Enhanced Interleukin-8 Release and Gene Expression in Macrophages after Exposure to *Mycobacterium tuberculosis* and Its Components

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Abstract

Mycobacterium tuberculosis **infection is accompanied by acute and chronic inflammatory infiltrates associated with necrotizing granulomas in lung tissue. The cellular infiltrate is characterized by inflammatory cells which include neutrophils, lymphocytes, and macrophages. In animal and in vitro models of mycobacterial infection, cytokines including tumor necrosis factor-α (TNF-α), interferon gamma (IFN-γ), and interleukin-1β (IL-1β) participate in granulomatous inflammation. We hypothesized that interleukin-8, a potent chemoattractant for neutrophils and lymphocytes, could be released by activated alveolar macrophages after exposure to *M. tuberculosis* or its components and contribute to granulomatous lung inflammation. A quantitative immunoassay revealed that IL-8 protein release was significantly elevated in supernatants of macrophages and in lavage fluid obtained from patients with pulmonary tuberculosis compared to normal controls. In addition, Northern blots demonstrated striking up-regulation of IL-8 mRNA in macrophages from these patients. *M. tuberculosis* and its cell wall components lipoarabinomannan (LAM), lipomannan (LM), and phosphoinositolmannoside (PIM) stimulated IL-8 protein release and mRNA expression in vitro from alveolar macrophages, but deacylated LAM did not. Neutralizing antibodies to TNF-α and/or IL-1-α and β blocked 83% of the stimulation. IL-8 synthesis and release is an early response of macrophages after phagocytosis of *M. tuberculosis*. Its production serves to attract both acute and chronic inflammatory cells of active infection and thus participates in the process of containment of the pathogen. (*J. Clin. Invest.* 95:586–592.) Key words: tuberculosis • cytokines • Interleukin-8**

Introduction

Tuberculosis is a major cause of morbidity and mortality with approximately 25 million infectious cases reported throughout the world annually (1–3). The decline in tuberculosis in the U. S. has been reversed by a new tuberculosis epidemic fueled by the crises of AIDS, intravenous drug use, homelessness, and dismantling of the public health infrastructure for tuberculosis programs (4). To develop new strategies to treat and prevent tuberculosis, we and others have focused on cytokine networks in the lung to understand more about the human host response (5–8). We have previously demonstrated that *Mycobacterium tuberculosis* and its cell wall components stimulate both mRNA for IL-1β and TNF-α and protein release. Transcription is enhanced by the nuclear transcription factor, NF-IL6 (5, 9–10). These data are supported by numerous other investigations on the release of these cytokines by *M. tuberculosis*, yet it is unclear whether in vivo, enhancement or inhibition of select cytokines is propitious (11–17) for containment of the infection. Friedland and colleagues (18) reported that IL-8 was produced after phagocytosis of *M. tuberculosis* by a monocytic cell line (THP-1 cells) and proposed that this cytokine was involved in granuloma formation possibly by acting as a T cell chemoattractant. We now demonstrate the in vivo release of IL-8 from alveolar macrophages obtained from patients with active tuberculosis and investigate the mechanism of this release.

IL-8 is a recently characterized cytokine that functions as a chemotactic factor for neutrophils (27), T lymphocytes (28) and basophils (29). It belongs to a family of 8-kD polypeptides (30, 31). Because IL-8 is a chemoattractant for both neutrophils and lymphocytes, it may modulate both acute and chronic inflammation. Neutrophils are sequestered within the lung in granulomatous as well as fibrotic interstitial lung diseases. Sequestration may be induced by IL-8 as well as a number of other chemoattractants including C5a (24), leukotriene B_4 (25) and transforming growth factor β (26). Granulomatous diseases are also characterized by an abundance of lymphocytes, many of which are found around the periphery of the granuloma. Thus IL-8, as both a neutrophil and lymphocyte chemoattractant, may be a pivotal cytokine in the control of the inflammatory response to *M. tuberculosis*. Therefore, overexpression of IL-8 during *M. tuberculosis* infection may be responsible for the neutrophil and lymphocyte infiltrations which lead to granuloma formation. In addition, overexpression of IL-8 may activate inflammatory cells and thus contribute to the necrotic destruction of the lung.

IL-8 is expressed by many monocytes/macrophages (32), as well as endothelial cells (33), fibroblasts, keratinocytes, and lymphocytes (for review see reference 34). Most cell types produce little, if any, IL-8 constitutively (34). However, a wide variety of cells have been shown to produce a large amount of IL-8 after stimulation with LPS, TNF-α, and IL-1 (33–35). We now demonstrate IL-8 production by alveolar macrophages (AMs)[1] derived from human patients with tuberculosis. Alveo-

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Received for publication 24 March 1994 and in revised form 29 August 1994.

