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Alveolar cell population in HIV infected patients

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Alveolar cell population in HIV infected patients. P. Palange, S. Carlone, M. Venditti, V.B. Antony, E. Angelici, S. Forte, F. Sorice, P. Serra.
ABSTRACT: Alveolar lymphocytosis, in the face of blood lymphopenia, is a common finding among patients with AIDS. We studied by bronchoalveolar lavage (BAL), the alveolar cell profile of 43 human immuno deficiency virus (HIV) seropositive patients divided into three groups involving the advanced stages of the disease: group A (n=9; CDC III), ambulatory individuals without systemic or respiratory symptoms; group B (n=15; CDC IV) patients admitted for evaluation of fever of unknown origin (FUO) without pulmonary involvement; group C (n=19; CDC IV), patients admitted for evaluation of an acute pulmonary condition. Sex, age and risk factor were comparable among the groups. Alveolar lymphocytosis was found in no group A patients, in 2 out of 15 group B patients (both with *P. carinii* lung infection) and in all group C patients, where pulmonary involvement was due to opportunistic infection or to nonspecific interstitial pneumonitis. Our findings suggest that in patients with advanced HIV infection alveolar lymphocytosis may be an expression of a concomitant process within the lungs either clinically manifest or inapparent, or possibly related to HIV primary lung involvement.
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BAL has been found to be a useful diagnostic tool aiding in establishing the etiology of opportunistic pulmonary infections in immunosuppressed patients, including patients with AIDS. BAL has also been used to define the patterns of alveolar cell populations to elucidate the pathogenetic mechanisms that underlie the increased susceptibility of these patients to pulmonary infections.

It has been established that in the BAL of AIDS patients with acute opportunistic pulmonary infections the most significant cellular abnormality is within the lymphocytic series, especially the increase in T-suppressor cells [1–3]. Some authors have interpreted the increase in lymphocytes merely as a response to the infectious state [4], while others have suggested that the immunosuppressed state results in a selective sequestration of these cells within the lung, perhaps through the release of specific chemotactic factors, independently of the presence/absence of an infectious process [2, 4].

However, still limited information is available concerning BAL cell population in asymptomatic patients with advanced stages of infection and/or in AIDS patients with an acute systemic illness not associated with a demonstrable respiratory infection [5, 7, 8]. Thus, knowledge of the behaviour of the alveolar cell population during the various stages of the HIV disease remains incomplete. The present study was undertaken to provide further information, as suggested by others [2], on the alveolar cell profile in the advanced stages of HIV infection in patients with and without clinically manifest pulmonary diseases.

Materials and methods

We studied 43 positive (by Elisa and Western Blot technique) patients, divided into 3 groups:

- Group A (n=9): patients with prolonged generalized lymphoadenopathy, Group III according with CDC [9], ambulatory individuals without fever and symptoms or signs of respiratory illness, recruited from our outpatient clinic;
- Group B (n=15): all individuals of CDC Group IV A (n=9) and Group IV C-1 (n=6), consecutively admitted to our hospital for evaluation of fever of unknown origin (FUO) [10], without evidence of pulmonary involvement by clinical, radiologic or arterial blood gas data (room air Po_2>11.3 kPa or 85 mmHg);
- Group C (n=19): all CDC Group IV C-1 individuals, consecutively admitted to our hospital for evaluation of an acute pulmonary ailment, evidenced by clinical (dyspnoea, tachypnoea) and radiologic (segmental or diffused pulmonary infiltrates) data in conjunction with hypoxaemia (Po_2<10 kPa or 75 mmHg).

All patients were evaluated as follows: clinical examination, chest X-ray, room air arterial pH, Po_2, Pco_2 (Blood gas analyser; IL 1603) and BAL. In Groups B and C all studies were performed simultaneously and always within 48 h of admission. For the BAL the standard fiberoptic procedure (BF-B2; Olympus Corporation) was followed [11], using injection of normal saline (220±4 ml). In Group C the pulmonary segment most involved on the chest radiograph was lavaged; in Groups A and B (normal chest X-ray), the right middle lobe or the lingula were utilized. The first aliquot of the lavage fluid was discarded; the fluid was then aspirated into a calibrated sterile siliconized container: the volume recovered equalled 50% or more of the amount injected in all instances.

The total cell count (filtered specimen) was determined by haemocytometer. For the differential count, fluid lavage was centrifuged at 1000 RPM x 10' and prepared with May-Grundwald-Giemsa and Papanicolau stains; differential percentages were obtained from total counts of 300 cells.

