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Activation of Peripheral Blood Neutrophils from Patients with Active Advanced Tuberculosis

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Activation of peripheral blood neutrophils (PMN) was investigated in order to determine whether they might contribute to the inflammatory process during active advanced tuberculosis. Receptors for the Fc portion of IgG (FcγR) (FcγRI, FcγRII, and FcγRIIIB), CD66 (degranulation marker), and receptors for tumor necrosis factor-α (TNF-R55 and TNF-R75) were analyzed on PMN obtained from normal controls and tuberculosis patients (TB-PMN). Functional parameters such as cytotoxicity, superoxide anion generation triggered by *N*-formyl-methionyl-leucyl-phenyl-alanine (FMLP), and TNF-α and IL-1β production were evaluated. A high expression of TNF-R55, CD66, and FcγRIIIB and the appearance of FcγRI were detected in TB-PMN. In addition, cytotoxicity, superoxide anion release, and TNF-α and IL-1β production were enhanced in TB-PMN. Thus, in tuberculosis, the activation of PMN outside the focus of infection strongly suggests the possibility of a systemic inflammation that could modulate the inflammatory response. © 2001 Academic Press

INTRODUCTION

Tuberculosis (TB) is a chronic infectious disease caused by *Mycobacterium tuberculosis*. It is well documented that cell-mediated immunity is required for protection in TB, but it may also be responsible for the tissue damage observed in the disease. The central role played by T cells in containing the infection is well established, but little is known about the role of polymorphonuclear neutrophils (PMN) during the inflammatory process in this human disease. A mycobacterial infection is followed by the influx of PMN (1), the first line of defense, which upon stimulation leave the circulation and enter into the inflammatory site, responding to different chemoattractants such as C5a factor, platelet-activating factor, leukotriene B4, and interleukin-8 (IL-8) (2). Once localized in the site of infection, PMN are able to phagocytose bacteria either spontaneously or through the receptors for the Fc portion of IgG (FcγRs) present in the neutrophil surface membrane (3, 4).

It has been shown that PMN participate in the early response against experimental *Mycobacterium avium* (5) and *M. tuberculosis* (6) infection by a mechanism that is not associated with the phagocytic activity of PMN. The increased recruitment of PMN in infected mice could be mediated through a T cell–macrophage-dependent mechanism. Furthermore, IL-1 and tumor necrosis factor-α (TNF-α) are able to induce the accumulation of neutrophils when inoculated into experimental animals by an indirect mechanism through the participation of macrophages (7).

In humans, it has been demonstrated that bronchoalveolar lavage fluid (BALF) from patients with advanced pulmonary TB, which is characterized by the presence of smear-positive sputum, shows a dramatic increase in PMN (8), which is proportional to BALF IL-8 content (9, 10). This cytokine is a potent chemoattractant signal of the alveolus and may be involved in host defense, triggering the secretion of other cytokines by PMN (11). Moreover, phagocytosis of *M. tuberculosis* and its major cellular wall component lipoarabinomanan (LAM) have been shown to be potent stimuli for IL-8 secretion by human monocytic cells *in vitro* (12), in part via the release of TNF-α and IL-1β (12, 13). These cytokines may mediate clinical manifestations of the infection such as fever, weight loss, anorexia, weakness, fatigue, night sweats, and tissue necrosis.

TNF-α, a well-known mediator in the pathophysiology of TB, plays an important role in the development of the protective response. It is necessary for the maintenance of the granuloma, sealing off the foci of infection and limiting dissemination of the bacteria. TNF-α synthesis coincides with the development of BCG-induced granuloma (14). Moreover, TNF-α can enhance

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PMN functions, including adherence to vascular endothelium, phagocytosis, degranulation, cytotoxcicity, and superoxide anion production (15, 16).

Most of the studies carried out in PMN from human TB are related to their activity at the site of infection. The recruitment of PMN may also allow contact with bacterial formyl peptides released during the infectious process, enhancing PMN activities and contributing to the pathophysiology. The present study was designed to acquire further evidence on the role of PMN, outside the focus of infection, during the course of the inflammatory response in active TB. For this purpose, we evaluated the role of peripheral PMN from patients with active pulmonary TB, analyzing expression of activation markers such as FcγRs, CD66b, and TNF-α receptors. In addition, functional parameters such as cytotoxicity, TNF-α and IL-1β production, and superoxide generation were evaluated. In the present report we demonstrate that circulating neutrophils from patients with active TB show parameters of activation. Whether this activation could play a deleterious or beneficial role in the inflammatory process is discussed.