J. Clin. Invest.

0021-9738/95/02/0586/07 $2.00
Volume 95, February 1995, 586–592

1. *Abbreviations used in this paper:* AM, alveolar macrophage; HRP, horseradish peroxidase; Hsp, heat-shock protein; LAM, lipoarabinomannan; LM, lipomannan; PIM, phosphoinositolmannoside.

lar macrophages from patients infected with *M. tuberculosis* had increased spontaneous IL-8 release; this increase was associated with an alveolitis in the lower respiratory tract. We have also demonstrated that mycobacterial components lipoarabinomannan (LAM), phosphoinositolmannoside (PIM), and lipomannan (LM) (36) are potent inducers for IL-8 production from alveolar macrophages and peripheral blood monocytes. A recombinant heat-shock protein (Hsp 65kD) is also a weak stimulus for IL-8 production.

Methods

Study population. The clinical protocol was approved by the Human Subjects Review Committees of New York University and Bellevue Hospital Center. There were 15 patients with pulmonary tuberculosis confirmed by a positive culture for *M. tuberculosis.* The patients, 14 males and 1 female, included 7 African-American, 3 Hispanic, and 5 Asian individuals. There were 8 nonsmokers, and 7 current smokers. All TB patients were HIV-. Their mean age was 41±4 yr. There were 5 normal volunteers with normal chest radiographs, pulmonary function tests, and physical examinations. They included 4 males, 1 female; 4 Caucasian, 1 Asian; 4 nonsmokers, 1 ex-smoker; mean age 28±5 yr and no HIV risk factors.

Materials. LPS was purchased from Sigma Chemical Co. (St. Louis, MO). Recombinant human TNF-α (specific activity, 4.8×10^7 U/ml) was kindly supplied by Dr. M. Tsujimoto (Suntory Institute for Biomedical Research, Osaka, Japan); recombinant human IL-1β (specific activity 3×10^7 U/mg) was kindly provided by Hoffman-LaRoche (Nutley, NJ). LAM, PIM, and LM deacylated LAM were gifts of P. Brennan (Colorado State University, Ft. Collins, CO). They were derived from a laboratory attenuated strain of *Mycobacterium sp.* (37). These reagents had been eluted through Detoxi-Gel columns using sterile, pyrogen-free water and stored in pyrogen-free vials that had removed any contaminating LPS. Only pyrogen-free water was used in reconstitution of this material. *M. bovis* heat-shock protein 65 kD (recombinant) was kindly provided by R. Van der Zee (National Institute of Public Health and Environmental Protection, Bilthoren, The Netherlands). Evaluation of tuberculosis reagents and lavage fluid for the presence of gram-negative bacterial endotoxin was done with the Amebocyte Lysate assay (E-toxate kit, Sigma Chemical Co.). Neutralizing polyclonal antibodies anti-IL-1α, anti-IL-1β, and anti-TNF-α (R & D Systems, Minneapolis, MN), and anti-IgG_2 isotype control antibodies (Coulter Immunology, Hialeah, IL) were used at a concentration of 10 μg/ml.

Bronchoalveolar lavage. Bronchoalveolar lavage was performed with a flexible fiberoptic bronchoscope with local Xylocaine anesthesia. Normal saline (6, 50-ml aliquots) was instilled and suctioned sequentially from two or three sites (including radiographically involved sites). The recovered fluid was filtered through sterile gauze. A total cell count was done in a hematocytometer, and cell differentials performed on cytocentrifuge slides stained with .Diff-Quick and 500 cells were counted. Cell viability was determined by trypan blue exclusion, and in all cases, recovered cells were > 90% viable. AMs were placed in 175-cc plastic culture flasks in RPMI, allowed to adhere for 1 h, and washed three times with RPMI to remove nonadherent cells. Adherent cells were scraped off with a sterile rubber policeman, and placed in 24-well tissue culture dishes at 10^6 AM/ml under serum-free conditions for 24 h. Viability remained > 90% after culture and > 98% of adherent cells were macrophages.

Isolation of human monocytes and cell culture. Mononuclear cells in buffy coat were separated by centrifugation over lymphocyte separation medium (Flow Laboratories, McLean, VA). The cells were washed, suspended in RPMI 1640 medium supplemented with 10% fetal bovine serum and seeded onto the plastic surface of a 175-cm^2 flask for 2 h at 37°C to let monocytes adhere. The flask was washed three times with RPMI 1640 to remove nonadherent cells and the monocyte-enriched population was detached by scraping with the aid of a rubber policeman. The isolated human peripheral monocytes were placed in 24-well plastic tissue culture plates (Falcon) with a density of 10^6 cells/well. The cells were stimulated with different reagents for 24 h. The culture supernatant was then collected and frozen at −70°C.

