Cytokine profiles in bronchoalveolar lavage fluid and blood in HIV-seropositive patients with *Pneumocystis carinii* pneumonia

R. M. PERENBOOM,† R. W. SAUERWEIN,† P. BECKERS,† A. C. H. W. VAN SCHIJNDEL,† R. P. VAN STEENWIJK,‡ J. C. C. BORLEFFS,§ R. VAN LEUSENF & J. W. M. VAN DER MEER* Department of *General Internal Medicine and †Medical Microbiology, University Hospital Nijmegen; ‡Department of Pulmonology, Academic Medical Center, Amsterdam; §Department of Internal Medicine, University Hospital Utrecht; ¶Department of Internal Medicine, Rijnstate Hospital, Arnhem, The Netherlands.

Received 14 June 1996; accepted 18 November 1996.

Abstract. Concentrations and *ex vivo* production of interleukin 1β (IL-1), tumour necrosis α (TNF), interleukin 6 (IL-6), interleukin-1 receptor antagonist (IL-1RA) and TNF soluble receptors (sTNF-receptors, P55 and P75) were measured in bronchoalveolar lavage (BAL) fluid and blood in 23 HIV-seropositive (HIV+) patients with *Pneumocystis carinii* pneumonia (PCP) and compared with values found in healthy HIV-seronegative (HIV-) controls and asymptomatic HIV+ subjects. Concentrations of the proinflammatory cytokine IL-1β were increased in BAL fluid of HIV+ patients with PCP (184 ± 47 pg mL⁻¹) compared with undetectable levels in healthy control subjects (P = 0·0001). In plasma of these patients higher concentrations of the anti-inflammatory cytokine IL-1RA were found during acute PCP than after recovery (2·1 ± 0·7 vs. 0·5 ± 0·2 ng mL⁻¹, P = 0·01). No correlations could be found between cytokine concentrations and clinical severity of the infection. Corticosteroid treatment did not influence cytokine concentrations in BAL or blood, nor did it suppress the production of tumor necrosis factor-α (TNF) by alveolar cells. In whole-blood cultures, however, lipopolysaccharide (LPS)-stimulated production was significantly suppressed for IL-1 (1·3 ± 0·5 vs. 5·5 ng mL⁻¹, P = 0·009) and for IL-6 (0·6 ± 2·5 ng mL⁻¹, P = 0·01). The overall data show that in HIV+ patients with PCP (similar to what we had found previously in HIV– patients with CP) proinflammatory cytokines are more prominently present in BAL, whereas anti-inflammatory reaction is predominant in the circulation.

Keywords. Cytokine, HIV, *Pneumocystis carinii* pneumonia, tumour necrosis factor α, soluble tumour necrosis factor receptor, interleukin 1, interleukin-1 receptor antagonist, interleukin 6.

Introduction

*Pneumocystis carinii* pneumonia (PCP) is the most frequently occurring severe opportunistic infection in patients infected with the human immunodeficiency virus (HIV). Clinical and experimental data suggest that CD4⁺ T lymphocytes are important for protection against PCP, although not directly as effector cells [1–3]. Macrophages have been shown to interact directly with *Pneumocystis carinii* (P. carinii) [4–7]. Cytokines are likely to be involved as soluble mediators in the host defence against *P. carinii*, as inducers of other mediators, as chemoattractants and as regulators of neutrophil adhesion, but their exact role remains to be established.

*P. carinii* is able to stimulate *in vivo* production of tumor necrosis factor-α (TNF) by alveolar macrophages [8–13]. *In vitro* studies suggest that *P. carinii* possesses binding sites for TNF and that this cytokine is capable of killing *P. carinii* [14,15]. In mice, TNF and interleukin 1β (IL-1) are important for the clearance of *P. carinii*, whereas interleukin 6 (IL-6) regulates pulmonary inflammation and antibody response during resolution [16–18]. Thus, similar to a variety of other infections, IL-1, IL-6 and TNF seem to play a role in the pathogenesis of and protection against PCP.