METHODS

Patients

A total of 17 hospitalized active tuberculosis patients (TB) was studied. Their informed consent for experimentation was obtained according to the Ethics Commission of the Hospital Francisco J. Muñiz. All of the patients were male and had an extensive pulmonary involvement confirmed by chest radiographs. According to the classification of the American Tuberculosis Society, 14 of them had bilateral disease with massive affectation and multiple cavities, and the 3 remaining had unilateral involvement of two or more lobes with cavities. All of the patients had a sputum smear positive for acid-fast bacilli and the presence of *M. tuberculosis* was confirmed by sputum-positive culture. All of them were seronegative for HIV, and those with other infectious or underlying disease were ruled out. Four patients had a previous history of TB and none of them had extrapulmonary tuberculosis. Ten patients were alcoholics, 8 were smokers, and none of them had used intravenous drugs. All were under therapy with anti-tuberculosis drugs (1–30 days). The mean age was 45 years (range from 23 to 73 years). Nine healthy volunteer blood donors (N) were studied. All N had received BCG vaccination in childhood and their tuberculin-test status was unknown; three of them were smokers.

Antigens

Lipopolysaccharide from *Escherichia coli* 0111:B4 (LPS) and *N*-formyl-methionyl-leucyl-phenyl-alanine (FMLP) were obtained from Sigma Chemical Co. (St. Louis, MO). The *M. tuberculosis* H37-Rv strain was kindly provided by the Mycobacteria service from Instituto Nacional de Enfermedades Infecciosas, ANLIS, Dr. C. G. Malbrán (Buenos Aires, Argentina). Stocks of these organisms were maintained in 7H11 agar (Difco Laboratories, Detroit, MI) at 37°C in 5% CO_2/95% air. A midlogarithmic phase of Mycobacteria was scraped from agar, suspended in phosphate-buffered saline free of pyrogen, and sonicated to break up clumps. After settling, the supernatant was transferred to a new tube, heated for 20 min at 80°C, and adjusted to an OD_{600} of 1, which corresponds to a bacterial suspension of $\sim 1 \times 10^8$ bacteria/ml. This antigenic preparation may contain not only particulate antigens but soluble antigens as well.

PMN Purification

Human PMN were isolated from heparinized venous blood by Ficoll–Hypaque gradient centrifugation (17) followed by sedimentation in 3% Dextran (Sigma). The PMN-rich supernatant was then collected and residual red blood cells removed by hypotonic lysis. The cells were washed immediately and resuspended at 3×10^6 cells/ml in RPMI 1640 (GIBCO, Life Technologies, Gaithersburg, MD) supplemented with 1% heat-inactivated fetal calf serum (GIBCO) and 50 μg/ml gentamicyn (CM). The purity of recovered PMN was about 96–98%, as determined by FACS light scatter patterns and by evaluation of cells fixed in methanol and stained with May–Grünwald–Giemsa, later examined by oil-immersion light microscopy at a 1000× final magnification. The viability was consistently >95%, as determined by trypan blue dye exclusion.

Target Cells

Chicken red blood cells (CRBC) were employed as target cells. Approximately 4×10^7 CRBC (20 μl) were labeled with 50 μCi of $Na_2{}^{51}CrO_4$ (NEN, Boston, MA) for 1 h at 37°C. The cells were then washed seven times and resuspended in CM at 5×10^6 CRBC/ml.

Antibody-Dependent Cellular Cytotoxicity

The cytotoxic assay was performed in triplicate by reacting 100 μl containing 3×10^5 neutrophils with 1×10^5 ^{51}Cr-CRBC at an effector to target cell ratio (E:T) of 3:1 into 96-well microtiter plates (Falcon, Becton Dickinson, Lincoln, NJ) in a final volume of 250 μl. Based on preliminary assays, subagglutinating amounts of rabbit anti-CRBC antibody were added to labeled target cells before antibody-dependent cellular cytotoxicity (ADCC) assay. The plates were centrifuged

at 50*g* for 5 min and incubated at 37°C in 5% CO_2 for 18 h. After centrifugation at 400*g* for 5 min, 100 μl of the supernatants was harvested and the radioactivity of supernatants and pellet from each well was measured using an automatic γ counter. The mean release of ^{51}Cr in triplicate samples was expressed as a percentage of cytotoxicity (%Cx) and was calculated as

$$\%Cx = \frac{A - B}{C - B} \times 100,$$

where A is the cpm of supernatants, *B* is the cpm of spontaneous release (target cultured with medium alone), and *C* is the cpm of total release. In the absence of antibodies, the spontaneous release was less than 5%. Cytotoxicity against nonsensitized CRBC was subtracted from the experimental data determined using antibody-coated CRBC.

