Inflammatory Responses in Blood Samples of Human Immunodeficiency Virus-Infected Patients with Pulmonary Infections

Natividad Benito,1* Asunción Moreno,1 Xavier Filella,2 José M. Miró,1 Julià González,3 Tomás Pumarola,3 María Eugenia Valls,1 Montserrat Luna,1 Felipe García,1 Ana Rañó,4 Antoni Torres,4 and José M. Gatell1

Infectious Diseases Service, 1 Biochemistry Service, 2 Microbiology Service, 3 and Pneumology Service, 4 Hospital Clinic Universitari-IDIBAPS, Barcelona, Spain

We analyzed the characteristics of the inflammatory response occurring in blood during pulmonary infections in human immunodeficiency virus (HIV)-infected patients. A prospective study of consecutive hospital admissions of HIV-infected patients with new-onset radiologic pulmonary infiltrates was carried out in a tertiary university hospital from April 1998 to May 2001. Plasma cyclic AMP receptor protein (CRP), interleukin 1β (IL-1β), IL-6, IL-8, IL-10, and tumor necrosis factor alpha (TNF-α) levels were determined at the time of admission and 4, 5, and 6 days later. Patients were included in a protocol addressed to study etiology and outcome of disease. A total of 249 episodes of infection were included, with the main diagnoses being bacterial pneumonia (BP) (118 episodes), Pneumocystis carinii pneumonia (PCP) (41 episodes), and mycobacteriosis (36 episodes). For these three patient groups, at the time of admission the median CRP and cytokine levels were as follows: CRP, 10.2, 3.8, and 5 mg/dl, respectively (P = 0.0001); IL-8, 19, 3, and 2.9 pg/ml (P = 0.045); and TNF-α, 46, 4, and 75 pg/ml, respectively (P = 0.029). There were no significant differences in levels of IL-1β, IL-6, or IL-10 among the patient groups. A total of 23 patients died. At the time of admission, HIV-infected patients with BP had higher plasma CRP and IL-8 levels than did PCP and mycobacteriosis patients. TNF-α levels were higher in patients with mycobacteriosis. An elevated IL-8 level (>61 pg/ml) at the time of admission was an independent factor associated with higher mortality (odds ratio, 12; 95% confidence interval, 1.2 to 235.5).

Pulmonary infiltrates are a frequent cause of morbidity and hospital admission of human immunodeficiency virus (HIV)-infected patients in the highly active antiretroviral therapy era, with an incidence of ~20 episodes per 100 hospital admission-years (5). The main diagnostic groups are bacterial pneumonia (BP), Pneumocystis carinii pneumonia (PCP), and mycobacteriosis, in that order. BP is currently the most common admission diagnosis in this population (2, 26). Pulmonary infections usually appear as pulmonary infiltrates (PIs) on chest radiographs and are frequently (but not always) associated with respiratory symptoms.

In recent years, the study of the host inflammatory response to infections has received considerable interest. One component of the host response to infection is the release of cytokines, intercellular signaling polypeptides produced by activated cells (13). A complex network of cytokines controls the generation and maintenance of innate and specific immunological responses (9). The cytokines that are produced during and participate in inflammatory processes are the chief stimulators of the production of acute-phase proteins, such as C-reactive protein (CRP). In pulmonary infections, the release of cytokines and other inflammatory mediators serves as a useful mechanism for the elimination of invading pathogens. However, excessive release can be harmful to the host, leading to respiratory distress syndrome, shock, multiorgan failure, or death. Thus, it has been suggested that the quantitation of the inflammatory response may have prognostic implications (22). The knowledge thus obtained may eventually lead to the development of new strategies for therapy of infectious diseases. Additionally, reports of different patterns of cytokine responses in different diseases raise the possibility that cytokine determinations may have diagnostic value (13).

The aims of this study were to evaluate the role of plasma proinflammatory cytokines (interleukin 1β [IL-1β], IL-6, IL-8, and tumor necrosis factor alpha [TNF-α]), anti-inflammatory cytokines (IL-10), and CRP in the diagnosis and outcome of HIV-1-infected patients with pulmonary infections with different etiologies.

(Presented in part at the XIV International AIDS Conference, Barcelona, Spain, 7 to 12 July 2002 [N. Benito, A. Moreno, X. Filella, J. M. Miró, J. González, T. Pumarola, M. E. Valls, M. Luna, A. Rañó, A. Torres, and J. M. Gatell, Abstr. XIV Int. AIDS Conf., abstr. WePeA5800, 2002.])

MATERIALS AND METHODS

A prospective study of consecutive hospital admissions of HIV-infected patients was carried out at Hospital Clinic Universitari, a 700-bed tertiary-level hospital in Barcelona, Spain.