THP-1 cells (myelomonocytic leukemia cell line) were obtained from American Type Culture Collection (Rockville, MD) and cultured in a 175-cm^2 flask in 10% fetal bovine serum until reaching a density of 5×10^5/ml. The cells were washed three times with RPM 1640 and cultured in 24-well plastic culture plates at a density of 10^6 cells/well for 24 h with various stimuli.

ELISA assay for IL-8. The assays were carried out with commercially available kits from R & D System (Minneapolis, MN). For the assay, the frozen supernatants were thawed out at room temperature and added to wells of rigid flat-bottomed microliter plates coated with murine monoclonal antibody to IL-8. After incubation of the samples, horseradish peroxidase (HRP)-conjugated antibody was added to the test wells. After a second incubation, the excess of the HRP-conjugated antibody was removed by washing. IL-8 was quantitated by a microtiter plate reader.

Isolation of RNA and Northern blot analysis. Normal alveolar macrophages were treated with test reagents for 4 h, collected by centrifugation and lysed by addition of 5.5 M guanidinium isothiocyanate buffer. Fresh alveolar macrophages from patients or controls were also processed directly for RNA extraction. Cytoplasmic RNA was isolated through $CsCl_2$ gradient ultracentrifugation. An equal amount of the obtained RNA was fractionated by electrophoresis through a 1% agarose–6% formaldehyde denaturing gel and transferred onto a nitrocellulose filter (BA 85; Schleicher and Schuell, Inc., Keene, NH). The baked filter was prehybridized in a solution containing 50% formamide, 0.5% SDS, 10× Denhardt, 2.5% herring sperm DNA, and 4× SSPE at 42°C for 6 h. For hybridization, the IL-8 cDNA (kindly provided by Jan Vilcek M.D. [New York University Medical Center]) was labeled with (alpha-^{32}P) dCTP (specific activity 3,000 Ci/mmol from DuPont-NEN, Boston, MA) by the random-priming technique using the kit purchased from Boehringer Mannheim (Indianapolis, IN). The hybridization was carried out at 42°C overnight. The filter was then washed in a solution containing 2× SSC and 0.5% SDS at room temperature for 20 min followed by 0.1× SSC plus 0.5% SDS at 65°C for 30 min. The filter was exposed at −70°C for 1–3 d.

Statistics. Data were assessed for normal distribution and analyzed with the Student's *t* test (unpaired) to compare mean IL-8 release and percent neutrophils from patients versus controls. Data was expressed as mean±standard error of the mean. A P value of < 0.05 was considered significant. When data were not normally distributed, nonparametric methods were used including the Wilcoxon test for BAL parameters.

Results

Evaluation of bronchoalveolar lavage for cell profile and IL-8 release. Both the cell concentration and cell differential differed between patients with active pulmonary tuberculosis and normals. Patients with active tuberculosis showed a twofold increase in cellularity ($641 \pm 165 \times 10^3$ cells/ml) versus normals ($314 \pm 39 \times 10^3$ cells/ml). A subgroup of the tuberculosis patients displayed increases in the percentage of lymphocytes or neutrophils (Fig. 1). Most of the patients with tuberculosis had a striking increase in neutrophils compared to controls (TB patients 37±10% versus normals 1±1%, $P < 0.05$).

To determine whether alveolar macrophages spontaneously released more IL-8 in patients with tuberculosis, AMs (10^6/ml) were cultured for 24 h and supernatants analyzed for IL-8 release. Alveolar macrophages from patients with tuberculosis released significantly more IL-8 into culture supernatants compared with normals (TB patients 87±15 ng/10^6 cells versus normals 9±1 ng/10^6 cells, $P < 0.01$, Fig. 2 *A*). IL-8 was also increased in the BAL fluid recovered from patients with tuberculosis (2,510±692 pg/mg protein versus normals 549±81

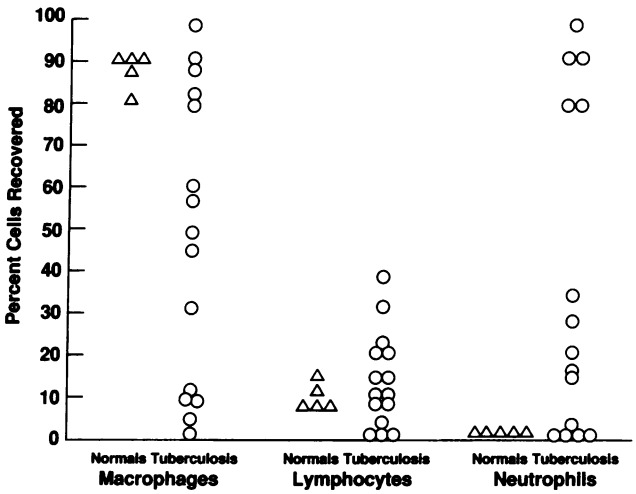

Figure 1. Percent cells recovered by bronchoalveolar lavage from patients with active tuberculosis and normal individuals. (△) Normals; (○)TB patients.

