Lung Compartmentalization of Increased TNF Releasing Ability by Mononuclear Phagocytes in Pulmonary Sarcoidosis

Mario Spatafora, M.D., F.C.C.P.* Anna Merendino, B.Sc.;† Giuseppina Chiappara, B.Sc.;‡ Mark Gjomarkaj, M.D.;† Mario Melis, M.D.;† Vincenzo Bellia, M.D.;‡ and Giovanni Bonsignore, M.D., F.C.C.P.†

The TNF is a monokine with cytotoxic and tumor-necrosing activities; in addition, TNF may play a role in inflammatory processes. The present study evaluates spontaneous and LPS-mediated release of TNF by AMs and autologous peripheral BMs of normal subjects and patients with pulmonary sarcoidosis. A recently developed cytotoxicity assay, specific for detection of TNF activity, was applied. This study demonstrates that (1) unstimulated mononuclear phagocytes released low levels of TNF with no differences between groups; (2) when effector cells were stimulated with LPS, AMs from patients with active pulmonary sarcoidosis released more TNF than AMs recovered from normal subjects and from patients with inactive disease; (3) this increase was compartmentalized to the lungs, since comparisons of TNF production by LPS-stimulated BMs failed to show any difference between study groups. These results suggest that TNF might play a role in the pathogenesis of the alveolitis of pulmonary sarcoidosis. (Chest 1999; 96:542-49)

Tumor necrosis factor is a mononuclear phagocyte-derived protein originally described as a mediator LPS-induced hemorrhagic necrosis of some murine sarcomas. Current concepts on mechanisms of TNF release suggest that the action of two factors in proper sequence is necessary for the occurrence of this phenomenon: the first one is a priming agent that causes activation of mononuclear phagocytes to become responsive to a second eliciting factor (such as LPS) that stimulates cells to release TNF. After TNF purification, sequencing and production by recombinant DNA technology, it became clear that cytotoxicity for some tumor cells is not the only biologic effect mediated by TNF. Indeed, TNF was shown to affect lipid metabolism by inhibiting lipoprotein-lipase activity, to modulate several functions of synovial and endothelial cells, and to influence collagen metabolism by providing myogenic stimuli for fibroblasts and by inducing collagenase and prostaglandin E2 synthesis. In addition, TNF has been suggested to play a role in inflammatory processes by virtue of its ability to attract and to activate inflammatory cells. and by inducing the production of other important mediators, such as interleukin 1 (IL-1). Pulmonary sarcoidosis is a chronic disease characterized by a mononuclear cell alveolitis, granuloma formation and, in some patients, interstitial fibrosis. The AMs, the resident mononuclear phagocytes of the lungs, currently are thought to play a key role in the pathogenesis of the disease by inducing replication and functional activation of lung T lymphocytes and by stimulating collagen production by lung fibroblasts. Since TNF may exert similar effects on the same target cells, the present study was carried out to investigate TNF production by AMs and autologous peripheral BMs isolated from normal subjects and from patients with active and inactive pulmonary sarcoidosis. The results presented here point out that, in the active form of the disease, the increased ability of mononuclear phagocytes to release TNF under a suitable stimulus is compartmentalized to the lungs.

METHODS

Study Population

Normal Subjects: Six normal nonsmokers (two female and four male subjects; age, 29.4 ± 2.5 years) were selected as controls. They had no history of pulmonary disease and had normal chest x-ray films and pulmonary function tests (Table 1).

Patients with Sarcoidosis: A diagnosis of pulmonary sarcoidosis was established in 20 nonsmoking patients (13 female and seven male subjects; age, 38.2 ± 3.1 years) according to previously described criteria, including transbronchial or open lung biopsies.
Table 1—BAL and 67Ga-Scan Data from Normal Subjects (n = 6) and Patients with Active (n = 12) and Inactive (n = 8) Pulmonary Sarcoidosis