Microbiologic studies on BAL material were performed according to the guidelines of the American Microbiologic Society [12]; the search for *P. carinii* cysts utilized a modified Gomori technique.

Both patient groups were subjected to other appropriate diagnostic procedures unless the BAL finding revealed the presence of an aetiologic agent.

All subjects signed an informed consent form prior to the study.

Unpaired Student t-test with appropriate Bonferroni correction was utilized for all statistical analyses.

Results

The pertinent characteristics of the three groups are presented in table 1. Table 2 shows the salient BAL findings of the study. In Group C there was a marked elevation (relative and absolute) of lymphocytes and a parallel reduction of macrophages. In Groups A and B the absolute as well the relative distribution of all cellular elements were within the normal range for our laboratory. The level of immunosuppression, at least as reflected by the serum CD4/CD8 ratio, was comparable among the three groups.

Table 1. – Characteristics of subjects

	Group A (n=9)	Group B (n=15)	Group C (n=19)
Age (yrs)	29±2	28±2	29±1
Sex (M/F)	6/3	9/6	14/5
Smoking history	+	+	+
Risk factors*			
-Heroin addiction	6	11	10
-Homosexuality	2	2	8
-Hemophilia	1	1	3
-Multiple transfusions	-	1	-
CD4/CD8	0.47±0.7	0.51±0.8	0.45±0.8

*Two patients in Group C had > 1 risk factor. Mean±SE.

Table. 2. – Distribution of BAL cells in the 3 groups

	Normal Range*	Group A (n=9)	Group B (n=15)	Group C (n=19)
Ratio volume fluid recovered/injected(%)		62±3	59±2	64±4
Total cells ($\times 10^6$/100 ml^{-1} lavage)	20.4±6.5	22.8±2.1	14.3±1.8	20.2±2.5
Macrophages (%)	92.8±3.5	95.0±0.8	87.0±2.6	69.4±1.7**
Lymphocytes (%)	5.9±2.7	3.7±0.3	9.2±2.1	22.8±1.6**
Polymorphs (%)	1.3±1.0	1.3±0.7	3.8±0.8	7.9±1.1

* for our laboratory, age matched individuals, smokers, n=10; ** $p<0.01$ *vs* all other groups. Mean±SE.

The aetiologic diagnosis of the acute illness was established in 9 of the 19 Group C patients, 8 by BAL and 1 by mediastinal node aspiration. The other 10 patients remained undiagnosed during the hospital stay and were treated empirically with trimethoprim-sulphamethoxazole. In 2 of the 15 Group B patients the cause of the fever could be attributed to *P.carinii:* in both instances the organism was identified in BAL fluid; only in these 2 patients was the number of BAL lymphocytes increased (>2 SD above the normal mean). In the remaining 13 patients the source of the fever could not be established by any means.

As shown in table 3, when the 2 Group patients with *P. carinii* in BAL fluid, (assumed to have an otherwise undetected lower respiratory tract infection) were moved to Group C, the distribution of cells in BAL remained essentially unchanged.

Table 3. – Distribution of BAL cells in the new groups** B and C

	Group B (n=13)	Group C (n=21)
Total cells ($\times 10^6$/100 ml^{-1} lavage)	14.6±1.9	19.4±2.3
Macrophages (%)	89.0±1.1	69.1±1.7*
Lymphocytes (%)	6.4±0.8	23.2±1.5*
Polymorphs (%)	4.3±0.8	7.9±1.0

*$p<0.01$ *vs* all other groups; **Group B = patients from Group B without *P. carinii* infection; Group C = patients from Group C plus patients from Group B with *P. carinii* infection. Mean±SE.

Discussion

HIV infected patients without evidence of acute respiratory tract infection by clinical and routine laboratory evaluation (Groups A and B) displayed a normal alveolar cell pattern; AIDS patients with acute respiratory infection (Group C) showed changes in alveolar lymphocytes and macrophages.

A normal alveolar cell pattern in asymptomatic homosexual individuals has been reported by YOUNG *et al.* [5, 6]: the value of these studies is limited because

seropositivity was not stated. By contrast, an abnormal alveolar cell pattern, *i.e.* lymphocytosis, was observed by VENET *et al* in a group of patients with chronic generalized lymphoadenopathy, CGL [7]: since the degree of lymphocytosis was comparable to that observed in their AIDS patients the authors implied that the alveolar lymphocytosis seen with HIV infection is related to the underlying defect in cellular immunity and not to an infectious process within the lungs. This implication would be valid if the CGL group (n=20) had comprised only non-infected patients and the AIDS group (n=63) only infected patients; in fact, at least 4 individuals in the former category were infected and at least 9 in the latter category were not infected.