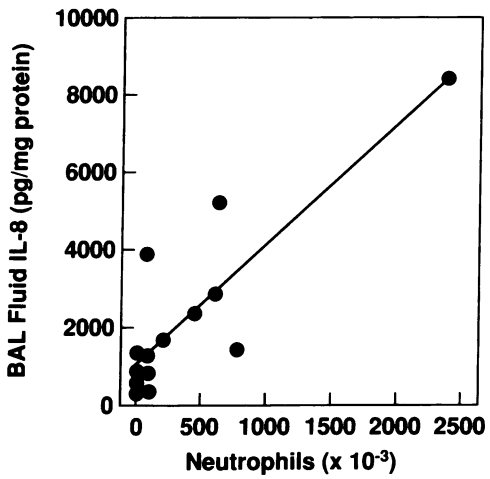

Figure 3. Linear association between IL-8 (pg/mg protein) in BAL fluid and neutrophils/ml recovered. The association was significant ($r^2 = 0.74$, $P < 0.01$).

pg/mg protein, $P = 0.06$, Fig. 2 *B*). LPS contamination of BAL fluid was negligible (14–50 pg/ml) and was not different between normals and TB patients. The linear association between the IL-8 in the BAL fluid and the neutrophils per milliliter recovered in the lavage was significant ($r^2 = 0.74$, $P < 0.01$, Fig. 3). In most TB patients there was a dramatic increase in the percentage of neutrophils, and IL-8 release and content in BAL fluid was significantly elevated consistent with the concept that IL-8 was a chemotactic factor for neutrophils in TB. Since alveolar macrophages were isolated by adherence to plastic and contaminating neutrophils and lymphocytes were removed by washing, and since adherent cells were confirmed to be AMs, the cell source of the IL-8 was most likely the alveolar macrophage.

To determine whether the increase in spontaneous IL-8 release was controlled at the gene level, we evaluated IL-8 mRNA expression in fresh alveolar macrophages from two normal individuals and three patients with TB who had > 80% macrophages in their BAL. Northern blot analysis revealed a single band of 1.8 kb in AM from each individual. In accordance with the measurement of IL-8 protein, alveolar macrophages consistently expressed increased mRNA for IL-8 in patients with TB compared with normal controls (Fig. 4). Thus AMs from patients with tuberculosis secrete increased amounts of IL-8, and this increase was determined in part, by enhanced mRNA expression.

Stimulation of IL-8 protein release and mRNA expression from alveolar macrophages with M. tuberculosis and its cell wall components. To determine the specific mechanism by which IL-8 release was stimulated, we evaluated the ability of *M. tuberculosis* H37Ra strain to stimulate the release of IL-8

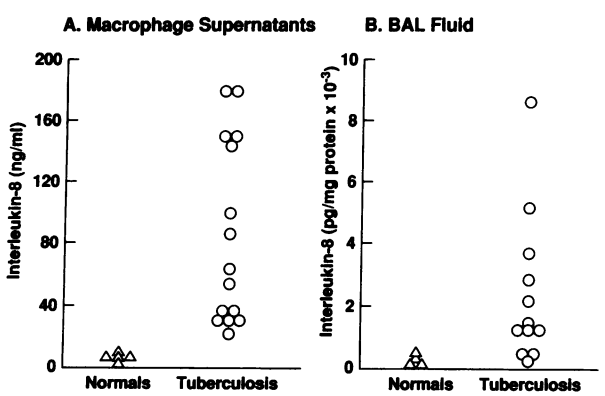

Figure 2. Spontaneous release of IL-8. (*A*) Macrophage supernatants from patients with active tuberculosis and normal individuals. (*B*) BAL fluid from patients with active TB and normals. (△) Normals; (○) TB patients. The spontaneous release of IL-8 was evaluated using an ELISA assay. ($n = 15$, one patient with 98% neutrophils had insufficient macrophages for assay.)