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Active</th>
<th>Inactive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Pulmonary Sarco</td>
<td></td>
</tr>
<tr>
<td>Fluid recovery (%)</td>
<td>53.5 ± 4.9</td>
<td>53.1 ± 2.4</td>
<td>56.0 ± 3.5</td>
</tr>
<tr>
<td>Total cells (× 10^6)</td>
<td>15.3 ± 1.8</td>
<td>41.8 ± 3.4</td>
<td>21.2 ± 2.8</td>
</tr>
<tr>
<td>T lymphocytes*</td>
<td>5.0 ± 2.1</td>
<td>39.8 ± 2.5</td>
<td>15.3 ± 2.2</td>
</tr>
<tr>
<td>67Ga scans†</td>
<td>No Data</td>
<td>128.3 ± 14</td>
<td>28.8 ± 2.9</td>
</tr>
</tbody>
</table>

*Data are expressed as percentage of recovered cells.
†Data are expressed as 67Ga index units calculated by the method proposed by Line et al.14

Patients were divided into two groups according to the activity of lung disease, as assessed by percentage of T lymphocytes in BAL and by 67Ga lung uptake. Patients were considered to have active pulmonary sarcoidosis if 25% or more of their BAL cells were T lymphocytes and 67Ga lung uptake was increased, as will be discussed further on, whereas patients having BAL T lymphocyte percentage <25 and normal 67Ga lung uptake were considered to have inactive disease. Although arbitrary, these criteria have been shown to be useful in reflecting the intensity of the inflammatory processes in the lung in this disorder.14 According to these criteria, 12 patients (eight female and four male subjects) were considered to be affected by active disease, while eight patients (five female and three male subjects) were considered as affected by inactive disease (Table 1).

67Ga Lung Scans

67Gallium lung scans were performed by a rectilinear scanner 48 h after the intravenous administration of 50 μCi/kg of body weight of 67Ga citrate. 67Gallium indexes were calculated according to the semiquantitative scoring method described by Line et al., the score of 50 units being considered as the threshold value. Scans were not performed in normal subjects for ethical reasons.

Culture Materials

Hypaque Ficoll and Percoll were from Pharmacia Fine Chemicals, Uppsala, Sweden. Phosphate buffered solution, Hank's balanced salt solution and RPMI 1640 (Gibco, Paisley, Scotland) were prepared by diluting 10× concentrated solutions with sterile pyrogen-free water (Don Baxter, Trieste, Italy). Complete medium consisted of RPMI 1640 supplemented with 10 percent aseptically collected FCS (Gibco) and penicillin and streptomycin for clinical use (Farmitalia, Milan, Italy, and Squibb, Rome, Italy). Trypsin and EDTA solutions were from Gibco.

All reagents were negative for endotoxin at a sensitivity of 0.02 ng/ml by LAL assay (Microbiological Associates, Walkersville, MD; lots AOB 210 and TDO 125). In order to avoid the occurrence of false-negative results due to the presence of endotoxin inhibitors, FCS samples were tested in the LAL assay after removal of proteins by perchloric acid precipitation and neutralization of supernatants with NaOH and found to be constantly negative.

Isolation of Mononuclear Phagocytes from Lungs and Blood

The AMs were obtained by BAL, using a total of 200 ml of 0.9 percent NaCl solution, as previously described.15 After fluid recovery, a small amount was taken for determination of cell number, cell viability and differential counting.15 Percentages of T lymphocytes with respect to total cell recovery were performed by a rosette-forming method, using neuraminidase-treated sheep red blood cells.16 The AMs were purified by adherence on plastic: cells obtained by BAL were resuspended in complete medium and allowed to adhere on plastic Petri dishes (Nunc, Roskilde, Denmark) at 37°C, 5 percent CO2; after 1 h nonadherent cells were removed by gently flooding dishes with PBS, while adherent cells were detached by 5 min exposure to EDTA. Enriched AMs preparation contained >95 percent AMs, as determined by counts made on nonspecific esterase-stained cytocentrifuge preparations; cell viability was >96 percent, as shown by the trypan blue method.

