophage function; 2) the presence of an increased percentage of activated CD8 cells (see below) might activate alveolar macrophages (AM), that in turn will release inflammatory cytokines resulting in altered permeability of the lung epithelium and endothelium [20–24]; and 3) AM infected by HIV-1 may exhibit compromised function. Indeed the number of infected AM is known to be very high, in particular in the patients with PCP where up to 58% of macrophages, both in BAL and tissues, have been found to express HIV-1 p18 [5, 8, 25].

There were no significant differences between the percentages of lymphocyte subsets found in the PB and BAL in either HIV-1+ group. Experiments reinfusing autologous radiolabelled ¹¹¹I CD8+ cells have shown that these cells migrate from the blood to the lung, and from there to other organs such as liver, spleen and bone marrow [26]. There is nevertheless a difference in the activation status of the PB

and BALF CD8+ T-cells in HIV infection where a higher proportion of CD8 cells expressed CD45RO and activation markers CD38 and CD7. In the present study, the proportion of CD45RO cells was lower than that described by other groups [11]. Whether the CD8 cells are activated locally or are preferentially selected still needs to be elucidated. This primed activated CD8+ population is also expanded in other viral diseases, and is a terminal population that loses its Bcl-2 expression and dies from apoptosis [27]. The Bcl-2 expression of CD8+ cells was very similar in PB and BAL suggesting that the proportion of these cells dying of apoptosis is very similar at both sites.

Another important finding is that around 70% of the CD8+ cells in the lung contain TIA-1 positive cytotoxic granules. This is in line with the observation that high frequencies of spontaneous cytotoxic cells to HIV infected macrophages have been described in the lung, especially before the establishment of opportunistic infections [10]. In contrast, with cells obtained from blood and lymph nodes, BAL CD8+ cells have cytotoxic capacity without further activation [7]. This might be due to a proportion of these cells in the lung already expressing activation markers. However, it has been shown that the specific cytotoxic capacity decreases with disease progression [10]. Again the loss of CD4 might contribute to the decreased CTL activity as these cells are dependent on CD4 help [10]. Furthermore, suppressor factors released by the CD8+ CD57+ population might also interfere with the cytotoxic function (see below). Another contributing factor to the loss of specific cytotoxic activity might be that CD8 cells from the lung can themselves carry HIV-1 [28]. This could contribute to the spreading of HIV infection, as cells from the lung can migrate to liver, spleen and bone marrow [26].

As described by other groups, we have also seen an expansion of the CD3 CD8 CD57 positive population in the BAL of HIV-infected people [6], though this was also paralleled by an expansion of this population in the blood in all but one patient where there was a fourfold increase of the cells in BAL when compared to PB. This patient was asymptomatic and had a splenectomy several years earlier. The CD8+ CD57+ T-cells in that patient had unusually high levels of Bcl-2 suggesting that it was a much longer lived population than the CD57-negative counterpart [27].

Within the lung the main differences between the HIVnrd and HIVp was a greater reduction of CD4 cells and an increase in the proportion of CD8 T-cells expressing activation markers CD45RO and CD38 in the symptomatics. An expansion of this population in the blood is known to be associated with a decline of CD4 cells [3]. The overlap seen between the percentages of activated CD8 cells in the two groups suggests that the changes are more likely to be the result of HIV-1 infection than a response to other organisms.

In summary, we found no significant lymphocytosis in the vast majority of human immunodeficiency virus-1 seropositive subjects and thus alveolar macrophages remain the main line of defence against opportunistic infections [20]. In some rare cases, there is an increase in primed activated CD8+ cells. Nevertheless, this infiltration is usually mild and reflects the changes seen in peripheral blood. This is in sharp contrast with what happens in the lymph nodes of human immunodeficiency virus-1 seropositive patients, where these CD8 cells massively invade the

nodes [29]. The lung microenvironment is capable of fully preventing opportunistic infections in the lung of human immunodeficiency virus-1 seropositive patients until they are seriously immunosuppressed. The loss of CD4 cells and alteration in the CD8+ population might affect macrophage function or vice versa. Further studies are clearly needed to elucidate why lung opportunistic infections cannot be controlled in late human immunodeficiency virus infection.