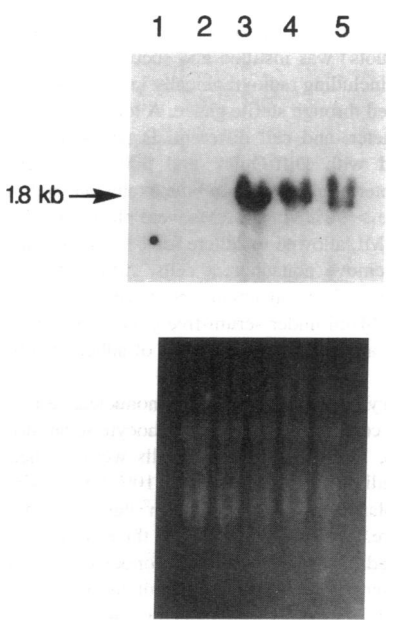

Figure 4. IL-8 steady state mRNA levels in alveolar macrophages from normal individuals or patients with active tuberculosis. Alveolar macrophages were collected by BAL from normal volunteers or active tuberculosis patients (> 80% macrophages) and total RNA was extracted. Northern blot analysis was carried out using a ^{32}P-labeled IL-8 cDNA probe and 30 μg of total RNA was placed in each lane. On the bottom of each figure are ethidium bromide stained RNA gels which demonstrated equal amounts of RNA in each lane. There is a striking increase in IL-8 mRNA expression in TB patients (lanes *3–5*) compared with normals (lanes *1–2*).

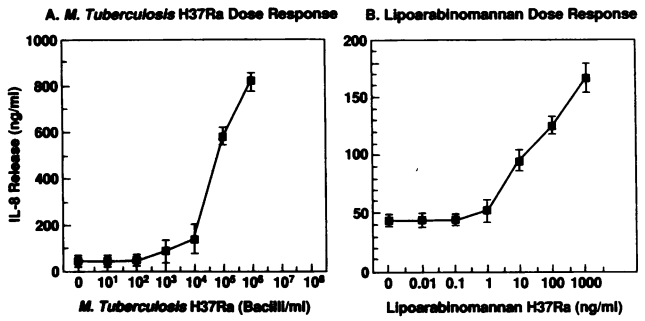

Figure 5. Dose-dependent stimulation of IL-8 production from alveolar macrophages by *M. tuberculosis* H37Ra or LAM. Alveolar macrophages from normal individuals (10^6/ml) were stimulated for 24 h. The culture supernatant was collected and IL-8 release was measured by ELISA. (*A*) *M. tuberculosis* H37Ra. (*B*) LAM.

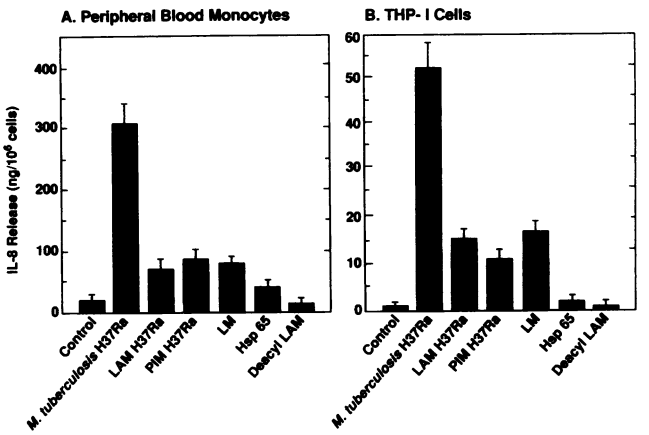

Figure 6. Stimulation of IL-8 production from peripheral blood monocytes (*A*) and THP-1 cells (*B*) with various stimuli. Cells (10^6/ml) were stimulated with different stimuli: *M. tuberculosis* (10^5/ml), LAM H37Ra (100 ng/ml), LAM Erdman (100 ng/ml), PIM H37Ra (100 ng/ml), LM (100 ng/ml), Hsp-65kD (1 μg/ml), and Deacyl LAM (100 ng/ml) for 24 h.

from alveolar macrophages from normal volunteers. Because of the similarity in function between *M. tuberculosis* H37Ra, and the cell wall component LAM derived from a laboratory attenuated strain of *Mycobacterium sp.* (37), we also examined the effect of LAM on IL-8 production. *M. tuberculosis* and LAM (H37Ra) stimulated a dramatic release of IL-8 from normal alveolar macrophages (24 h) in a dose-dependent manner (Fig. 5 *A* and *B*). Unstimulated AMs released 10–30 ng/10^6 cells of IL-8. LAM at a dose of 10–100 ng/ml enhanced IL-8 release to levels observed in BAL supernatants from patients with pulmonary tuberculosis. The decreased potency of LAM alone for IL-8 release compared to the whole *M. tuberculosis* bacillus suggests that multiple components are capable of stimulating IL-8 release. We also performed experiments with the Erdman strain which was a far weaker stimulus of IL-8 release from AMs compared to H37Ra strain (10-fold less, data not shown).