Peripheral BMs were obtained as described elsewhere.17 Heparized venous blood samples drawn the same day of bronchoscopy were diluted in a ratio of 1:3 with sterile saline solution, and mononuclear cells were obtained by Ficoll-Hypaque density gradient centrifugation (400 g, 30 min). The upper interface layer of mononuclear cells was collected and cells were washed twice with PBS. The BMs were enriched by fractionation on a one-step discontinuous Percoll gradient. The Percoll solution (9.25 parts of concentrated Percoll + 0.75 parts of PBS, 10× (vol/vol)) was adjusted to an osmolality of 285 mosmol/L with concentrated PBS and mixed to isoosmotic complete medium to give a 46 percent (vol/vol) Percoll solution. The latter was delivered at the bottom of 15 ml conical tubes (Falcon, Oxnard, CA) and 3 ml of the mononuclear cells suspension was carefully layered on the top. After centrifugation (550 g, 30 min) the BM fraction was collected at the interface, while lymphocytes were pelleted at the bottom. The BMs were further enriched by adherence on plastic using the same procedure described for purification of AMs, as mentioned previously. After these procedures, the BM-enriched fraction (hereafter referred to as BMs) contains >95 percent of viable BMs, as determined by nonspecific esterase staining performed on cytocentrifuge preparations and trypan blue method. The BMs were washed twice in PBS and resuspended in complete medium for subsequent use.

Culture Conditions

Activation of AMs and BMs was performed by incubating cells with Escherichia coli LPS (Sigma Chemical Co, St. Louis, MO). Briefly, cells to be stimulated were resuspended in complete medium with LPS at various concentrations ranging from 1 to 1,000 ng/ml and incubated in 15 ml conical tubes (37°C, 5 percent CO2) for variable times. In preliminary experiments the LPS concentration of 100 ng/ml and the stimulation time of 2 h were demonstrated as those causing maximal stimulation of both normal and sarcoid mononuclear phagocytes without any cell toxicity. Therefore, these exposure parameters were chosen for subsequent experiments, in agreement with previously reported contributions.18 Unstimulated mononuclear phagocytes were incubated for 2 h in LPS-free complete medium. After incubation, both unstimulated and LPS-stimulated cells were washed twice with PBS, counted in a hemocytometer and resuspended in complete medium at the concentration of 2.5×10^6 cells/ml for use in cytotoxicity assays.

TNF Assays

TNF activity was detected by applying a 6-h 51Cr release cytotoxicity assay using as targets actinomycin D-pretreated WEHI 164 murine sarcoma cells (DDCC), as previously described.19 Target cells were detached by trypsinization (0.025 percent, 2 min) and, after washing with complete medium, 6×10^6 cells/ml were incubated (3 h, 5 percent CO2, 37°C) with 1 μg/ml of Actinomycin D (Sigma, St. Louis, MO) solution. After incubation, cells were washed in complete medium, resuspended in a small volume and labeled with 50 μCi of Na-51CrO4 (Amersham International, Amersham, GB). After 1 h (37°C, 5 percent CO2) cells were washed twice, resuspended in complete medium at the concentration of 5×10^6 cells/ml and 0.1 ml-aliquots of the target cell suspension were delivered in round bottomed 96-well plates (Nunc). The AMs and BMs at effector-target cell ratios ranging from 50:1 to 1:5.1
were added to target cells to give a total final volume of 0.2 ml/well (direct DDCC assay).

In order to demonstrate that cell-to-cell interaction is not mandatory for the occurrence of cytotoxicity, being lytic effect accounted for by a soluble factor released by cells, mononuclear phagocytes' culture supernatants were used as effectors in the cytotoxicity assays (indirect DDCC), as previously described. Briefly, after purification and stimulation procedures mononuclear phagocytes, at the concentration of 2.5 × 10⁶ cells/ml, were incubated (37°C, 5 percent CO₂) in 24-well flat-bottom plates (Nunc). After 4 h, culture media were harvested, centrifuged (600 g, 10 min) and kept frozen at −20°C until used. Lytic supernatants, at serial twofold dilutions ranging from 1:2 to 1:64 (final volume = 0.1 ml) were added to target cell suspension to give a total volume of 0.2 ml/well. To confirm that cytotoxicity was accounted for by TNF and that other mononuclear phagocyte-derived toxic agents (such as oxidants) were not involved in DDCC, experiments of inhibition of cytotoxicity were made by using an anti-TNF monoclonal antibody (Roche, Basel, Switzerland; final dilution-1:2,000) and two antioxidants, namely SOD (Sigma; 500 U/ml) and catalase (Sigma; 1,100 U/ml). Anti-TNF monoclonal antibody or antioxidants were suspended in 0.01 ml of medium and added to the test wells before delivery of LPS-stimulated effector cells (for direct DDCC) or undiluted lytic supernatants (for indirect DDCC). After 6 h of incubation (37°C, 5 percent CO₂), assay plates were gently centrifuged (40 g, 3 min) and 0.1 ml aliquots were harvested from each well and counted in a gamma counter (Beckman S5000, Irvine, CA).