Immune Complex-Dependent Cytotoxicity

Immune complexes (IC) were prepared with rabbit IgG anti-ovoalbumin and chromatographically purified ovoalbumin (Cappel Lab., Detroit, MI). These IC were added at 2, 4, 6, or 8 ng to 3×10^5 neutrophils in a final volume of 150 μl 5 min before the addition of 1×10^5 nonsensitized ^{51}Cr-CRBC (E:T = 3:1). After 18 h at 37°C in 5% CO_2–95% humidified air, the culture plates were centrifuged, the radioactivities of the supernatant and pellet were measured, and the percentage of lysis was calculated as mentioned above.

Nitroblue Tetrazolium Reduction Assay

The nitroblue tetrazolium (NBT) reduction assay, which is a measure of respiratory burst activity, was performed in order to determine the superoxide anion release NBT assay (18). Briefly, 100 μl of a PMN suspension at 20×10^6 cells/ml was incubated with 20 μl of different concentrations of FMLP (10^{-6}, 10^{-7}, 10^{-8}, 10^{-9} M) in the presence of 200 μl of 0.1% NBT. After a 30-min incubation at 37°C, the reaction was stopped by adding 0.5% HCl. The cells were centrifuged and the pellet was resuspended in 300 μl of dimethyl sulfoxide and read at 550 nm in a microtiter plate reader.

Cytokine Determination

One milliliter of a PMN suspension (3×10^6 cells) was cultured in plastic tubes at 37°C in a humidified 5% CO_2 incubator and stimulated with LPS (1 μg/ml) (*E. coli* 0111:B4, Sigma) or heated *M. tuberculosis* (1×10^6 bacteria/ml). Culture supernatants of both stimulated and nonstimulated PMN were harvested, centrifuged, and kept frozen (−70°C) until use. TNF-α and IL-1β were measured in cell-free culture supernatants by using commercial specific enzyme-linked immunosorbent assay (ELISA) kits (Immunotech, Marseille, France) and processed according to the manufacturer's specifications. Cytokine levels are expressed as pg/ml of protein and the detection limits of the assay were 10 and 15 pg/ml, respectively.

Surface Antigen Analysis

Cell surface markers for FcγRs, TNF-αRs, and CD66b antigens were determined. For this purpose, PMN were washed and resuspended in phosphate-buffered saline (PBS) at 5×10^5/100 μl and then labeled with anti-FcγRIII (anti-CD16), anti-FcγRII (anti-CD32), anti-FcγRI (anti-CD64), anti-TNF-R55 (anti-CD120a), anti-TNF-R75 (anti-CD120b), or anti-CD66b monoclonal antibodies (MoAb). FITC–anti-CD16 and anti-CD64 were obtained from Immunotech, and PE–anti-CD120a, anti-CD120b, and anti-CD32 were purchased from Caltag (Burlingame, CA). FITC or PE-labeled isotype controls were used for each MoAb. Labeling was performed in the dark at 4°C for 30 min, followed by two washes with PBS. Cells were then fixed in 500 μl of 1% paraformaldehyde. Using FACScan (Becton Dickinson), 10,000 events were collected in linear mode for forward scatter and side scatter and log amplification for FL-1 and FL-2. Analysis was performed using CellQuest software (Becton Dickinson Immunocytometry Systems, San Jose, CA), and subclass isotype controls were used to determine autofluorescence and nonspecific staining.

Statistical Analysis

All values are presented as the means ± SEM of a number of independent experiments. The data were evaluated statistically using the paired or unpaired Student *t* test; *P* values < 0.05 were considered significant.

RESULTS

Cytotoxic Mechanisms Mediated by FcγRs Are Enhanced in TB-PMN

Antibody-dependent cell-mediated cytotoxity and IgG immune complex-mediated cytotoxicity (IC-Cx) were two FcγR-dependent functions evaluated in TB and normal (N) PMN. IC-Cx evaluates a deleterious mechanism for bystander cells due to oxygen radical generation and is dependent on NADPH oxidase activity. On the other hand, ADCC is a FcγR-dependent but NADPH oxidase-independent cytotoxic mechanism (19).