To investigate the role of these proinflammatory cytokines in patients with PCP, we studied the profiles of IL-1, TNF, IL-6 and their inhibitors, IL-1 receptor antagonist (IL-1RA) and the soluble TNF receptors (sTNF-R) in blood as well as in bronchoalveolar lavage (BAL) fluid.

Material and methods

Patients

Twenty-three adult HIV-positive (HIV+) patients with PCP who underwent BAL according to a standardized protocol (see below) at the four participating centres (three University Hospitals and one regional referral Hospital) were included in the study between October...
Table 1. Clinical characteristics of patients and control subjects

<table>
<thead>
<tr>
<th></th>
<th>HIV+ PCP</th>
<th>HIV+ asymptomatic</th>
<th>Healthy control subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number</td>
<td>23</td>
<td>25</td>
<td>8</td>
</tr>
<tr>
<td>Male (%)</td>
<td>23 (100)</td>
<td>20 (80)</td>
<td>2 (25)</td>
</tr>
<tr>
<td>Age (years) (SEM)</td>
<td>35·8 (6·0)</td>
<td>37·4 (8·8)</td>
<td>23·3 (1·6)</td>
</tr>
<tr>
<td>Smokers (%)</td>
<td>9 (39)</td>
<td>12 (48)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>CD4 cells mm⁻³ (median and range)</td>
<td>30 (10–440)</td>
<td>100 (10–760)</td>
<td>ND</td>
</tr>
<tr>
<td>Corticosteroids (%)</td>
<td>8 (35)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Body temperature (°C) (SEM)</td>
<td>38·4 °C (1·0)</td>
<td>&lt;37·5 °C</td>
<td>&lt;37·5 °C</td>
</tr>
<tr>
<td>WBC x 10⁻⁹ L⁻¹ (SEM)</td>
<td>5·6 (0·5)</td>
<td>4·2 (0·4)</td>
<td>5·0 (0·5)</td>
</tr>
<tr>
<td>Per cent Neutrophils (SEM)</td>
<td>76 (3)</td>
<td>57 (2·2)</td>
<td>57 (3·6)</td>
</tr>
<tr>
<td>Per cent Neutrophils in BAL (SEM)</td>
<td>15 (2·7)</td>
<td>ND</td>
<td>0·9 (0·6)</td>
</tr>
<tr>
<td>Per cent Macrophages in BAL (SEM)</td>
<td>68 (4·4)</td>
<td>ND</td>
<td>95 (1·1)</td>
</tr>
</tbody>
</table>

Data, except CD4 cells, are given in number and per cent or means and SEM. HIV+, HIV seropositive; PCP, *Pneumocystis carinii* pneumonia; SEM, standard error of the means; ND, not done; BAL, bronchoalveolar lavage fluid.

1993 and November 1994. All patients were under the care of specialists in internal medicine dealing with HIV+ patients. BAL was performed by a chest physician. The diagnosis PCP was based on demonstration of *P. carinii* organisms on silver- and/or Giemsa-stained BAL specimens [19].

Blood samples for measurements of circulating and *ex vivo*-produced cytokines and inhibitors were taken at the time of BAL and after full recovery.

Respiration rate and arterial oxygen pressure (Po₂) on admission were used as parameters for severity of infection. In the four participating centres patients were given adjunctive corticosteroids when the Po₂ was equal to or less than 8 kPa, a lower threshold than mentioned in the NIH consensus [20].

Control subjects

Eight healthy non-smoking subjects who volunteered to undergo BAL were used as control subjects [21]. None of them used medication or had evidence of lung disease or a history of recent respiratory tract infection. Before BAL, a blood sample was taken for measurement of cytokines and white blood cell count (WBC).