For both direct and indirect DDCC assays, CI were calculated as: CI = (A − B)/(C − B) × 100, where A is the amount of ⁵¹Cr release (expressed in counts per minute) under the experimental conditions, B is the spontaneous ⁵¹Cr release as determined by incubation of target cells with medium alone and C is the maximum releasable ⁵¹Cr as determined by incubation of target cells with 1 percent sodium dodecyl sulfate solution. For indirect DDCC assay, data presented here are expressed as LU, where 1 LU is defined as the reciprocal of the diulture of lytic supernatants needed to determine a CI value of 10 percent. In all experiments, spontaneous ⁵¹Cr release was <2.5 percent/h and no significant difference was detected between untreated and Act D-pretreated WEHI 164 sarcoma cells. Negligible levels of cytotoxicity were always observed when untreated WEHI 164 sarcoma cells were used as targets, as previously described. All tests were run in triplicate and variability between replicates did not exceed 15 percent.

**Statistical Analysis**

Data are expressed as means ± SEM; comparisons were made by using Student's t test for unpaired data.

**RESULTS**

**BAL Data**

No bronchoscopic abnormalities were detected in the tracheobronchial trees before BAL procedures; the recovered fluid was sterile in all subjects and was constantly free of endotoxin contamination, as assessed by the LAL assay. Table 1 shows BAL and ⁶⁷Ga scan data of the subjects included into the study; as expected, patients with active pulmonary sarcoidosis had significantly higher total cell recovery, percentage of T lymphocytes and ⁶⁷Ga lung uptake (p<0.01, for all comparisons).

**Ability of BMs and AMs from Normal Subjects and Patients with Pulmonary Sarcoidosis to Exert Direct DDCC**

The cytotoxic activities of LPS-stimulated AMs and autologous BMs on Act D-treated WEHI 164 cells (direct DDCC) are shown on Figure 1. In different samples comparable levels of cytotoxicity were exerted by LPS-stimulated BMs on drug-sensitized targets at any effector-target cell ratio (normal subjects [n = 4]: CI = 36.2 ± 4.2 percent; active pulmonary sarcoidosis [n = 5]: CI = 40.2 ± 2.8 percent; inactive pulmonary sarcoidosis [n = 5]: CI = 39.7 ± 4.2 percent; [data relevant to effector-target cell ratio = 50:1]; p>0.2) (Fig 1, A). In contrast, AMs isolated from patients with active pulmonary sarcoidosis were more cytotoxic than AMs from normal subjects and from patients with the inactive form of the disease; this difference was statistically significant at 12.5:1 and 25:1 effector-target cell ratios (p<0.05), but was even more marked.
at a 50:1 effector-target cell ratio (normal subjects: CI = 35.4 ± 3.7 percent; active pulmonary sarcoidosis: CI = 58.7 ± 4.8 percent; inactive pulmonary sarcoidosis: CI = 41.5 ± 3.2 percent; p < 0.01) (Fig 1, B).

Ability of BMs and AMs from Normal Subjects and Patients with Pulmonary Sarcoidosis to Exert Indirect DDCC