From April 1998 to March 2000, all HIV-infected patients with new-onset radiologic PIs on admission or with PIs that developed during hospitalization were included. Patients were followed up until radiologic resolution of the infiltrates or death secondary to the pulmonary event.

The following variables were recorded: demographic features; HIV risk factors; antiretroviral therapy; CD4 lymphocyte count per cubic millimeter and
TABLE 1. Demographic features, HIV infection risk factors, immunologic status (CD4 lymphocyte count), and VL of HIV patients with PIs

| Parameter                     | Value                  |
|-------------------------------|------------------------|
| No. of episodes/patients      | 249/220                |
| No. of male patients          | 180 (73%)              |
| Mean age (SD, range) (yr)     | 39 (10, 22–77)         |
| No. with HIV risk factor      |                        |
| Injecting drug user           |                        |
| Heterosexual                  | 118 (54%)              |
| Homosexual                    | 45 (20%)               |
| Transfused or unknown         | 44 (20%)               |
| No. with HIV diagnosis during pulmonary event |                |
| Median CD4 cell count per cubic millimeter (interquartile range) | 139 (273) |
| No. of episodes with CD4 count of <200 cells/mm³ | 103 (41%) |
| No. of episodes with VL of <200 copies | 36 (15%) |
| No. of episodes in patients receiving highly active antiretroviral therapy | 109 (44%) |

HIV-1 plasma viral load (VL) before the pulmonary event; clinical, analytic and radiologic data at admission; microbiologic results; final etiologic diagnosis; antimicrobial treatment; and outcome.

All patients were included in a protocol that addressed studying the etiology and outcome of the pulmonary complication, as previously described (5). Definitions of definitive etiologic diagnosis, probable etiologic diagnosis, BP, and undiagnosed PI have also been previously reported (5).

In all these episodes, plasma CRP, IL-1β, IL-6, IL-8, IL-10, and TNF-α levels were determined at admission and 4 to 6 days later.

Plasma CRP levels were estimated by means of a nephelometric method (Dade Behring Inc., Newark, Del.). This assay uses monoclonal anti-CRP antibodies. Polystyrene particles are coated with the specific antibodies, which form a complex with CRP present in the measured study sample. The amount of scattered light is directly proportional to the size of the antigen-antibody complex and reflects the CRP concentration present in the study sample. The calibration range was 0.35 to 22 mg/dl. The lower limit of detection was 0.0175 mg/dl.

For assays to determine plasma IL-1β, IL-6, and TNF-α concentrations (Biosource Europe, Nivelles, Belgium), as well as IL-8 and IL-10 concentrations (Assay Designs, Ann Arbor, Mich.), blood samples were collected in sterile tubes not containing anticoagulants. The samples were centrifuged at 3,000 rpm for 10 min, and the plasma was stored at −70°C until it was processed. The Biosource tests are solid-phase enzyme-amplified sensitivity immunoassays performed on microtiter plates. These assays are based on an oligosaccharidic system in which mixtures of monoclonal antibodies directed against distinct epitopes of the corresponding cytokine are used. Standards used in the calibration curve and samples containing the cytokine react with capture monoclonal antibodies with which the microtiter well is coated. After incubation, excess antigen is removed by washing. A second monoclonal antibody labeled with horseradish peroxidase is then added. After incubation, the microtiter plate is washed and bound enzyme-labeled antibodies are measured through a chromogenic reaction. The reaction is stopped with the addition of H₂SO₄ as a stop solution. The Assay Designs tests use a monoclonal antibody directed against IL-8 or IL-10, with which the wells of a microtiter plate are precoated. IL-8 or IL-10 present in the standards or in the samples binds to the antibody on the coated well. After the cytokine is bound to the immobilized antibody, a second monoclonal antibody labeled with streptavidin is added to the wells and allowed to bind to a different epitope on the same cytokine. After the plates are washed, tetramethylbenzidine is added. A stop solution ends the reaction, and the absorbance is measured. In both cases, colorimetric determination was done by means of a polychromic reader (Vmax EASIA reader; Medegen Diagnostics). Concentrations of cytokines from samples were determined by comparing the optical densities of the samples to the standard curves. The results are expressed in picograms per milliliter of serum. The sensitivity of the technique allows the detection of levels as low as 2 pg/ml and first quartile (25th percentile) is the interquartile range, a measure of the spread of the data. The middle line represents the median. Lines extend from the central box to the minimum and the maximum values, excluding outliers values, which are displayed as separate points: an outside value is defined as a value that is smaller than the first quartile minus 1.5 times the interquartile range or larger than the third quartile plus 1.5 times the interquartile range (inner fences). A far-out value is defined as a value that is smaller than the first quartile minus three times the interquartile range or larger than the third quartile plus three times the interquartile range (outer fences), (29). The cutoff of the studied CRP and cytokines that maximized the ability to make a correct diagnosis was established by means of receiver operating characteristic curves. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of this cutoff were calculated with the prevalence estimated in the sample (12). Linear correlations between quantitative variables were analyzed with the Spearman test. A multiple-regression analysis model was used to evaluate factors independently associated with quantitative variables. Analysis of factors independently associated with mortality was performed with logistic regression. The results are given as an odds ratio (OR). The confidence interval (CI) was established at 95%. A probability value of <0.05 was considered to be significant.