As previously described (20), low levels of IC-Cx were seen in normal human neutrophils triggered by IC stimuli. However, as shown in Fig. 1A, TB-PMN exhibit a significant increase in IC-Cx (190 ± 85%) compared to N-PMN at every concentration of IC tested ($P < 0.01$). The mean value of ADCC, after 18 h of incubation, was higher in patients (50 ± 6.2%) than in controls (34 ± 5.4%) ($n = 11$, $P < 0.005$) (Fig. 1B). Thus, the functional parameters that depend on FcγRs were found elevated in these patients.

Upregulation of FcγRs on TB-PMN

It is known that FcγRII and FcγRIIIB are constitutively expressed on PMN. In order to determine whether the higher cytotoxic activity observed in TB-PMN was linked to overexpression of FcγRs, these receptors were evaluated in circulating PMN of normal controls and TB patients. Surface expression of FcγRIIIB was significantly elevated in TB compared with internal normal controls [mean fluorescence intensity (MFI) of N: mean ± SEM, 178 ± 26, $n = 11$, $P < 0.02$]. Surprisingly, FcγRI, which is only observed in normal PMN after stimulation with IFN-γ (15), was detected in TB-PMN from the 16 patients studied (range of MFI, 20–105). No significant differences were observed in FcγRII expression. Figure 2 shows a representative experiment of 16.

Enhanced Degranulation of TB-PMN

Degranulation, another parameter that correlates with PMN activation, was evaluated by measuring the upregulation of CD66b on the surface membrane of PMN. This molecule resides in the specific granules of resting neutrophils and appears on the membrane upon degranulation (21). The expression of CD66b was enhanced in TB-PMN compared to in N-PMN (% MFI of N: 153 ± $P < 0.03$). One representative experiment is depicted in Fig. 2.

Respiratory Burst Is Enhanced in TB-PMN

It is well known that priming of PMN with FMLP, a prototypic bacterial formyl peptide, triggers the release of an active oxygen-derived metabolic product (22). As an additional parameter of PMN activation, we evaluated whether superoxide anion production was enhanced in TB-PMN. For this purpose, an NBT reduction assay, which is a measure of respiratory burst activity, was performed by incubating PMN with different concentrations of FMLP (10^{-6}, 10^{-7}, 10^{-8}, 10^{-9} M). The results in Fig. 3 show that FMLP was capable of inducing enhancement of superoxide anion release from TB-PMN compared with normal PMN ($P <$ 0.02), even at concentrations of FMLP that are chemoattractant but do not induce secretion.