To verify that cytotoxicity on drug-sensitized targets was accounted for by a chemically stable soluble factor released by cells after LPS stimulation, the indirect DDCC assays were performed. Comparisons of cytotoxic activity exerted by culture supernatants of unstimulated BMs did not show any difference between study groups (BM: normal subjects [n = 5] = 1.9 ± 0.7 LU; active pulmonary sarcoidosis [n = 6] = 2.7 ± 0.7 LU; inactive pulmonary sarcoidosis [n = 5] = 1.6 ± 0.5 LU; p > 0.2). After stimulation of BMs with LPS, culture supernatants exerted significant levels of cytotoxicity on targets, with no difference between study groups (normal subjects [n = 5] = 6.7 ± 2.4 LU; active pulmonary sarcoidosis [n = 12] = 6.7 ± 1.1 LU; inactive pulmonary sarcoidosis [n = 8] = 6.9 ± 1.5 LU; p > 0.2) (Fig 2A). Similarly, no difference was shown in comparing lytic activity of supernatants collected from unstimulated AMs (AMs: normal subjects = 1.9 ± 0.4 LU; active pulmonary sarcoidosis = 2.1 ± 0.6 LU; inactive pulmonary sarcoidosis = 2.0 ± 0.7; p > 0.2). In marked contrast, supernatants collected after stimulation of perforated AMs recovered from patients with active pulmonary sarcoidosis were significantly more cytotoxic for targets than lytic supernatants collected after stimulation of AMs of normal subjects and patients with inactive pulmonary sarcoidosis (normal subjects = 6.2 ± 2.5 LU; active pulmonary sarcoidosis = 29.8 ± 5.6; inactive pulmonary sarcoidosis = 6.7 ± 0.3; p < 0.005) (Fig 2B). The compartmentalization of cytotoxic activity in the lower respiratory tract was clearly observed when the ratios between LU of LPS-stimulated AMs and autologous BMs were calculated. In fact, the latter ratio was close to one in normal subjects and in patients with inactive pulmonary sarcoidosis, thus demonstrating that AMs and BMs have comparable ability to exert indirect DDCC under LPS-stimulation; in contrast, calculation of LU ratios in individual patients with active pulmonary sarcoidosis confirmed the increased lytic ability of AMs with respect to autologous BMs (LU ratio, AMs/BMs: normal subjects [n = 5] = 0.9 ± 0.1; active pulmonary sarcoidosis [n = 12] = 4.7 ± 0.6; inactive pulmonary sarcoidosis [n = 8] = 1.3 ± 0.3; p < 0.005) (Fig 3).

Ability of anti-TNF Monoclonal Antibody to Inhibit DDCC

To demonstrate that the lytic factor involved in DDCC was related to TNF and that no role was played by oxidants, inhibition experiments were made by using anti-TNF monoclonal antibody, SOD, and catalase in both direct and indirect DDCC assays. The direct DDCC exerted by LPS-stimulated AMs from five patients with active pulmonary sarcoidosis was completely suppressed by the addition of the anti-TNF monoclonal antibody to the test wells before delivery of effector cells, while no protection was provided by both antioxidants (active pulmonary sarcoidosis AMs alone: CI = 56.3 ± 5.0; + SOD: CI = 54.5 ± 3.9; + catalase: CI = 52.3 ± 6.2; + anti-TNF: CI = 2.5 ± 2.0; p < 0.001) (Fig 4A). The same results were obtained when other effector cells (ie, BMs or inactive pulmonary sarcoidosis AMs) were used in the direct DDCC inhibition assays (data not shown). In order to confirm that indirect DDCC was
actually due to the release of TNF in the conditioned culture media, further experiments were made by using lytic supernatants of LPS-stimulated AMs from five patients with active pulmonary sarcoidosis, three patients with inactive pulmonary sarcoidosis and four normal subjects. Indeed, while DDCC exerted by lytic supernatants of LPS-stimulated AMs from patients with active pulmonary sarcoidosis was unaffected by antioxidants, anti-TNF monoclonal antibody completely inhibited cytotoxicity (baseline: 25.0 ± 6.6 LU; + SOD: 23.6 ± 5.8; + catalase: 23.4 ± 5.1; + anti-TNF: 0.5 ± 0.2; p<0.001) (Fig 4, B). The same results were obtained when other lytic supernatants were used as effectors in the indirect DDCC inhibition assays (data not shown).

**DISCUSSION**

The TNF is a cytokine produced by LPS-stimulated mononuclear phagocytes that may play a role in inflammatory processes. The present study demonstrates that, after LPS-stimulation, the cytotoxic activity exerted by AMs and that exerted by BMs isolated from normal subjects on Act D-treated WEHI 164 sarcoma cells (DDCC) are comparable, whereas LPS-stimulated AMs isolated from patients with active pulmonary sarcoidosis, are significantly more cytotoxic than autologous BMs for drug-sensitized targets.