Blood was obtained for cytokine measurements from 25 asymptomatic ambulant HIV+ individuals with varying CD4 counts who visited the HIV clinic of the University Hospital Nijmegen. They were the asymptomatic HIV+ control group.

Bronchoalveolar lavage

After topical lidocaine anaesthesia of the oropharynx and bronchial tree, a flexible fibreoptic bronchoscope was introduced into the bronchial tree and, after inspection, wedged in a subsegmental bronchus serving the area of greatest radiological abnormality. In the control subjects the lavage was performed in the right middle lobe. BAL was performed by injecting and aspirating 6–8 aliquots of 20 mL of sterile saline. In patients most of the BAL fluid was used for diagnosis, the remaining fluid (in general 10–20 mL) for cytokine assessment.

Processing BAL fluid

Immediately after BAL, 30 µL was taken for cell count and cell viability was assessed by trypan blue dye exclusion. The remaining fluid was centrifuged at 5000×g for 15 min. The supernatant was removed and one percentage of bovine serum albumin (Sigma, St Louis, MO, USA) was added. The fluid was then filter sterilized, aliquoted and frozen at −80°C until assay.

The pellet was resuspended in Dulbecco's modified Eagle medium at a concentration of 6·5×10⁶ viable macrophages mL⁻¹. 100 µL was taken for preparing cytocentrifuged Giemsa-stained preparations and half of the remaining fluid was incubated in 4-mL closed polystyrene tubes (Greiner, Alphen, The Netherlands) without any addition (unstimulated culture) whereas the other half was incubated with lipopolysaccharide (LPS, *E. coli* serotype 055:B5, Sigma) at a final concentration of 10 µg mL⁻¹. All incubations were carried out at 37°C for 24 h. Following incubation the tubes were centrifuged at 1200×g for 10 min and the supernatant and cell pellets separately frozen at −80°C until cytokine analysis.

Processing whole blood

Venous blood was drawn from patients and control subjects for cytokine assessment in whole blood as described previously [22]. Briefly, venous blood was collected aseptically into three 5-mL-draw sterile vacuum tubes, containing 7·2 mg of lyophilized EDTA and 250 µL of aprotinin (Trasyloil 2500 KIU, Bayer, Leverkusen, Germany; final concentration 625 KIE mL⁻¹).

One tube was immediately centrifuged at 1250×g for 10 min. Plasma was transferred to a polypropylene tube (Eppendorf, Sarstedt, Nümbrecht, Germany) and centrifuged at 15000×g for 1 min to remove platelets.
Figure 1. Concentrations of cytokines in BAL (A and B) and plasma (C and D) in HIV+ patients with PCP during the acute phase (AC) and recovery (REC), in asymptomatic HIV+ subjects (AS) and in healthy control subjects (CO). Bars indicate the medians. The asterisks indicate statistical significance (*P* ≤ 0.05, Wilcoxon rank-sum test with Kruskal-Wallis chi-square approximation for unpaired and signed-rank test for paired samples).

Platelet-poor plasma was pipetted into another sterile polypropylene tube and frozen at −80°C until cytokine analysis.

In one of the two remaining tubes 50 µL of LPS was added (final concentration 10 µg mL⁻¹) to stimulate cytokine production. This tube and the third tube were then incubated at 37°C for 24 h. After incubation the tubes were centrifuged and the plasma frozen at −80°C as described above.

Cytokine assays

Plasma and BAL samples were analysed by specific radioimmunoassays for IL-1, IL-1 receptor antagonist (IL-1RA) and TNF [23,24]. An Enzyme-linked binding assay (Hoffman-la Roche, Basel, Switzerland) was used for soluble receptors of TNF (sTNF-R;P55, P75), and an enzyme-linked immunoassay (ELISA) as described before for IL-6 (Inotherapy, Besançon, France) [25].

The detection limits of the various assays were: IL-1, 20 pg mL⁻¹; IL-1RA, 800 pg mL⁻¹; TNF, 20 pg mL⁻¹; sTNF-R;P55, 80 pg mL⁻¹, P75, 300 pg mL⁻¹; and IL-6, 20 pg mL⁻¹.