RESULTS

There were 1,016 consecutive hospital admissions of adults with HIV infection during the study period. Two hundred forty-nine episodes of PI (25%) in 220 patients were diagnosed. Demographic features, HIV infection risk factors, immunologic status (CD4 lymphocyte counts), and virologic status are shown in Table 1. The three main diagnostic groups were mycobacteriosis (148 cases), PCP (191 episodes), and mycobacteriosis (36 cases) (Table 2).

The median levels of CRP and the studied cytokines for BP, PCP, and mycobacteriosis at admission are shown in Table 3. The median levels of plasma CRP and IL-8 at admission were significantly higher in BP (10.2 mg/dl and 19 pg/ml, respectively) than in mycobacteriosis (5.1 mg/dl and 0) and PCP (3.8 mg/dl and 3 pg/ml, respectively). The median level of plasma TNF-α in mycobacteriosis (75 pg/ml) was statistically higher than in the other two diagnostic groups (46.5 pg/ml in BP and 44 pg/ml in PCP). These differences in the medians of plasma levels of CRP and the studied cytokines at admission among the diagnostic groups remained when patients were classified according to the CD4 lymphocyte count (higher or lower than 200/mm³) (Fig. 1). No other statistically significant differences were found in the studied cytokines among the three diagnostic groups at admission. There was no association between etiologies and plasma levels of cytokines and CRP determined on the fourth to sixth days after admission.
TABLE 2. Diagnosis of 249 episodes of pulmonary infiltrates in HIV-infected patients

| Infectious etiology | No. of episodes (% of total episodes) |
|---------------------|--------------------------------------|
| Bacteria            | 118 (47.4)                           |
| Streptococcus       | 55                                   |
| Haemophilus         | 8                                    |
| Staphylococcus      | 6                                    |
| Legionella pneumonia| 5                                    |
| Escherichia coli     | 2                                    |
| Acinetobacter baumanii | 1                                 |
| Without identification | 41                                 |

The sensitivity, specificity, PPV, and NPV with a CRP level of ≥10 mg/dl and an IL-8 level of ≥20 pg/ml for the diagnosis of BP versus PCP and mycobacteriosis were 69, 83, 71, and 82%, respectively. A value for TNF-α of ≥60 pg/ml had a sensitivity, specificity, PPV, and NPV of 85, 70, 31, and 97%, respectively, for the mycobacteriosis diagnosis versus the other two diagnoses.

Linear correlations between CRP and the studied cytokines and the CD4 lymphocyte count, as well as between CRP and the studied cytokines and the HIV plasma VL were analyzed in order to evaluate the possible association among immunologic and virologic statuses of HIV patients and plasma levels of CRP-cytokines (Table 4). A positive linear correlation was found between plasma CRP and the CD4 lymphocyte count per cubic millimeter (P = 0.007), and a negative correlation was found between CRP and the HIV-1 plasma VL (P < 0.001). However, these associations were no longer significant when a current diagnosis of BP was also included in a multiple-regression analysis model. This can be explained by the fact that the CD4 lymphocyte median in the BP group (263/mm³ [interquartile range, 320]) was significantly higher than the CD4 median in the patients with other diagnoses (46/mm³) (P < 0.001). In fact, having BP was the only independent factor significantly associated with the levels of CRP (P = 0.001). Similarly, the HIV-1 plasma log₁₀ VL was lower in the BP group (3.7 versus 5.3) (P < 0.001). A positive correlation was found between the HIV-1 VL and IL-10 (P = 0.031) and TNF-α (P = 0.001). There was a negative correlation between the CD4 lymphocyte count and TNF-α (P = 0.033). These associations remained significant when a current diagnosis of BP, a current diagnosis of mycobacteriosis, and a current diagnosis of PCP were also included in a multiple-regression analysis model. Mycobacteriosis did not seem to be a confounding factor in the correlation between TNF-α and the CD4 lymphocyte count, since the median CD4 lymphocyte count in mycobacteriosis cases (164 cells/mm³ [interquartile range, 207]) was no different than that in the rest of the cases (156 cells/mm³ [interquartile range, 287]) (P = 0.780). No others significant correlations were found between the studied cytokines and these variables showing the immunologic and virologic status of patients.