Several lines of evidence strongly support the concept that the lytic factor involved in DDCC is TNF. First, the cytotoxic activity was exerted by effector cells (and by lytic supernatants) only after stimulation with LPS, a factor known to be the most important inducer of TNF release by mononuclear phagocytes. In this regard, Nissen-Meyer et al recently demonstrated that the “cytotoxic factor” of DDCC shares identical biological and physicochemical properties with recombinant TNF. Second, the anti-TNF monoclonal antibody completely suppressed the cytotoxicity exerted by effector cells and by lytic supernatants. Third, the addition of antioxidants, such as SOD and catalase, was unable to reduce cytotoxicity, thus demonstrating that other toxic substances releasable by activated mononuclear phagocytes (such as oxidants) are not involved in DDCC. In agreement with the latter concept, Colotta et al demonstrated that Act D-treated WEHI 164 cells do not trigger any measurable release of oxidants by BMs and that PMA, a
powerful inducer of toxic oxygen intermediates release by phagocytes, is unable to augment cytotoxic activity exerted by BMs on these targets cells. Therefore, from all these pieces of evidence and from the results of other studies\cite{18,20} it is clear that TNF is the effector factor involved in DDCC.

**Evaluation of TNF Release by AMs and BMs**

The finding that normal AMs and BMs release, under LPS stimulation, comparable amounts of TNF is in contrast with the results of a study by Martinet and co-workers\cite{33} demonstrating the increased TNF-releasing capacity of normal AMs with respect to autologous BMs. These discrepancies might be explained, at least in part, by the different experimental conditions and, in particular, by the different parameters chosen for LPS stimulation of effector cells. The exposure parameters chosen in our study are in agreement with previously reported contributions. First, studies at the molecular level demonstrated that maximum expression of the TNF gene is reached after 2 h of stimulation of macrophages with 100 ng/ml of LPS.\cite{24} Second, the immunofluorescence staining technique for cytoplasmic TNF (allowing the identification of single TNF-producing BMs) demonstrated that TNF synthesis induced by LPS (1 µg/ml) already is apparent after 1 h, culminates after another one to two h and is followed by a strong decline during the next two to three h.\cite{25,26} Third, Kornbluth and Edgington\cite{18} demonstrated that TNF-mediated cytotoxicity is maximal after 2 h of incubation of BMs with LPS.

**Evaluation of TNF Release by Sarcoid Mononuclear Phagocytes**

Current concepts of pulmonary sarcoidosis suggest that AMs play a pivotal role in the pathogenesis of alveolitis by releasing a variety of important mediators.\cite{18,19} In the present study, we have demonstrated that the ability of AMs to release TNF after LPS-stimulation is related to activity of the alveolitis, as assessed by percentage of BAL T lymphocytes and $^{67}$Ga lung uptake.\cite{14} In this regard, although comparisons of spontaneous TNF release by AMs and autologous BMs did not show any difference between study groups, LPS-stimulated AMs isolated from patients with active pulmonary sarcoidosis released significantly more TNF than BMs from normal subjects and from patients with inactive disease. In contrast, no difference was shown in comparing TNF production by LPS-stimulated peripheral BMs. The results presented here suggest that in pulmonary sarcoidosis AMs are locally "primed" to become more responsive than normal to a TNF-eliciting factor, such as LPS. In this context, the increased ability to release TNF under LPS stimulation might be considered an addi-

\[\text{Trophic marker of functional activation of mononuclear phagocytes that is compartmentalized to the lungs. Our findings confirm and extend the data reported by Bachwich and co-workers}\cite{39} \text{who demonstrated the enhanced capacity of sarcoid AMs to produce TNF. However, no reliable inference about the relationship of this phenomenon with the natural history of the disease could be derived from that study since the disease activity was evaluated only on a clinical basis. In addition, the procedures used for purification of effector cells and for the assessment of TNF release were different from those used in the present study. Moreover, since no comparison of TNF-releasing ability of AMs and autologous BMs was reported in that study, it remained to be determined whether the increased ability of sarcoid mononuclear phagocytes to release TNF is a systemic phenomenon or is compartmentalized to the sites of disease activity. The results of the present study lend support to the second hypothesis. This finding is in agreement with the concept that AMs and autologous BMs are functionally different, at least in active disease, as reported in a variety of studies. For example, previous investigations have shown that, while AMs from patients with active pulmonary sarcoidosis have increased capacity, with respect to normal AMs, to present antigens to autologous T lymphocytes, the antigen-presenting ability of sarcoid BMs is not higher than normal.\cite{27} Moreover, sarcoid AMs, but not sarcoid BMs, spontaneously release gamma-interferon (gamma-IFN), a mediator that is known to up-regulate inflammatory and immune processes.\cite{38} The spontaneous production of gamma-IFN by sarcoid AMs (and sarcoid lung T lymphocytes) may account, at least in part, for the activation of AMs to release TNF under LPS stimulation, as shown in the present study. Gamma-IFN was shown to increase the production of TNF by mononuclear phagocytes in response to endotoxin\cite{40} by enhancing TNF-gene transcription and TNF-mRNA translation.\cite{30} Interestingly, this effect further can be enhanced by incubation with interleukin 2 (IL-2).\cite{39} Since sarcoid lung T lymphocytes spontaneously release IL-2 within the alveolar spaces,\cite{31,32} it could be postulated that the two mediators (ie, gamma-IFN and IL-2) act synergistically in enhancing TNF-releasing capacity of sarcoid AMs.