Statistical analysis

Values are reported as medians and ranges. Differences between groups were analysed with the Wilcoxon rank-sum test. For paired samples the signed-rank test was used. Correlations between variables were estimated with Spearman’s rank correlation coefficient. *P*-values of 0.05 or less were considered significant.

The study was approved by the Hospital Ethics Committee and informed consent was obtained from all patients and healthy control subjects.

Results

Clinical characteristics

Clinical characteristics of patients and control subjects are shown in Table 1. PCP prophylaxis was used by four patients only (17%), all of them used monthly pentamidine. Mean duration of symptoms was 5 (± 5) weeks. Mean arterial oxygen pressure was 8.6 (± 1.5) kPa. Eight (35%) patients had cytomegalovirus early antigen in BAL fluid. Two (9%) patients needed a respirator and two (9%) died. Four of the 23 HIV+ patients with PCP (17%) had concomitant infections (two had cerebral
PCP Control

Receptors were low in BAL fluid.

In three HIV+ patients (> 10 ng/mL⁻¹), the difference in the acute phase vs. 0.5 ± 0.2 ng/mL⁻¹ during recovery, P = 0.03; 8.5 vs. 5.8 ng/mL⁻¹, P = 0.01, Fig. 1D).

Compared with healthy control subjects, HIV+ patients with PCP had significantly higher concentrations of proinflammatory cytokines (P < 0.002, Fig. 1C) and of sTNF-receptors (P < 0.0006, Fig. 1D), whereas IL-1RA did not differ between the two groups.

**Ex vivo production of cytokines**

**Alveolar cells.** During the acute phase of PCP, LPS-stimulated production of proinflammatory cytokines by alveolar cells of HIV+ patients did not differ from the production of healthy control subjects (Fig. 2A), whereas LPS-stimulated production of IL-1RA was significantly higher (29.8 ± 4.9 vs. 10.4 ± 1.8, P = 0.01, Fig. 2A). Similarly, unstimulated ex vivo production showed higher production of IL-1RA in HIV+ patients with PCP, median 12.5, range 1.6–95 ng/mL⁻¹ vs. median 6.5, range 0.8–9.7 ng/mL⁻¹ in healthy control subjects (P < 0.05).

**Whole-blood cultures.** LPS-stimulated production of IL-6 and TNF was suppressed in the acute phase of PCP, and restored during recovery (Fig. 2B). Although the same trend was seen for production of IL-1, the difference was not significant. Production of IL-1RA tended to be higher in the acute phase than during recovery, the difference was however, not significant. Compared with asymptomatic HIV+ persons, proinflammatory cytokine production was slightly lower in the acute phase of PCP and higher during recovery. Compared with healthy control subjects, LPS-stimulated production of TNF in HIV+ patients in the acute phase of PCP was significantly suppressed (P = 0.007, Fig. 2B) and IL-1RA production capacity increased (P = 0.01, Fig. 2B).

Unstimulated production of proinflammatory cytokines was higher in HIV+ persons than HIV- control subjects and not influenced by the presence of PCP. Median concentrations in all HIV+ persons were 110 pg IL-1 mL⁻¹ (range < 20–640 pg/mL⁻¹) and 102 pg TNF mL⁻¹ (range 60–282 pg/mL⁻¹), whereas concentrations in healthy control subjects were < 20 pg IL-1 mL⁻¹ (range < 20–280 pg/mL⁻¹) and 80 pg TNF mL⁻¹ (range 60–120 pg/mL⁻¹).

**Effects of glucocorticosteroids and/or concomitant infections on cytokines in HIV+ patients with PCP**

Corticosteroids (40–60 mg of prednisone) were given to eight patients, 2–24 h before BAL. Concentrations of cytokines in BAL and plasma did not differ from the 15 patients who were not treated with corticosteroids. LPS-stimulated production in alveolar cells also did not differ significantly between these two groups (data not shown).