Twenty-three (10%) patients died: seven PCP patients, six patients without etiologic diagnoses, four with BP, one with mycobacteriosis, two with cytomegalovirus pneumonia, two patients with *Aspergillus fumigatus* infection, and one patient with

**TABLE 3. Median levels and interquartile ranges at admission of CRP and cytokines (IL-1β, IL-6, IL-8, IL-10, and TNF-α) in main diagnostic groups**

| Parameter       | Value for diagnostic group¹ | p²  |
|-----------------|-----------------------------|-----|
|                 | PCP (n = 41) | Mycobacteriosis (n = 36) | BP (n = 118) |
| CRP (NV<0.8)    | 3.8 (8.6) | 5.1 (6.9) | 10.2 (11.7) | <0.001 |
| IL-1β (NV<15)   | 8 (31.3) | 7.5 (25) | 3 (11.3) | NS |
| IL-6 (NV<5)     | 29.5 (90) | 35 (124) | 45 (75.3) | NS |
| IL-8 (NV<10)    | 3 (24.5) | 0 (8) | 19 (38) | 0.012 |
| IL-10 (NV<10)   | 8 (22) | 5 (55.5) | 6 (8.8) | NS |
| TNF-α (NV<20)   | 44 (36.8) | 75 (29) | 46.5 (30.5) | 0.016 |
| CD4 lymphocyte count per mm³ | 23 (43) | 164 (207) | 263 (320) | <0.001 |
| HIV-1 plasma log₁₀ VL | 5.62 (0.76) | 4.82 (1.68) | 3.67 (2.56) | <0.001 |

¹ Median level of CRP (milligrams per deciliter) and cytokines (picograms per milliliter) (interquartile range) at admission, CD4 count, and HIV plasma VL. Differences among medians have been assessed using the nonparametric test of medians (7). NV, normal value.

² NS, not significant.

Boldface type indicates differences that are statistically significant (P < 0.05).

Downloaded from http://cvi.asm.org/ on April 27, 2019 by guest
FIG. 1. Distribution of plasma CRP, IL-8, and IL-10 levels at admission in the main diagnostic groups (BP, PCP, and mycobacteriosis) in patients classified according to a CD4 lymphocyte count higher or lower than 200/mm$^3$. In the basic box-and-whisker plot, the central box represents the values from the 25th to the 75th percentile and therefore contains the middle half of the scores in the distribution. The numerical difference between the third quartile (75th percentile) and first quartile (25th percentile) is the interquartile range, a measure of the spread of the data. The middle line represents the median. Lines extend from the central box to the minimum and the maximum values, excluding outlier values, which are displayed as separate points; an outside value is defined as a value that is smaller than the first quartile minus 1.5 times the interquartile range or larger than the third quartile plus 1.5 times the interquartile range (inner fences). These values are plotted with a round marker. A far-out value is defined as a value that is smaller than the first quartile minus three times the interquartile range or larger than the third quartile plus three times the interquartile range (outer fences).
Strongyloides stercoralis infection. Admission to the intensive care unit and mechanical ventilation were required in 5 out of 118 (4%) episodes and in 4 cases (3%) of BP, with 3% mortality. The levels of CRP and the studied cytokines at admission to patients who died and survivors are shown in Table 5. In the univariate analysis, elevated CRP and IL-6 values were associated with higher mortality, and there was an almost-significant association between higher plasma TNF-α levels and mortality. In a multivariate analysis, when CRP and all the studied cytokines were included—categorized as having a value higher or lower than the median value in the group of patients that died—an IL-8 level of >61 pg/ml (OR = 27; 95% CI, 2 to 384.8) and an IL-10 level of >22 pg/ml (OR = 11; 95% CI, 1.2 to 176.5) were the variables significantly associated with higher mortality in HIV-1-infected patients with PIs. When the CD4 lymphocyte count, the HIV-1 VL, and the diagnostic group were included in the model as potential confounding factors, CD4 lymphocyte count and HIV plasma VL showed higher mortality in HIV-1-infected patients with PIs. When the CD4 lymphocyte count, the HIV-1 VL, and the diagnostic group were included in the model as potential confounding factors, CD4 lymphocyte count and HIV plasma VL showed higher mortality in HIV-1-infected patients with PIs. CRP and IL-8 levels at admission than patients with PCP and mycobacteriosis; (ii) the plasma TNF-α value is higher in HIV patients with mycobacteriosis than in HIV patients with BP or PCP; (iii) plasma IL-8 is an independent factor associated with mortality in HIV-infected patients with pulmonary infections.