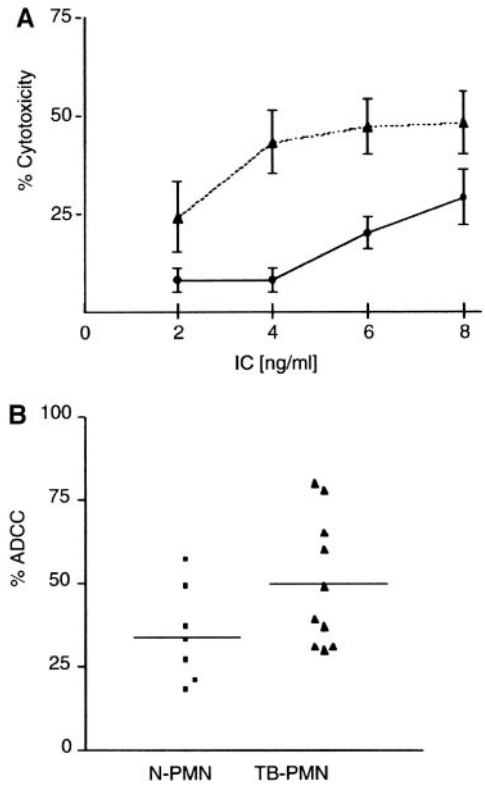

FIG. 1. (A) Enhanced IC-Cx in TB-PMN. Human PMN (3×10^5/ml) from TB patients (--▲--) and normal individuals (-●-) were treated with different concentrations of Ova-anti-Ova-IC for 5 min and used as effector cells (E). Then, E were incubated with 1×10^5 nonsensitized ^{51}Cr-CRBC target cells (T) at an E:T ratio of 3:1 for 18 h. The radioactivity of supernatants and pellets was measured and the percentage of lysis calculated as mentioned under Methods. Results are expressed as percentages of cytotoxicity and data are means ± SEM of separate experiments. The statistical difference for each point of the curve for TB-PMN vs N-PMN was $P < 0.01$. (B) Enhanced ADCC in TB-PMN. Human PMN (3×10^5/ml) were incubated with 1×10^5 ^{51}Cr-CRBC at an E:T ratio of 3:1 for 18 h at 37°C in 5% CO_2. The radioactivity of supernatant and pellet from each well was measured and the mean release of ^{51}Cr in triplicate samples was expressed as the percentage of cytotoxicity as mentioned under Methods. Data are means ± SEM of separate experiments. Statistical difference of TB-PMN vs N-PMN was $P < 0.004$.

TNF-α and IL-1β Production Is Enhanced in TB-PMN

As can be seen in Fig. 4A, secretion of TNF-α was significantly increased in TB-PMN when either LPS (TB, 684 ± 92; N, 357 ± 88 pg/ml) or heated *M. tuberculosis* (TB, 469 ± 107; N, 265 ± 45 pg/ml) ($P < 0.05$) were employed as stimuli. Moreover, although no significant differences were observed between TB and N nonstimulated PMN, the secretion of TNF-α by TB-

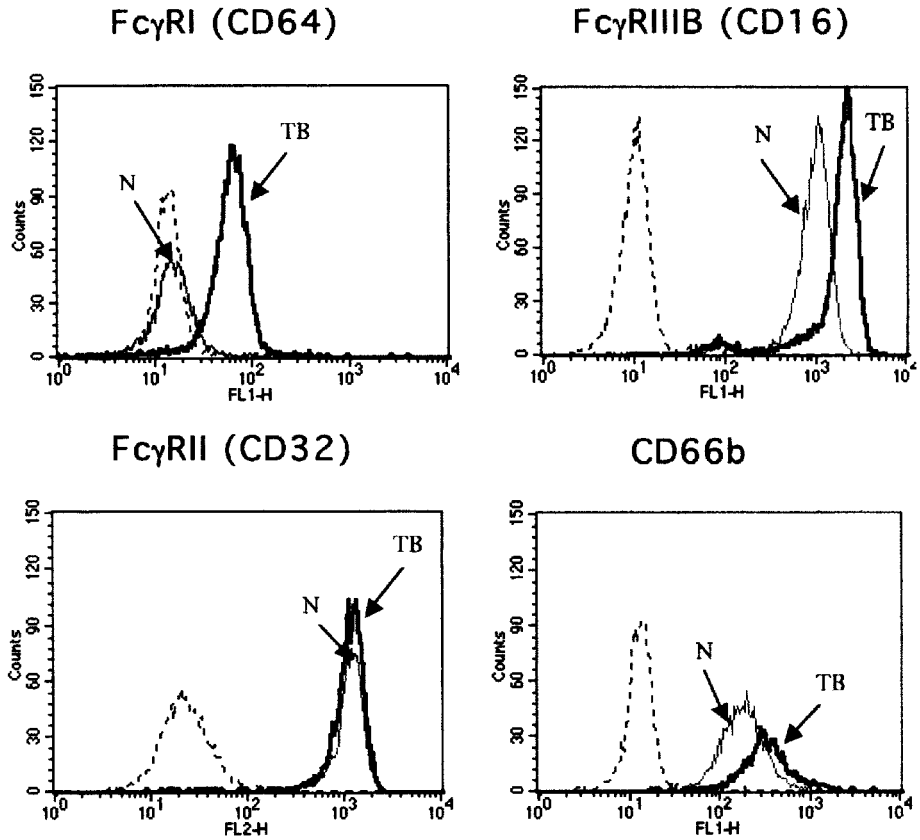

FIG. 2. Enhanced expression of FcγRs (FcγRI, FcγRII, FcγRIIIB) and CD66b on TB-PMN. Fresh PMN from TB and N were labeled with PE–anti-FcγRII, FITC–anti-FcγRIII, FITC–anti-FcγRI, or FITC–anti-CD66 mAb. FITC or PE-labeled and control isotype. A representative experiment is shown.

PMN tended to be higher than that of N-PMN (TB, 360 ± 51; N, 276 ± 95 pg/ml). As it is shown in Fig. 4B, when LPS was employed as a stimulus secretion of IL-1β was significantly enhanced in TB-PMN compared to that in N-PMN (TB, 625 ± 101; N, 321 ± 35 pg/ml) ($P < 0.01$). Moreover, a higher secretion of IL-1β was observed even in nonstimulated PMN obtained from TB patients (TB, 131 ± 50; N, 45 ± 12 pg/ml). However, heated *M. tuberculosis* induced IL-1β secretion in TB as well as in N-PMN (TB, 131 ± 50; N, 45 ± 13 pg/ml) ($P < 0.05$).