To test whether other cell wall components of *M. tuberculosis* also induced IL-8 release, we evaluated the following: PIM, LM, and deacylated LAM derived from *Mycobacterium sp.* and heat-shock protein 65 kD (Hsp-65kD). *M. tuberculosis* H37Ra, LAM, LM, and PIM were potent inducers of IL-8 release from peripheral blood monocytes (Fig. 6 *A*) and THP-1 cells (Fig. 6 *B*). There was a sixfold greater release of IL-8 from blood monocytes compared with THP-1 cells. LAM and PIM from the *M. tuberculosis* Erdman strain stimulated minimal release of IL-8. This is consistent with previous reports demonstrating that extensive mannosyl capping is associated with less cytokine release from macrophages (38). The Hsp-65kD from *M. bovis* also stimulated minimal release of IL-8. Last, deacylated LAM was unable to stimulate IL-8 release, a finding consistent with previous reports demonstrating the necessity of the phosphoinositol cell wall anchor of LAM to stimulate the release of cytokines (8, 38).

To determine whether IL-8 mRNA level was also increased by *M. tuberculosis* and its components, we performed Northern blot analysis of normal AM. Whole *M. tuberculosis* and its components LAM, PIM, and LM from H37Ra as well as Hsp-65kD stimulated a significant increase in IL-8 mRNA level in alveolar macrophages after 4 h of stimulation (Fig. 7).

To demonstrate that these results were not due to contamination with LPS, all test reagents were evaluated for LPS contamination using the *Limulus Amebocyte* assay. Less than 10 pg/mg was found for each test reagent (LAM, LM, PIM, Hsp-65kD) in all lots tested. To further guard against LPS contamination, the experiments in Figs. 5 and 6 were performed in the presence of polymyxin B 10–100 mg/ml with no significant alteration of the results (data not shown). These doses of polymyxin B completely blocked the stimulatory effect of LPS on IL-8 (14).

Effect of anti-cytokine antibodies on IL-8 release stimulated by M. tuberculosis. We (6) and others (7–8, 14–15) have previously described that LAM, PIM, LM, or Hsp-65kD stimu-

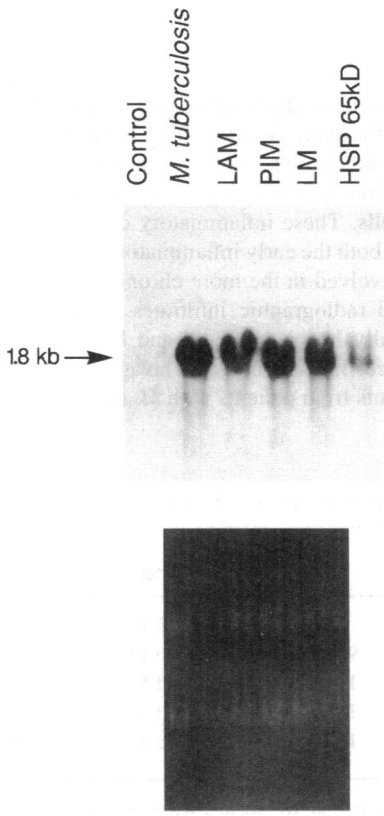

Figure 7. Northern analysis of IL-8 steady-state mRNA from alveolar macrophages after stimulation. Alveolar macrophages from normal individuals were stimulated with *M. tuberculosis* (10^5/ml), LAM H37Ra (100 ng/ml), PIM H37Ra (100 ng/ml), LM H37Ra (100 ng/ml), or Hsp 65kD (1 μg/ml) for 4 h. RNA was extracted, 30 μg was placed in each lane, and Northern blot analysis was carried out as described in Methods. On the bottom of each figure are ethidium bromide stained RNA gels which demonstrated equal amounts of RNA in each lane.

Table I. Effect of Anti-TNF-α and/or Anti-IL-1α and β Antibodies on the Release of IL-8 (ng/ml) from THP-1 Cells

	Control	Anti-TNF-α	Percent inhibition	Anti-IL-1	Percent inhibition	Anti-IL-1α and β and anti-TNF-α	Percent inhibition
Unstimulated cells	0.6±1	0.6±1	—	0.5±1	—	0.5±1	—
LAM H37Ra	52±8	26±3	49	38±7	26	20±2	61
PIM H37Ra	43±7	25±3	43	28±5	36	9±1	80
LM H37Ra	60±10	29±5	52	40±8	33	11±3	82

THP-1 cells were plated at 10^6/ml in 24-well tissue culture plates and stimulated for 24 h with LAM (100 ng/ml), PIM (100 ng/ml), and LM (100 ng/ml) in the absence or presence of antibodies against TNF-α, IL-1α, and/or IL-1β. The supernatants were collected and IL-8 rlease was measured using IL-8 ELISA. The numbers in the table are calculated with three independent experiments and expressed as mean±SEM.