Although the role played by TNF in the pathogenesis of pulmonary sarcoidosis is still unknown, some speculations might be made. The TNF might account, together with other mediators,\cite{33} for the increased recruitment of peripheral BMs in the alveolar spaces of sarcoid patients\cite{35} by providing chemotactic stimuli for circulating BMs.\cite{9} In addition, since TNF was shown to increase the binding of lymphocytes to endothelial cells, it was suggested that it may cause enhanced migration of lymphocytes from blood into the tissues;\cite{40} actually, the compartmentalization of T
cell population within the lungs represents a hallmark of active pulmonary sarcoidosis. The TNF also could act in increasing lymphocyte migration to the lungs by stimulating the production of IL-1 by both endothelial cells and macrophages and by enhancing the production of gamma-IFN by activated T lymphocytes. In this regard, previous studies have shown that in active pulmonary sarcoidosis, AMs release IL-1 in the alveolar spaces and that both AMs and lung T lymphocytes spontaneously release gamma-IFN.

Therefore, TNF might play a role in the pathogenesis of pulmonary sarcoidosis by influencing, either by itself or in cooperation with other mediators, both AMs and T lymphocyte components of the alveolitis. Moreover, TNF might contribute, along with other mediators such as fibronectin and AM-derived growth factor for fibroblasts, to the interstitial fibrosis that represents, in some cases, the outcome of the disease. In agreement with the latter concept, TNF has been shown to stimulate the growth of both skin and lung fibroblasts in a dose-dependent manner. Thus, from all these pieces of evidence and from the results of the present study, the possibility that TNF is involved in the pathogenesis of pulmonary sarcoidosis might be inferred. However, it is still to be determined whether the increased capacity of LPS-stimulated AMs to release TNF is a condition specific for pulmonary sarcoidosis or if it is shared by other diseases characterized by the activation of AMs (such as idiopathic pulmonary fibrosis). In addition, since demonstration of in vivo production of TNF within the lungs is still lacking, further studies are required to investigate whether sarcoïd AMs actually and continuously release TNF or are just capable of releasing great amounts of TNF after minimal stimulation. In any case, since AMs constantly are under stimulation by inhaled antigens, both possibilities are consistent with the concept that the increased capacity of TNF production by sarcoïd AMs might play a role in the pathogenesis of this disease.

ACKNOWLEDGMENTS: The writers thank Dr. A. Mantovani (Istituto M. Negri, Milan, Italy) and Dr. G. A. Rossi (Ospedale S. Martino, Genoa, Italy) for their valuable suggestions. Dr. C. Spinnato for support in the statistical evaluation of the results and Drs. A. M. La Rocca, E. Sacco and D. Buzzone (Ospedale V. Cervello, Palermo, Italy) for performing bronchoscopies.

REFERENCES


548

Pulmonary Sarcoidosis (Spatafora et al)
intermediates and is suppressed by protease inhibitors. J Immunol 1985; 134:3524-29
24 Adams DO, Hamilton TA. Molecular transductional mechanisms by which gamma IFN and other signals regulate macrophage development. Immunol Rev 1987; 97:5-27
37 Hunninghake GW. Release of interleukin 1 by alveolar macrophages of patients with active pulmonary sarcoidosis. Am Rev Respir Dis 1984; 129:569-72