References

- Gottlieb MS, Schroff R, Schanker HM, Weisman JD, Fan PT, Wolf RA. *Pneumocystis carinii* pneumonia and mucosal candidiasis in previously healthy homosexual men: evidence of a new acquired cellular immunodeficiency. *N Engl J Med* 1981; 305: 1425–1431.
- Phillips AN, Lee CA, Elford J, Janossy G, Bofill M, Timms A, Kernoff PB. Prediction of progression to AIDS by analysis of CD4 lymphocyte counts in a haemophilic cohort. *AIDS* 1989; 3: 737–741.
- Bofill M, Mocroft A, Lipman M, *et al.* Increased numbers of primed activated CD8+CD38+CD45RO+ T-cells predict the decline of CD4+ T cells in HIV-1-infected patients. *AIDS* 1996; 10: 827–834.
- Borthwick NJ, Bofill M, Gombert WM, *et al.* Lymphocyte activation in HIV-1 infection II. Functional defects of CD28- T-cells. *AIDS* 1994; 8: 431–441.
- Donaldson YK, Bell JE, Ironside JW, Brettell RP, Robertson JR, Simmonds P. Redistribution of HIV outside the lymphoid system with onset of AIDS. *Lancet* 1994; 343: 383–385.
- Sadat-Sowti B, Debre P, Idziorek T, *et al.* A lectin-binding soluble factor released by CD8+CD57+ lymphocytes from AIDS patients inhibits T-cell cytotoxicity. *Eur J Immunol* 1991; 21: 737–741.
- Hoffenbach A, Langlade-Demoyen P, Dadaglio G, *et al.* Unusually high frequencies of HIV-specific cytotoxic T-lymphocytes in humans. *J Immunol* 1989; 142: 452–462.
- Plata F, Autran B, Martins LP, Wain-Hobson S, *et al.* AIDS virus-specific cytotoxic T-lymphocytes in lung disorders. *Nature* 1987; 328: 348–351.
- Guillon JM, Autran B, Denis M, *et al.* Human immunodeficiency virus-related lymphocytic alveolitis. *Chest* 1988; 94: 1264–1270.
- Walker BD, Plata F. Cytotoxic T-lymphocytes against HIV. (Review). *AIDS* 1990; 4: 177–184.
- Agostini C, Chilosi M, Zambello R, Trentin L, Semenzato G. Pulmonary immune cells in health and disease: lymphocytes. *Eur Respir J* 1993; 6: 1378–1401.
- Saukkonen JJ, Kornfeld H, Berman JS. Expansion of a CD8+CD28- cell population in the blood and lung of HIV-positive patients. *J Acquir Immune Defic Syndr* 1993; 6: 1194–1204.
- Rosen MJ, Lou Y, Kvale PA, *et al.* Pulmonary function tests in HIV-infected patients without AIDS. Pulmonary Complications of HIV Infection Study Group. *Am J Respir Crit Care Med* 1995; 152: 738.
- Poulter LW, Norris A, Power C, Condez A, Schmekel B, Burke C. T-cell dominated inflammatory reactions in the bronchi of asthmatics are not reflected in matched bronchoalveolar lavage specimens. *Eur Respir J* 1992; 5: 182–189.
- Hoffman RA, Kung PC, Hansen WP, Goldstein G. Simple and rapid measurement of human T-lymphocytes and their subclasses in peripheral blood. *Proc Natl Acad Sci USA* 1980; 77: 4914–4917.
- Janossy G, Amlot P. Immunofluorescence and immunohistochemistry. *In:* Klaus G, ed. *Lymphocytes: A Practical Approach*. Oxford, IRL Press, 1987; pp. 67–108.
- Poulter LW, Norris A, Power C, *et al.* T-cell dominated inflammatory reactions in the bronchioles of asymptomatic asthmatics are also present in the nasal mucosa. *Postgrad Med J* 1991; 67: 747–753.
- Saltini C, Kirby M, Trapnell BC, Tamura N, Crystal RG. Biased accumulation of T-lymphocytes with "memory"-type CD45 leucocyte common antigen gene expression on the epithelial surface of the human lung. *J Exp Med* 1990; 171: 1123–1140.
- Pauza CD. HIV persistence in monocytes leads to pathogenesis and AIDS. *Cell Immunol* 1988; 112: 414–424.
- O'Riordan C, Rosoff PM. Reconstitution of a T-cell receptor-stimulated plasma membrane calcium transporter: lack of dependence on inositol phosphates. *Cell Calcium* 1993; 14: 119–133.
- Agostini C, Trentin L, Zambello R, *et al.* Release of granulocyte-macrophage colony-stimulating factor by alveolar macrophages in the lung of HIV-1-infected patients. A mechanism accounting for macrophage and neutrophil accumulation. *J Immunol* 1992; 149: 3379–3385.
- Twigg HL, Iwamoto GK, Soliman DM. Role of cytokines in alveolar macrophage accessory cell function in HIV-infected individuals. *J Immunol* 1992; 149: 1462–1469.
- Buhl R, Jaffe HA, Holroyd KJ, *et al.* Activation of alveolar macrophages in asymptomatic HIV-infected individuals. *J Immunol* 1993; 150: 1019–1028.
- Semenzato G, Agostini C. HIV-related interstitial lung diseases. *Curr Opin Pulmon Med* 1995; 1: 383–391.
- Clarke JR, Williamson JD, Mitchell DM. Comparative study of the isolation of human immunodeficiency virus from the lung and peripheral blood of AIDS patients. *J Med Virol* 1993; 39: 196–199.
- Ho M, Armstrong J, McMahon D, *et al.* A phase 1 study of adoptive transfer of autologous CD8+ T-lymphocytes in patients with acquired immunodeficiency syndrome (AIDS)-related complex or AIDS. *Blood* 1993; 81: 2093–2101.
- Akbar AN, Borthwick N, Salmon M, *et al.* The significance of low bcl-2 expression by CD45RO T cells in normal individuals and patients with acute viral infections. The role of apoptosis in T cell memory. *J Exp Med* 1993; 178: 427–438.
- Semenzato G, Agostini C, Ometto L, Zambello R, Trentin L, De Rossi A. CD8+ T-lymphocytes in the lung of acquired immunodeficiency syndrome patients harbor human immunodeficiency virus type 1. *Blood* 1995; 85: 2308–2314.
- Bofill M, Gombert W, Borthwick NJ, *et al.* Presence of CD3+CD8+Bcl-2(low) lymphocytes undergoing apoptosis and activated macrophages in lymph nodes of HIV-1+ patients. *Am J Pathol* 1995; 146: 1542–1555.