The local and systemic inflammatory response in BP, with special emphasis on the patterns of cytokines and levels of CRP, has been studied in recent years in the immunocompetent population (20, 22, 23, 27). An increase in proinflammatory cytokines, such as IL-1β, IL-6, and TNF-α, as well as acute-phase proteins, such as CRP, has been described in BP (20, 27). IL-8 seems to be another inflammation-associated cytokine. It is a chemokine with neutrophil-activating capacity that has been shown to be increased in the pneumonia lung, but not in the nonpneumonic lung (1, 9, 11, 24). IL-10 is an anti-inflammatory cytokine that inhibits the production of proinflammatory cytokines by monocytes and macrophages (9). In recent studies, a relationship between IL-10 levels and the severity of community-acquired pneumonia has been demonstrated (15). The inflammatory response in BP has not been studied in HIV-infected patients.

In our study, HIV patients with BP had elevated levels of the proinflammatory cytokines IL-6, TNF-α, and IL-8 in plasma. Additionally, the plasma CRP level was elevated. Overall, these results are similar to those found in the general population with BP, although we found neither high serum IL-1β levels nor high levels of the anti-inflammatory cytokine IL-10. Interestingly, although IL-8 in BP is usually compartmentalized in the involved lung and rarely spills over into the serum (6), in our study there were detectable plasma IL-8 levels in 75% of HIV-infected patients with BP. In a previous study of BP in the general population, serum IL-8 was detectable in only 25% of patients (6). In relation to the proinflammatory cytokines IL-1β, IL-6, and TNF-α, Dehoux et al. found that only levels of IL-6 were mildly increased in the sera of patients with BP in the general population, whereas the increased levels of the other cytokines were confined to the lungs alone (10). However, other studies have documented the presence of systemic inflammation in patients with more severe BP and found that serum IL-6 and TNF-α levels correlated with the severity of pneumonia and mortality (3, 22, 25, 27). Thus, in one study of consecutive patients with BP, serum IL-6 and TNF-α were increased.

### DISCUSSION

The most significant findings of our study can be summarized as follows: (i) HIV patients with BP have higher plasma CRP and IL-8 levels at admission than patients with PCP and mycobacteriosis; (ii) the plasma TNF-α value is higher in HIV patients with mycobacteriosis than in HIV patients with BP or PCP; (iii) plasma IL-8 is an independent factor associated with mortality in HIV-infected patients with pulmonary infections.

### TABLE 4. Linear correlations between CRP and studied cytokines (IL-1β, IL-6, IL-8, IL-10, and TNF-α) and variables showing immunologic and virologic status of patients (CD4 lymphocyte count and HIV plasma VL)

| CRP or cytokine | Linear correlation | Confounding factors |
|-----------------|--------------------|--------------------|
| CD4 lymphocyte counts | Positive | Negative |
| HIV plasma VL | Negative | Positive |
| BP | None |

* Only statistically significant linear correlations found between CRP and studied cytokines and variables showing the immunologic and virologic status of patients (CD4 lymphocyte count and HIV plasma VL) are shown.

### TABLE 5. Median levels (interquartile range) at admission of CRP and cytokines (IL-1β, IL-6, IL-8, IL-10, and TNF-α) in patients who died and survivors

| Parameter | Value for group |
|-----------|----------------|
| CRP (NV < 0.8) | 6.8 (12.2) |
| IL-1β (NV < 15) | 3 (14) |
| IL-6 (NV < 5) | 36 (67) |
| IL-8 (NV < 10) | 8.5 (27.8) |
| IL-10 (NV < 10) | 6 (8.25) |
| TNF-α (NV < 20) | 46 (35) |
| CD4 lymphocyte count per mm³ | 180 (284) |
| HIV-1 plasma VL log₁₀ | 4.66 (2.94) |

| | Survivors (n = 23) | Patients who died (n = 226) | p*b |
|-----------------|-----------------|-----------------|-----|
| CRP (NV < 0.8) | 6.8 (12.2) | 10.3 (12.3) | 0.028 |
| IL-1β (NV < 15) | 3 (14) | 24.5 (56.8) | 0.432 (NS) |
| IL-6 (NV < 5) | 36 (67) | 95 (686) | 0.016 |
| IL-8 (NV < 10) | 8.5 (27.8) | 61 (220.5) | 0.620 (NS) |
| IL-10 (NV < 10) | 6 (8.25) | 22 (197.5) | 0.103 (NS) |
| TNF-α (NV < 20) | 46 (35) | 70 (66) | 0.068 (NS) |
| CD4 lymphocyte count per mm³ | 180 (284) | 20.5 (44.5) | 0.004 |
| HIV-1 plasma VL log₁₀ | 4.66 (2.94) | 5.22 (0.9) | <0.001 |

* Median levels of CRP (milligrams per deciliter) and cytokines (picograms per milliliter) (interquartile range) at admission, CD4 count, and HIV VL. Differences among medians have been assessed using the nonparametric test of medians (7). NV, normal value.