late the release of TNF-α and IL-1β, cytokines which also induce IL-8 release in monocytes/macrophages. Thus, we determined whether IL-8 release by *M. tuberculosis* components was mediated by TNF-α and/or IL-1. The addition of anti-TNF-α and anti-IL-1α and anti-IL-1β antibodies alone or in combination significantly reduced the release of IL-8 from THP-1 cells after 24 h of stimulation by LAM, PIM, or LM (H37Ra) (Table I). Anti-TNF-α antibody alone blocked the production of IL-8 stimulated by LAM, PIM, or LM by 49, 43, and 52%, respectively. Neutralizing antibodies against IL-α and β inhibited IL-8 production by these stimuli to a slightly lesser extent (26, 36, and 33%, respectively). The presence of all three neutralizing antibodies to TNF-α and IL-1α and β reduced secretion to 61, 80, and 82%, respectively. We performed identical experiments with normal alveolar macrophages and obtained very similar results (Table II). However, AMs release much more IL-8 than THP-1 cells and are similar to peripheral blood monocytes (Fig. 6). Thus, cell wall components of *M. tuberculosis* elicit release of IL-8 in part via the release of TNF-α and IL-1α and β.

Discussion

Pulmonary tuberculosis has been characterized by the presence of necrotizing granuloma for many decades. We have now used the research tool of bronchoalveolar lavage to demonstrate an alveolitis in the lower respiratory tract characterized by an abundance of inflammatory cells. These inflammatory cells consist of neutrophils involved in both the early inflammatory response, as well as lymphocytes involved in the more chronic response. Most of our patients had radiographic infiltrates in multiple lobes, and we preferentially lavaged middle and lower lobes that appeared less involved. Bronchoalveolar lavage fluid and AM culture cell supernatants from patients with *M. tuberculosis* had significantly increased amounts of IL-8 in comparison with normals. In addition, Northern blots demonstrated a striking up-regulation of IL-8 mRNA expression in alveolar macrophages. Thus, the BAL cells were activated and participated in the granulomatous inflammation.

Of interest, we demonstrated that *M. tuberculosis* was more potent for IL-8 production than any single cell wall component, including the LAM from the laboratory attenuated avirulent mycobacterial strain. We suspect that this may be due to the coordinated effect of multiple stimuli in the cell wall of the intact mycobacteria or to altered presentation of the antigenic compound. Interestingly, LAM, live bacilli, and PIM from the avirulent strain H37Ra were more potent inducers for IL-8 than those from the virulent strain *M. tuberculosis* Erdman. Similar observations have been made with induction of other cytokines, such as TNF-α and IL-1β (8, 16). Chatterjee et al. (16) postulated that mannose side chains effectively capped the arabinose polysaccharides in the Erdman strain resulting in less TNF-α release by Erdman LAM, PIM, and LM. Despite the diminished potency of the virulent Erdman strain compared to the avirulent H37Ra strain of *M. tuberculosis* in the in vitro experiments, the in vivo studies demonstrate clear potency of the live bacillus. We observed exaggerated spontaneous IL-8 release from AMs lavaged from patients with active tuberculosis which was even greater than that seen in the in vitro studies obtained from the avirulent Erdman strain; these results demonstrate the difficulty in modeling the human experience with animal models and laboratory strains of *M. tuberculosis.*

IL-8 is a known chemotactic agent for both neutrophils and T cells at nanomolar and picomolar concentrations respectively; in this regard, IL-8 may be of central importance in granuloma formation and necrotic changes of granulomas (28). IL-8 has been implicated as a chemotactic factor in bronchoalveolar la-

Table II. Effect of Anti-TNF-α and/or Anti-IL-1α and β Antibodies on the Release of IL-8 (ng/ml) from Alveolar Macrophages

	Control	Anti-TNF-α	Percent inhibition	Anti-IL-1	Percent inhibition	Anti-IL-1α and β and anti-TNF-α	Percent inhibition
Unstimulated cells	33±1	31±1	—	34±2	—	37±3	—
M. tuberculosis H37Ra	995±57	526±15	47	562±17	44	197±5	80
LAM H37Ra	112±8	65±5	44	69±1	38	39±2	65
PIM H37Ra	124±6	63±3	50	68±1	46	44±1	65
LM H37Ra	182±11	79±3	57	88±4	52	45±1	75

Alveolar macrophages were plated at 10^6/ml in 24-well tissue culture plates and stimulated as THP-1 cells.

vage fluid or supernatants in a variety of inflammatory and interstitial lung diseases ranging from idiopathic pulmonary fibrosis (22, 23), pneumocystis carinii pneumonia (40), nonspecific interstitial pneumonitis associated with HIV infection (40), and inorganic dusts. IL-1 and TNF-α both stimulate IL-8 release and correlate with increased BAL neutrophils (41). These two cytokines have also been implicated as stimuli for IL-8 release from mononuclear phagocyte and bronchial epithelial cells (42, 43). Not only can bronchial epithelial cells release IL-8, but McCain et al have shown that neutrophils can synthesize and release IL-8 after stimulation by leukotriene B_4 (44). IL-1, TNF-α, and PMA activate the IL-8 gene rapidly and directly in the absence of new protein synthesis probably by a common serine kinase (41). Thus several cytokines and cell types contribute to IL-8 release in the locally inflamed lung.