Enhanced Expression of TNF-R55 in TB-PMN

Taking into account that TNF-R55 mediates signaling for the respiratory burst in PMN, while TNF-R75 is essential for the maximal response to TNF-α (23), we evaluated the expression of the two TNF-α receptor chains, the 55-kDa TNF-R55 and the 75-kDa TNF-R75, in TB and N-PMN. The expression of TNF-R55 was significantly increased in TB compared to expression in N-PMN (% MFI of N, 188 ± 34%, $n = 9$, $P < 0.02$), while no significant difference was found in TNF-R75 expression. However, no differences were found in the percentages of cells bearing any of the TNF-α receptor chains. Figure 5 shows one representative experiment of nine.

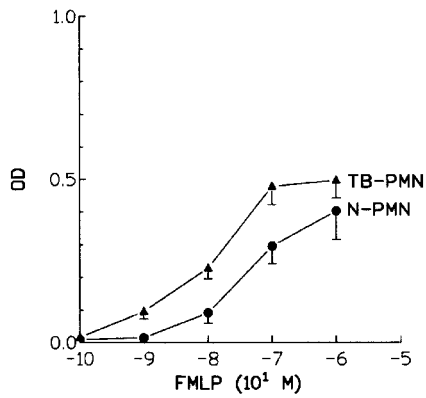

FIG. 3. Respiratory burst is enhanced in TB-PMN. An NBT reduction assay was evaluated as a measure of the respiratory burst activity. OD represents the superoxide anion release from TB-PMN (▲) ($n = 17$) and N-PMN (●) ($n = 7$). PMN (2×10^6/ml) were incubated with 20 μl of different concentrations of FMLP in the presence of 0.1% NBT. The reaction was measured at 550 nm in a microtiter plate reader. Significant differences for TB-PMN vs N-PMN were $P < 0.02$.

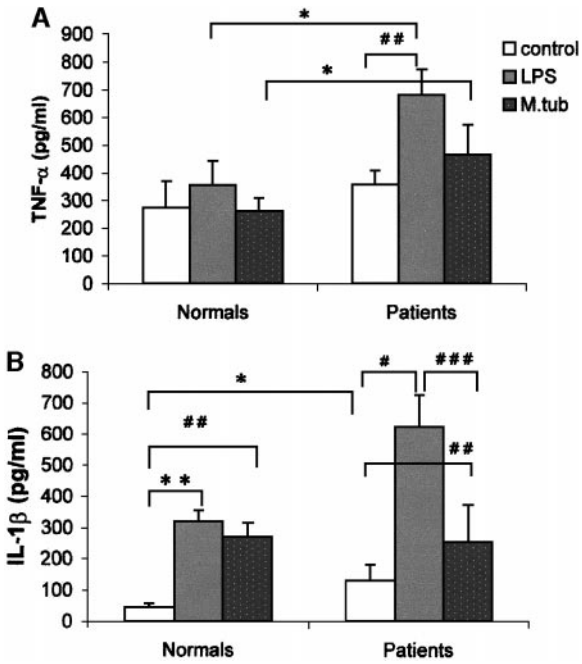

FIG. 4. TNF-α and IL-1β production are enhanced in TB-PMN. PMN (3×10^6 cells) were cultured with medium alone, LPS (1 μg/ml), or *M. tuberculosis* (1×10^6 bacteria/ml). TNF-α (A) and IL-1β (B) were measured in cell-free culture supernatants by commercial ELISA kits. Significant differences: $*P < 0.05$; $**P < 0.0005$; $\#P < 0.0001$; $\#\#P < 0.005$; $\#\#\#P < 0.01$.

DISCUSSION

Although the role of PMN during the course of human tuberculosis is not well understood, the presence of these cells in the lung may represent a response to local tissue injury. The precise mechanisms by which *M. tuberculosis*, as well as its components, interacts with PMN are poorly understood. PMN are generally the first cell type to reach the site of inflammation, and it is accepted that they meet their fate *in situ* because there is little evidence that they return to the blood from the inflammatory site. Thus, the migration of PMN is closely related to local chemoattractants and *in situ* expression of adhesion molecules (24). Although the inflammatory response observed during the chronic course of tuberculosis is characterized by a monocyte/macrophage infiltrate in the lung, a persistent influx of PMN extravased from the blood is observed.