* Boldface type indicates differences that are statistically significant (P < 0.05).
present only in 23 and 41% of patients, respectively, whereas in another study of patients with severe pneumonia requiring mechanical ventilation, IL-6 and TNF-α were detected in all patients (3, 23). In the present study, a cytokine pattern similar to that found in patients with severe pneumonia was observed in HIV-infected patients with BP: 99 and 100% of patients had detectable plasma IL-6 and TNF-α levels, respectively. However, our patients with BP did not show clinical criteria of severity: only 5 out of 118 (4%) required admission to the intensive care unit, and 4 (3%) required mechanical ventilation, with 3% mortality. In any case, the serum cytokine and CRP profile found in HIV patients with BP versus the other main diagnostic groups—PCP and mycobacteriosis—is quite characteristic and allows us to distinguish BP from the other processes with good sensitivity, specificity, and predictive values.

In this study, the plasma TNF-α levels were elevated in patients with BP, mycobacteriosis, and PCP. However, they were significantly higher in mycobacteriosis than in the other diagnostic groups (Table 3). Indeed, a TNF-α value of ≥60 pg/ml had a very high NPV for the diagnosis of mycobacteriosis (97%). It is known that TNF-α contributes to the host defense mechanisms in mycobacterial infection (8, 28, 30). Studies of mice have demonstrated that TNF-α is important in granuloma formation and in controlling the extent of mycobacteriosis (8, 28). In humans, the use of anti-TNF-α monoclonal antibodies, such as infliximab, has been associated with increased rates of tuberculosis reactivation (19). In immunocompetent tuberculosis patients, TNF-α production is present at the site of disease but is seldom found in the circulation (18, 30). Nevertheless, recent reports have indicated that the plasma TNF-α levels may be correlated with tuberculosis severity and activity (4). It has also been demonstrated that *Mycobacterium tuberculosis* phagocytosis induces greater TNF-α production in HIV-infected macrophages than in uninfected cells (17). In patients with HIV infection and active tuberculosis, serial measurement of plasma TNF-α levels correlated with the response to antituberculosis treatment (16). However, a possible role of plasma TNF-α levels in the diagnosis of mycobacteriosis in HIV-infected patients has not been reported. The high incidence of mycobacteriosis and the frequent association of mycobacteriosis with other pulmonary infections in HIV patients, as well as the epidemiological relevance of this diagnosis, support the importance of excluding mycobacteriosis at admission (5). However, direct microbiologic diagnosis is not always possible at admission, and acid-fast smears in sputum are not always positive in patients with mycobacteriosis. Since TNF-α has a very high NPV, it could be used as a tool to exclude the mycobacteriosis diagnosis in these patients.

Could some of the differences in cytokine levels observed in this study be due to different stages of HIV disease itself and not necessarily all be due to BP or mycobacterial infections? Immune dysregulation increases with more advanced HIV infection, even in the absence of opportunistic infections, and is associated with increased levels of proinflammatory cytokines, including IL-1β, IL-6, and TNF-α. Additionally, decreased production of important Th1 immunoregulatory cytokines (such as IL-2 and gamma interferon, which are critical for cell-mediated immune responses) and increased production of the Th2 cytokines, such as IL-10, which are important in humoral immunity, are observed in more advanced HIV infection (7, 21). However, we were able to demonstrate that a high level of plasma CRP at admission is associated with BP, independent of the immunologic and virologic status of HIV-infected patients. We observed that plasma levels of IL-10 and TNF-α have a linear correlation with CD4 lymphocyte counts, and therefore, confirmed that they are correlated with more advanced HIV infection. Nevertheless, TNF-α is also independently associated with mycobacterial infections, since there are no differences in CD4 lymphocyte counts between patients with and without mycobacterial infections.