Figure 2. LPS-stimulated ex vivo cytokine production in alveolar cells (Fig. 2A) and whole-blood cultures (B) in HIV+ patients with PCP during the acute phase (AC) and recovery (REC), in asymptomatic HIV+ subjects (AS) and in healthy control subjects (CO). Bars indicate the medians. The asterisks indicate statistical significance (P ≤ 0.05, Wilcoxon rank-sum test with Kruskal–Wallis chi-square approximation and signed-rank test for paired samples).

cytokines and anti-inflammatory mediators did not differ between the acute and recovery phase in HIV+ patients with PCP (Fig. 1D).

Toxoplasmosis, one cryptococcal pneumonia and one salmonella septicemias). Fourteen patients (61%) were given anti-*Pneumocystis carinii* therapy within 24 h before BAL, in the others treatment was started after BAL.

**Cytokine concentrations in BAL fluid and in plasma**

BAL. BAL was performed in the acute phase of PCP and in healthy control subjects. Concentrations of all three proinflammatory cytokines were generally higher in the acute phase of PCP than in healthy control subjects, but significance was only obtained for IL-1 (184 ± 47 pg mL⁻¹ vs. no IL-1 detected, P = 0.0001, Fig. 1A). Although concentrations of IL-1RA were high in three HIV+ patients (> 10 ng/mL⁻¹), the difference with healthy control subjects did not reach statistical significance (Fig. 1B). Both types of soluble TNF receptors were low in BAL fluid.

**Plasma.** With the exception of IL-1RA (2.1 ± 0.7 in the acute phase vs. 0.5 ± 0.2 ng/mL⁻¹ during recovery, P = 0.01), plasma concentrations of proinflammatory cytokines did not differ between the acute and recovery phase in HIV+ patients with PCP (Fig. 1D).
However, LPS-stimulated \textit{ex vivo} production in whole blood showed significantly reduced IL-1 (median 300, range 100–7400 pg mL\(^{-1}\)) and IL-6 (median 180, range 30–2960 pg mL\(^{-1}\)) production in corticosteroid-treated patients as compared with untreated patients (IL-1 mean 5700, range 700–30300 pg mL\(^{-1}\), \(P = 0.009\); and IL-6 mean 2060, range 160–4950 ng mL\(^{-1}\), \(P = 0.01\)); there was no difference in TNF and IL-1RA synthesis.

In two patients circulating concentrations of cytokines were not different before and within 24 h after steroid administration. However, profound effects were seen on whole-blood cytokine production in one patient, whereas in the other patient the production was already reduced before the administration of corticosteroids (Table 2).

Unexpectedly, cytokine concentrations and production in the four HIV+ patients with PCP and another opportunistic infection (cerebral toxoplasmosis in two, cryptocoecal pneumonia in one and salmonella septicemia in one) were not different from those without concomitant infections (data not shown). We were also unable to show that cytomegalovirus in BAL fluid, use of prophylactic pentamidine inhalations or prior use of anti-\textit{Pneumocystis carinii} therapy affected cytokine profiles.

**Table 2. Effect of corticosteroids on (anti-)inflammatory mediators.** Plasma concentrations and LPS-stimulated production of (anti-)inflammatory mediators were measured in two HIV+ patients with PCP before administration of 40 mg of prednisone (C–) and 24 h after the first dose (C+). Data are presented in ng mL\(^{-1}\).