GUILLON *et al.* [8] observed alveolar lymphocytosis in most of 276 HIV patients regardless of the stage of HIV disease and of the presence of opportunistic lung infection. Contrary to our findings, such alveolar lymphocytosis was observed in 27 (59%) of 46 patients without respiratory symptoms and with normal chest roentgenograms. However, given the small size of the population analysed, in the present study we could have not enrolled patients with initial HIV pulmonary involvement that may manifest alveolar lymphocytosis without any other symptom or sign of pulmonary disease. Moreover, distributions of risk factors for HIV infection that might have affected pulmonary cell population were also different. In fact intravenous drug addiction was observed in 27 (63%) of our 43 patients, *versus* 21 (7.6%) out of 276 patients in Guillon's series.

Our data indicate that the concentration of alveolar lymphocytes may not be related to the state of immunosuppression; to the extent that the serum CD4/CD8 ratio reflects immunologic competence [13], our three groups of patients were comparably immunosuppressed; yet, alveolar lymphocytosis was observed only in Group C. Thus, this study supports the view that changes in alveolar lymphocytosis may be related to intercurrent respiratory diseases.

The recovery of the aetiologic agent in only 9 of the 19 Group C patients is in keeping with the reports of others, reflecting the presence of a nonspecific interstitial pneumonitis [14] likely caused by HIV induced immunodysfunction or by HIV load in the lungs.

The two Group B patients with positive BAL for *P. carinii* deserve a special comment. They presented with an acute systemic illness, presumably infectious in origin; in view of the BAL findings, we assumed that their infection was located within the respiratory tract since we know that *P. carinii* can be found in the lungs of immunosuppressed, asymptomatic individuals [15]. When these two patients were moved into the category with clinical respiratory infection (Group C), Group B resembled more closely Group A, while Group C remained unaffected.

In conclusion, our data lend support to the view that in HIV patients the abnormal alveolar cell pattern is related to clinically evident superimposed respiratory infections or to nonspecific interstitial pneumonitis. Such abnormal pattern may be observed also in individuals without clinically apparent pulmonary disease [8]. Further studies are necessary to clarify a possible role of HIV infection in the pathogenesis of alveolar lymphocytosis.

In addition, the observation that in two acutely ill patients with no detectable pulmonary pathology and increased alveolar lymphocytes a potential pathogen was recovered from the lung fluid points to the need for large scale clinical trials in this area: conceivably, it may be possible to show that a high alveolar lymphocyte count in HIV patients with an acute systemic illness is always an indicator of pulmonary pathology even when the clinical and routine laboratory data fail to indicate that the lung is the affected organ.

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Population cellulaire alvéolaire chez les sujets infectés par le VIH. P. Palange, S. Carlone, M. Venditti, V.B. Antony, E. Angelici, S. Forte, F. Sorice, P. Serra.

RÉSUMÉ: Une lymphocytose alvéolaire, associée à une lymphopénie sanguine, est une caractéristique commune chez les patients atteints de SIDA. Nous avons étudié, par lavage broncho-alvéolaire, le profil cellulaire alvéolaire de 43 patients séro-positifs VIH, divisés en trois groupes, comportant les stades avancés de la maladie. Le groupe A (n=9; CDC III), était constitué de patients ambulatoires sans symptômes systémiques ou respiratoires; le groupe B (n=15; CDC IV) comporte des patients hospitalisés pour évaluation d'une fièvre d'origine indéterminée (FUO) sans atteinte pulmonaire; le groupe C (n=19; CDC IV), correspond à des patients admis pour l'évaluation d'une atteinte pulmonaire aiguë. Le sexe, l'âge et les facteurs de risque, étaient comparables dans les trois groupes. La lymphocytose alvéolaire n'a été observée chez aucun des patients du groupe A, chez 2 des 15 patients du groupe B (tous deux avec une infection pulmonaire à *Pneumocystis carinii*), et chez tous les patients du groupe C, où l'atteinte pulmonaire était due à une infection opportuniste ou à une pneumopathie interstitielle non spécifique. Nos observations suggèrent que chez les patients avec une infection à VIH à un stade avancé, la lymphocytose alvéolaire peut être l'expression d'un processus concomitant au niveau pulmonaire, qu'il soit cliniquement manifeste ou inapparent, ou être en relation avec une atteinte pulmonaire primaire due au VIH.

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