There were not enough death events in each diagnostic group to analyze prognostic factors in each of them. In the overall group of HIV-infected patients with PIs, elevated plasma IL-8 levels (higher than 61 pg/ml) were independently associated with higher mortality. Although etiological diagnosis does not seem to be a factor related to this association, this cannot be asserted absolutely. In patients who died, all the studied serum cytokines and CRP were higher than in survivors (Table 5). Because of the profound cytokine dysregulation associated with HIV infection, some of these findings could actually be due to an advanced HIV status. However, when virologic and immunologic statuses were also considered, IL-8 was independently related to higher mortality. This finding underlines the importance of the systemic inflammatory response of the host in the prognosis of pulmonary infections in this group of patients.

In conclusion, differences in the pattern of systemic inflammatory responses found in HIV-infected patients with PIs could be very useful noninvasive and early tools in the initial diagnosis of BP and mycobacteriosis. Thus, a CRP value of ≥10 mg/dl plus an IL-8 value of ≥20 pg/ml would allow the establishment of a diagnosis of BP versus PCP or mycobacteriosis with a PPV and NPV of 71 and 82%, respectively. Moreover, a TNF-α value of ≥60 pg/ml could be used for early exclusion of the mycobacteriosis diagnosis in these patients based on its high NVP (97%). Direct microbiologic diagnosis is not always possible at admission, and acid-fast smears in sputum are not always positive in patients with mycobacteriosis, which underlines the importance of having other quick and reliable diagnostic tools. The high incidence of mycobacteriosis and the frequent association of mycobacteriosis with other pulmonary infections in HIV patients, as well as the epidemiologic relevance of this diagnosis, support the importance of excluding mycobacteriosis at admission (5). More prospective studies are needed to evaluate the role of IL-8 in the outcome of each diagnostic group and to establish if the plasma IL-8 level could have value in the decision about initial treatment of PIs in HIV-infected patients.

ACKNOWLEDGMENTS

During the study period, N. Benito was the recipient of a research grant from the Fundación Privada Máximo Soriano Jiménez. J.M.M. was the recipient of a research grant from the Institut d’Investigacions Biomèdiques August Pi i Sunyer, Barcelona, Spain.

REFERENCES

1. Abul, H., A. Abul, I. Khan, T. C. Mathew, A. Ayed, and E. Al-Athary. 2001. Levels of IL-8 and myeloperoxidase in the lungs of pneumonia patients. Mol. Cell Biochem. 217:107–112.
2. Altés, J., M. Guadarrama, L. Force, A. Tapiz, J. Vilaró, I. García, and the Catalanian Hospital's HIV Infection Study Group. 1999. The impact of highly active antiretroviral therapy on HIV-related hospitalizations in 17 county hospitals in Catalonia, Spain. AIDS 13:1418-1419.

3. Bauer, T. T., C. Montón, A. Torres, H. Cabello, X. Filella, A. Maldonado, et al. 1999. Comparison of systemic cytokine levels in patients with acute respiratory distress syndrome, severe pneumonia, and controls. Thorax 55: 46-52.

4. Bekker, L. G., G. Maartens, L. Steyn, and G. Kaplan. 1998. Selective increase in plasma necrosis factor-alpha and comitant clinical deterioration after initiating therapy in patients with severe tuberculosis. J. Infect. Dis. 178:1328-1333.

5. Benito, N., A. Rañó, A. Moreno, J. González, M. Luna, C. Agustí, C. Danes, T. Pumarola, J. M. Miro, A. Torres, and J. M. Gatell. 2001. Pulmonary infiltrates in HIV-infected patients in the highly active antiretroviral therapy era in Spain. J. Acquir. Immune Defic. Syndr. 27:35-43.

6. Bottun, A., M. S. Dehoux, N. Seta, J. Ostinelli, P. Venembre, B. Crestani, et al. 1996. Compartmentalized IL-8 and elastase release within the human lung in unilateral pneumonia. Am. J. Respir. Crit. Care Med. 153:336-342.

7. Cohen, O. J., A. Kinter, and A. S. Faucy. 2001. The many faces of host factors in the pathogenesis of HIV disease. Immunol. Rev. 199:31-48.

8. Collins, H. L., and S. H. E. Kaufmann. 1997. The host immune response to Mycobacterium tuberculosis. Clin. Microbiol. Rev. 10:435–457.

9. Curfs, J. H. A. J., J. F. G. M. Meis, and J. A. A Hoogkamp-Korstanje 1997. A primer on cytokines: sources, receptors, effects, and inducers. Clin. Microbiol. Rev. 10:742-780.

10. Dehoux, M. S., A. Bottun, J. Ostinelli, N. Seta, M. C. Dombre, and B. Crestani. 1994. Compartmentalized cytokine production within the human lung in unilateral pneumonia. Am. J. Respir. Crit. Care Med. 150:710–716.