In this study we demonstrated that the surface expression of FcγRIIIB was significantly increased in TB patients and that FcγRI expression was detected. Therefore, functions that depend on FcγR such as IC-Cx and ADCC were increased in peripheral TB-PMN. In addition, high superoxide anion generation (Fig. 3) was triggered even at chemoattractant concentrations of FMLP, suggesting that PMN released reactive oxygen intermediates even if they were not located at the site of infection.

It is well known that cytokines may affect the expression of FcγRs that are involved in ADCC (15). As previously demonstrated, FcγRII and FcγRIIIB are observed in normal PMN, while FcγRI is almost exclusively restricted to mononuclear phagocytes, except when PMN are exposed to IFN-γ (25). Concomitant with induction of FcγRI, PMN enhanced their ability to lyse sensitized CRBC through this receptor, becoming functional by induction with IFN-γ or G-CSF (4, 25, 26). The fact that overexpression of FcγRIIIB and FcγRI was enhanced in circulating TB-PMN may suggest either that abnormally high levels of those cytokines have been released into the circulation or that circulating TB-PMN have been in contact with those cytokines at the site of infection. In this sense, increased levels of circulating IFN-γ, as well as IL-18, an

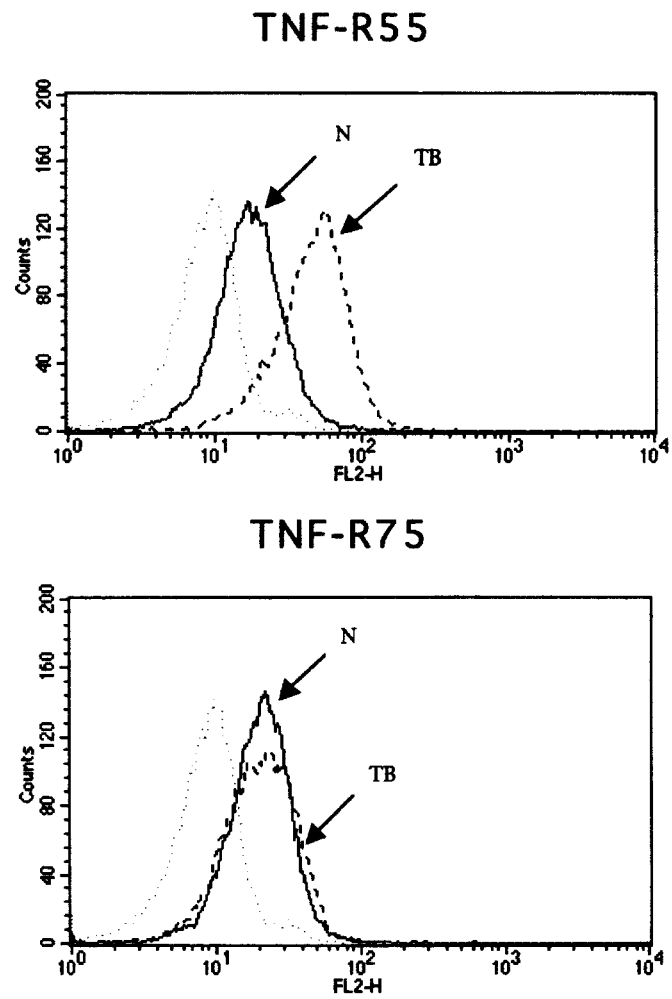

FIG. 5. Enhanced expression of TNF-R55 in TB-PMN. Fresh PMN from TB patients and N controls were labeled with PE–anti-TNF-R55 or PE–anti-TNF-R75 and PE-isotype-matched control MoAbs. A representative experiment is shown.

IFN-γ-inducing factor, have been found in TB patients (27). In this way, increased superoxide anion generation by PMN has been demonstrated by treatment with lymphokines such as TNF-α, G-CSF, and IL-1β (28). TNF-α also mediated neutrophil degranulation (29), and an active role for CD66 has been demonstrated in the modulation of neutrophil functional responses as well as β2-integrin-mediated spreading adhesion after CD66 ligation (30). On the other hand, in experimental *M. avium* infection, *in vivo* and *in vitro* activation with G-CSF stimulates PMN anti-mycobacterial activity and induces neutrophils to produce macrophage-activating cytokines such as IL-1β, TNF-α, and IL-12 (31, 32). Therefore, the significant increase in TB-PMN-mediated ADCC and IC-Cx, as well as the appearance of FcγRI, may be the result of lymphokine-induced activation of PMN, and the degranulation of neutrophils that we observed in TB-PMN (Fig. 2) could be mediated by TNF-α.