<table>
<thead>
<tr>
<th>Plasma concentration</th>
<th>Ex vivo production</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C–</td>
</tr>
<tr>
<td><strong>Patient A</strong></td>
<td></td>
</tr>
<tr>
<td>IL-1</td>
<td>0.12</td>
</tr>
<tr>
<td>IL-6</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>TNF</td>
<td>0.11</td>
</tr>
<tr>
<td>IL-1RA</td>
<td>1.0</td>
</tr>
<tr>
<td>sTNF-R, p. 55</td>
<td>3.0</td>
</tr>
<tr>
<td>sTNF-R, p. 75</td>
<td>7.5</td>
</tr>
<tr>
<td><strong>Patient B</strong></td>
<td></td>
</tr>
<tr>
<td>IL-1</td>
<td>0.10</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.01</td>
</tr>
<tr>
<td>TNF</td>
<td>0.09</td>
</tr>
<tr>
<td>IL-1RA</td>
<td>1.2</td>
</tr>
<tr>
<td>sTNF-R, p. 55</td>
<td>4.75</td>
</tr>
<tr>
<td>sTNF-R, p. 75</td>
<td>11.5</td>
</tr>
</tbody>
</table>

Discussion

HIV+ patients with PCP showed higher concentrations of the proinflammatory cytokine IL-1 in BAL fluid than healthy control subjects. In the circulation, higher concentrations of the anti-inflammatory cytokine IL-1RA were found compared with the situation during recovery.

It is tempting to hypothesize that high concentration of anti-inflammatory mediators in the circulation ensures that the effect of proinflammatory cytokines remains limited to the focus of infection. The host is subsequently protected against the deleterious systemic effects of proinflammatory cytokines, including damage to the endothelium, shock and mortality. At the site of infection, these cytokines are important as early response mediators regulating cellular function, activating the host response against \textit{P. carinii} and dictating events leading to repair of tissue injury. Their presence in BAL in patients with PCP is in accordance with the findings in \textit{in vitro} and animal studies showing that TNF and IL-1 play a role in the acute phase of PCP [12,13,17,18].

Increased concentrations of proinflammatory cytokines in BAL fluid and lungs were also found in HIV-negative (HIV–) immunocompromised patients and steroid-treated rats with PCP [21,26]. TNF was significantly increased in BAL fluid of HIV– patients, IL-6 in BAL fluid of rats. In lung homogenates of rats, concentrations of IL-1 were increased. Thus, in PCP at least one of the proinflammatory cytokines is present at the site of infection, although this is not always the same mediator. Why different proinflammatory cytokines are present in BAL in the various PCP models (HIV+ patients, immunosuppressed HIV– patients and steroid-treated rats) needs further study. The explanation for these differences is most probably multifactorial and may reflect different underlying immune defects or disease stages. It is remarkable that TNF, which is most frequently mentioned in relation to HIV and \textit{P. carinii}, is only increased in BAL in HIV– patients. These data do not corroborate findings by Millar \textit{et al.} [27], who reported increased production of TNF by alveolar macrophages in HIV+ patients with PCP. Differences in cell populations (adherent cells vs. unselected BAL cells in our study), incubation time (4 vs. 24 h) and assays (immunoassay vs. radioimmunoassay) might be responsible for these discrepancies. Our data are, however, in accordance with the results by Kandil \textit{et al.} [13]. They show that HIV infection modulated the response to \textit{P. carinii}; monocyte-derived macrophages \textit{in vitro} infected with HIV and

co-incubated with P. carinii, produce less TNF and IL-1 than non-HIV infected cells.

Anti-inflammatory mediators in BAL fluid of HIV− and HIV+ patients with PCP show low concentrations of s-TNF receptors, whereas IL-1RA is significantly increased in the HIV− patients and marginally increased in HIV+ patients. Although in some HIV+ patients the increased concentrations of IL-1 were accompanied by a local increase in IL-1RA, this was not the case in all patients. Why in some HIV+ patients with PCP IL-1RA levels were not further increase these plasma concentrations and proinflammatory cytokine production [32-35]. PCP did not support the latter hypothesis [26].