11. Dinarello, C. A. 2000. Proinflammatory cytokines. Chest 118:503–508.

12. Doménech Massons, J. M., and R. Granero. 2000. Fundamentos de la teoría de la probabilidad. Pruebas diagnósticas, p. 5–90. In J. M. Doménech Massons (ed.), Fundamentos de diseño y estadística. Gráficas Signo, S.A., Barcelona, Spain.

13. Galbáy, C., and I. Kushner. 1999. Acute-phase proteins and other systemic responses to inflammation. N. Engl. J. Med. 340:448-454.

14. Gibbons, J. D. 1985. Nonparametric statistical inference, 2nd ed. Marcel Dekker, New York, N.Y.

15. Glynn, P., R. Coakley, I. Kilgallen, N. Murphy, and S. O'Neill. 1999. Circulating interleukin 6 and interleukin 10 in community acquired pneumonia. Thorax 54:51-55.

16. Hsieh, S. M., C. C. Hung, M. Y. Chen, W. H. Sheng, and S. C. Chang. 1999. Dynamics of plasma cytokine levels in patients with advanced HIV infection and active tuberculosis: implications for early recognition of patients with poor response to anti-tuberculosis treatment. AIDS 13:935-941.

17. Imperiali, F. G., A. Zaninoni, L. La Maestra, P. Tarsia, F. Blasi, and W. Barcellini. 2001. Increased Mycobacterium tuberculosis growth in HIV-1-infected human macrophages: role of tumour necrosis factor-α. Clin. Exp. Immunol. 125:435-442.

18. Joffermans, N. P., A. Verbon, S. J. J. van Deventer, H. van Deutekom, P. Speelman, and T. van der Poll. 1998. Tumor necrosis factor and interleukin-1 inhibitors as markers of disease activity of tuberculosis. Am. J. Respir. Crit. Care Med. 157:1328-1331.

19. Keane, J., S. Gershon, R. P. Wise, E. Mirabile-Levens, J. Kasznica, W. D. Schnieterman, et al. 2001. Tuberculosis associated with infliximab, a tumor necrosis factor alpha-neutralizing agent. N. Engl. J. Med. 345:1098-1104.

20. Kosmas, E. N., C. N. Baxevanis, M. Papamichall, and T. Kordossi. 1997. Daily variation in circulating cytokines and acute-phase proteins correlates with clinical and laboratory indices in community-acquired pneumonia. Eur. J. Clin. Invest. 27:308-315.

21. Lucey, D. R., M. Clerici, and G. M. Shearer. 1996. Type 1 and type 2 cytokine dysregulation in human infectious, neoplastic, and inflammatory diseases. Clin. Microbiol. Rev. 9:532-562.

22. Montón, C., A. Torres, M. El-Eliyari, X. Filella, A. Xautet, and J. Puig de la Bellacasa. 1999. Cytokine expression in severe pneumonia: a bronchoalveolar lavage study. Crit. Care Med. 27:1745–1753.

23. Moussa, K., H. J. Michie, I. A. Cree, A. C. McCafferty, J. H. Winter, D. P. Dhillon, et al. 1994. Phagocyte function and cytokine production in community acquired pneumonia. Thorax 49:107–111.

24. Niederman, M. S., and Q. A. A. Ahmed. 1999. Inflammation in severe pneumonia: act locally, not globally. Crit. Care Med. 27:2030–2032.

25. Ortvist, A., J. Hedlund, B. Wretlind, A. Carlstrom, and M. Kalin. 1995. Diagnostic and prognostic value of interleukin-6 and C-reactive protein in community acquired pneumonia. Scand. J. Infect. Dis. 27:457–462.

26. Paul, S. H. M. Gilbert, W. Ziecheck, J. Jacobs, and K. A. Septowitz. 1999. The impact of potent antiretroviral therapy on the characteristics of hospitalized patients with HIV infection. AIDS 13:415–418.

27. Puranen, A. J., C. Feldman, N. Savage, P. J. Becker, and C. Smith. 1995. Patterns of cytokine expression in community-acquired pneumonia. Chest 107:1342-1349.

28. Schlüter, N. W., and W. N. Rom. 1998. The host immune response to tuberculosis. Am. J. Respir. Crit. Care Med. 157:679–691.

29. Tukey, J. W. 1977. Exploratory data analysis. Addison-Wesley Publishing Company, Reading, Mass.

30. van Crevel, R., T. H. M. Ottenhoff, and J. W. M. van der Meer. 2002. Innate immunity to Mycobacterium tuberculosis. Clin. Microbiol. Rev. 15:294–309.