It has been demonstrated that IL-1 and TNF-α stimulate IL-8 release from mononuclear phagocytes and bronchial epithelial cells (9, 33). An exaggerated spontaneous IL-8 release from alveolar macrophages that correlates with increased BALF-PMN has been found in patients with active TB (8, 9). Furthermore, cytokines such as IL-1β, IL-6, IL-8, IL-12, IFN-γ, and TNF-α have been detected at higher concentrations in more affected lungs (34, 35), suggesting that these mediators are associated with the degree of tuberculosis disease and may be related to the intensity of inflammatory phenomena. The release of IL-1β upon stimulation with LPS even in nonstimulated PMN, demonstrates an activation of PMN far from the site of infection. In addition, heated *M. tuberculosis* triggered IL-1β production to a lesser extent than LPS, although we could not rule out that LAM, lipoprotein, or cell wall component was the stimulus for IL-1β and TNF-α secretion. The production of IL-1β in response to cytokines may have several important roles in the regulation of inflammation; inasmuch as IL-1β is important in the recruitment of PMN to sites of tissue injury, PMN-derived IL-1β may help to amplify this early response when monocytes are not prominent.

We also showed that TNFα secretion is enhanced in circulating TB-PMN, and, in contrast to Djeu *et al.* (36), we did not observe LPS-triggered in N-PMN. In addition, in TB-PMN TNF-α secretion was enhanced by heated *M. tuberculosis*. In this context, it has been shown that LAM and LPS possibly activate PMN through the same receptor, since cytokine induction by LAM and LPS was inhibited by the LPS receptor CD14 (12). Furthermore, LAM also induces IL-8 and the specific chemokine GRO-α in PMN at the mRNA and protein levels (37). Thus, common receptor components for LAM and LPS may be expressed on PMN, including the CD14 signaling system (38). Even though the main sources of TNF-α are activated mononuclear phagocytes, the participation of other cell types cannot be ruled out.

The presence of TNF-α in the pleural effusions and a high concentration of soluble TNF-α receptors in serum have been documented in patients with active disease (32, 39). TNF-R55 mediates ADCC and also the signal for PMN respiratory burst, whereas TNF-R75 is essential for a maximal response to TNF-α (23, 40). Thus, higher expression of TNF-R55 on TB-PMN could explain the higher capability to respond to stimulation mentioned above, making PMN more susceptible to TNF-α-induced activation of the respiratory burst. The multicomplex enzyme NAPH oxidase, the activation of which requires PMN activation and involves assembly from cytoplasm and integral membrane subunits, catalyzes this process. The first event in oxidase activation is the phosphorylation of the cytoplasm protein $p47^{phox}$ by both the p38 mitogen-activated protein kinase (MAPK) and extracellular-regulated protein kinase. p38 is involved in TNF-α expression of PMN stimulated by LPS (41), as in FMLP-induced leukocyte migration and IL-8-induced activation of PMN. In addition, expression of IL-8 requires p38 MAPK following stimulation with TNF-α, GM-CSF, FMLP, and LPS (42). In a model of aerosol infection with *M. tuberculosis*, it has been demonstrated recently that mice lacking the $p47^{phox}$ gene had a significant increase in bacterial growth over the early period of infection. The lack of superoxide anion correlated with an increase in the neutrophilic infiltrates within the granuloma (43). Once in the lung, activated PMN could subsequently enhance the localized inflammatory response by releasing these toxic metabolites, resulting in damage to the surrounding tissue. On the contrary, in the bloodstream oxidants might inactivate chemotactic factors, destroying their biological activity (44). Thereby, this process may serve to help terminate neutrophil influx, acting as a negative feedback loop.

Our results indicate the possibility of a systemic inflammation in active TB patients. The presence of these systemic PMN abnormalities indicates that during TB the chronic inflammatory response could no longer be considered solely as a local lung inflammation. Prevention of the influx of PMN into sites of chronic inflammation, or limiting their activation out of the site of infection, could be beneficial for the host by controlling the inflammatory process and thus diminishing tissue damage.

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