M. tuberculosis has previously been demonstrated to elicit the release of IL-8. Friedland et al. demonstrated a 10-fold greater release of IL-8 by THP-1 cells following phagocytosis of *M. tuberculosis* than after LPS (18). Whereas these investigators observed IL-8 mRNA accumulated gradually over 24 h in THP-1 cells, they found that secretion was identical using virulent or avirulent strains. Using a monoclonal antibody to TNF-α, they also failed to demonstrate a change in IL-8 release. We have now demonstrated that both AM and PBM release IL-8 in response to *M. tuberculosis* and *Mycobacterium sp.* cell wall components. In contrast to Friedland et al., our polyclonal antibody elicited a 47–57% decrease in IL-8 release. Thus AM and PBM release IL-8 in a paracrine/autocrine manner in response to *M. tuberculosis*. Subcomponents LAM, LM, and PIM, had similar stimulatory capabilities, but removing the phosphoinositol anchor in the cell wall by deacylation abrogated these activities. We found AM released more IL-8 than PBM in response to live *M. tuberculosis* but similar amounts of IL-8 were released in response to using cell wall components. Both AM and PBM released approximately sixfold more IL-8 than THP-1 cells with each of the stimuli. The difference in the response to LAM between THP-1 cells and AM or PBM may be due to culture differences; THP-1 cells are cultured in suspension whereas AM and PBM are adherent. In addition, cell lines may be less responsive than primary cultures.

Transcripts for mRNA from two receptors for IL-8 have been described in PMN (45–47) and IL-8 binds to two proteins (p70 and p44) with high affinity. Thus the enhanced presence of IL-8 in response to *M. tuberculosis* may function to recruit and activate neutrophils at sites of pulmonary infections. Jones and colleagues have demonstrated that neutrophils kill *M. tuberculosis* (48). The toxic effect is mediated by nonoxidative means because neither free radical inhibitors (catalase, superoxide dismutase) nor deferoxamine impaired killing (48). Moreover, neutrophils from a patient with chronic granulomatous disease with defective NADPH pathway, were able to kill *M. tuberculosis* (48). *M. tuberculosis* is also capable of producing superoxide dismutase (the 23-kD antigen), and catalase (49). The 23-kD superoxide dismutase is the major protein released by *M. tuberculosis* in logarithmic growth constituting a very important factor for virulence (49). In addition, LAM is a potent scavenger of oxygen radicals (50). Although neutrophil nonoxidative mechanisms including defensins and bactericidal permeability increasing protein may contribute to host defense, they may be inadequate to control infection of mycobacteria. Neutrophils in an animal model of pleural TB release chemotaxins for monocytes that may then participate in granuloma formation (51).

In contrast to neutrophils, lymphocytes express only a single mRNA transcript for IL-8R2 receptor (46) and its expression is less than that in neutrophils. In addition, only a subpopulation of lymphocytes respond to IL-8. These lymphocytes require prior activation and are enriched for CD45RO (47). Indeed, preincubation of lymphocytes to purified protein derivative of *M. tuberculosis* results in a phenotypically distinct population of lymphocytes with an enhanced response to IL-8 (52).

IL-8 may also mediate the healing of granuloma. In a manner similar to basic and acidic fibroblast growth factor, IL-8 is angiogenic and also binds to heparin (53). Thus IL-8, like TNF-α, may participate in the angiogenesis observed in the healing process, particularly that seen at the margin of tuberculosis cavities.

We propose that the release of IL-8, as well as that of TNF-α and IL-1β from alveolar macrophages is rapid upon ingestion of the *M. tuberculosis*. These cytokines form an autocrine regulatory loop. Although the neutrophils are rapidly recruited, oxidant and nonoxidant mechanisms of killing may be overwhelmed by *M. tuberculosis*, LAM and scavenging enzymes. These data demonstrate the enhanced presence of IL-8 in pulmonary tuberculosis in humans. The potent effect of IL-8 on both neutrophils and lymphocytes make this a pivotal cytokine.

Acknowledgments

The authors thank Natalie Little for editorial assistance.

This work was supported by grants M0100096 (General Clinical Research Center) from National Institutes of Health (NIH), Aaron Diamond Foundation, NIH grants AI-35233, HL-51494, HL-51631, and NIOSH Cooperative Agreement U60/CCU206153.

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