For cytokine patterns in the lung, we had to compare HIV+ patients with healthy control subjects because a BAL procedure was not considered eligible during recovery or in asymptomatic HIV+ persons. For cytokines in the circulation, acute phase was compared with recovery in the same patients to match for the presence of HIV infection. These data must be interpreted with caution because of the use of different control subjects. Another problem in our study was the uneven distribution of smokers. Smoking has been shown to decrease IL-1, which are able to induce IL-1Ra. Smoking was supported by the Netherlands Program coordination committee for AIDS research (PccAO).

In both HIV+ patients with PCP (this study) and HIV− patients with PCP (our previous study, ref. 21) circulating concentrations of anti-inflammatory mediators were higher in the acute phase of PCP than during recovery.

For cytokine patterns in the lung, we had to compare HIV+ patients with healthy control subjects because a BAL procedure was not considered eligible during recovery or in asymptomatic HIV+ persons. For cytokines in the circulation, acute phase was compared with recovery in the same patients to match for the presence of HIV infection. These data must be interpreted with caution because of the use of different control subjects. Another problem in our study was the uneven distribution of smokers. Smoking has been shown to decrease IL-1, which are able to induce IL-1Ra. Smoking was supported by the Netherlands Program coordination committee for AIDS research (PccAO).

In both HIV+ patients with PCP (this study) and HIV− patients with PCP (our previous study, ref. 21) circulating concentrations of anti-inflammatory mediators were higher in the acute phase of PCP than during recovery.

For cytokine patterns in the lung, we had to compare HIV+ patients with healthy control subjects because a BAL procedure was not considered eligible during recovery or in asymptomatic HIV+ persons. For cytokines in the circulation, acute phase was compared with recovery in the same patients to match for the presence of HIV infection. These data must be interpreted with caution because of the use of different control subjects. Another problem in our study was the uneven distribution of smokers. Smoking has been shown to decrease IL-1, which are able to induce IL-1Ra. Smoking was supported by the Netherlands Program coordination committee for AIDS research (PccAO).

Our data from steroid-induced PCP in a rat model do not support the latter hypothesis [26].

Both HIV− patients and HIV+ patients with PCP treated with steroids showed suppressed production of proinflammatory cytokines in whole-blood cultures in the acute phase. In contrast, proinflammatory cytokine production was not reduced in the HIV+ patients with PCP who did not receive steroids. Production in the latter group did not differ from that in recovery, nor from that in asymptomatic HIV+ individuals. The high dosages of corticosteroids were only given to patients with $P_{O_2}$ ≤ 8 kPa. Thus, severe infection could also be responsible for suppression of proinflammatory cytokine production [31,37,38]. In four of four patients with PCP (two HIV+ from the present series and two HIV− [20]), blood was taken before and after high-dose corticosteroids; in three of these patients we found that cytokine production was already suppressed before administration of steroids. As all four patients had severe PCP with low $P_{O_2}$, severe infection rather than corticosteroids seems to lead to down-regulation of systemic proinflammatory cytokine production.

Acknowledgments

We thank Dr P. Reiss from NATEC and Professor Dr S. Danner from the Department of Internal Medicine, Academic Medical Center, Amsterdam for their help in facilitating the start of this study, Dr J. Wijdenes (Inotherapie, Besançon, France) for the anti-IL-6 antibodies, Dr H. Gallati (Hoffmann-La Roche, Basel, Switzerland) for the sTNF-R ELISA. We greatly appreciated the statistical support from H. J. J. van Lier M.Sc. from the Department of Medical Statistics, University of Nijmegen. This study was supported by the Netherlands Program coordination committee for AIDS research (PccAO).

References


15 Dona ST, Pesanti EL. Tumor necrosis factor-α binds to specific receptors on Pneumocystis carinii (abstract). Clin Res 1990;38:352 A.


34 Breen EC, Rezai AR, Kajima K et al. Effect of corticosteroids on IL-10 and TNF-a release by alveolar macrophages from patients with AIDS and PCP. Chest 1991;100:1060–6.