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Mycobacterium tuberculosis Alters Expression of Adhesion Molecules on Monocytic Cells

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The host response to *Mycobacterium tuberculosis* is characterized by interactions between mononuclear cells, with recruitment and fusion of these cells culminating in granuloma formation. In addition, the host response to *M. tuberculosis* requires CD4$^+$ T-cell reactivity, mediated by antigen-independent as well as antigen-dependent mechanisms. Thus, we hypothesized that cell adhesion molecules such as intercellular adhesion molecule 1 (ICAM-1; CD54) would participate in the response to infection with *M. tuberculosis*. Exposure of THP-1 cells derived from a monocyte/macrophage cell line to *M. tuberculosis* (1:1 bacterium/cell ratio) elicited a sustained increase (660% ± 49% above resting level) in the expression of ICAM-1 that continued for at least 72 h. Neither the expression of vascular cell adhesion molecule 1 (VCAM-1; CD106) nor that of the integrins lymphocyte function-associated antigen 1 (LFA-1; CD11a/CD18) or CR3 (CD11b/CD18) was increased to a similar extent at corresponding time points. The increase in ICAM-1 protein expression was accompanied by an increase in steady-state mRNA (Northern [RNA] analysis). Neutralizing monoclonal antibodies directed against tumor necrosis factor alpha but not interleukin 1α or interleukin 1β substantially abrogated the response to *M. tuberculosis* consistent with a paracrine or autocrine response. Continuous upregulation of the expression of ICAM-1 on mononuclear phagocytes induced by *M. tuberculosis* may mediate the recruitment of monocytes and enhance the antigen presentation of *M. tuberculosis*, thus permitting the generation and maintenance of the host response.

In the past few years, there has been a dramatic increase in the number of cases of active tuberculosis diagnosed around the world. In 1990, there were an estimated 8 million new cases of tuberculosis diagnosed, with 2.9 million deaths due to the disease (6). The host response to infection with *Mycobacterium tuberculosis* involves a complex interplay between inflammatory cells and their cytokines. *M. tuberculosis* invades and multiplies within macrophages, eliciting a T-cell response to an antigen presented by an accessory cell (antigen-presenting cell [APC]) in the context of major histocompatibility complex (MHC) class II molecules (17). Early granulomas consist predominantly of macrophages in association with CD4$^+$ T cells. Mature granulomas contain giant cells and large multinucleated cells, formed by either fusion of macrophages or incomplete cell division, as well as CD4$^+$ and CD8$^+$ lymphocytes around the periphery (13, 26).

The development of the host response to *M. tuberculosis* is enhanced by the generation of diverse cytokines which include interleukin-1 (IL-1) and tumor necrosis factor alpha (TNF-α). These cytokines are expressed sequentially in the formation of granuloma: initial development of the granuloma involves the production of IL-1 (predominantly IL-1β), followed by the increasing ability of macrophages of the granuloma to secrete TNF-α (17). The macrophage response is further modified by the generation of lymphokines, specifically gamma interferon (IFN-γ) (10, 21). Whereas the role of select cytokines in the host response to *M. tuberculosis* is being elucidated, the sequelae of cytokine production remain even less well understood. Cytokines control the display of surface-adhesive molecules which mediate the antigen-independent interactions required for antigen-dependent T-cell stimulation. The interaction of lymphocyte function-associated antigen 1 (LFA-1; CD11a/CD18), a member of the integrin family, with intercellular adhesion molecule 1 (ICAM-1; CD54), a member of the immunoglobulin superfamily, is critical in determining conjugate formation between the APC and T cell as well as in the activation of T cells (18). Indeed, ICAM-1 augments T-cell proliferation and is pivotal in recall antigen responses of T cells to purified protein derivative of *M. tuberculosis* (3, 37). The expression of select adhesion molecules has also been demonstrated in some granulomatous diseases. Granulomas from both tuberculous leprosy and lepromatous leprosy display ICAM-1 expression (36). ICAM-1 expression has also been described in alveolar macrophages derived from patients with sarcoid (19).

Thus, we hypothesized that ICAM-1 would amplify and enhance the immune and inflammatory responses to *M. tuberculosis*. To begin to understand the role of ICAM-1 expression in the host response to *M. tuberculosis*, we investigated the mechanisms by which ICAM-1 expression was controlled in response to *M. tuberculosis*. We asked whether enhanced ICAM-1 expression in APCs was a direct effect of stimulation with *M. tuberculosis*. Our data demonstrate that THP-1 cells which are derived from a monocyte/macrophage cell line, increased surface expression of ICAM-1 in a sustained and selective manner upon stimulation with *M. tuberculosis*. Select cell wall components of a mycobacterium were also capable of eliciting this response. In addition, the enhanced expression of ICAM-1 in response to *M. tuberculosis* was mediated in a paracrine or autocrine manner predominantly via TNF-α. We propose that induction of the expression of ICAM-1 in APCs

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by *M. tuberculosis* is involved in the host response that results in recruitment and activation of mononuclear cells with subsequent formation of granulomas.

MATERIALS AND METHODS

Culture of *M. tuberculosis*. *M. tuberculosis* H37Ra (American Type Culture Collection) was maintained in Lowenstein-Jensen medium slants (Becton Dickinson Microbiology Systems, Cockeysville, Md.). The mycobacteria were subcultured 7 days prior to their use in 8 ml of Middlebrook 7H9 broth supplemented with 0.05% polysorbate 80 (Becton Dickinson). Prior to the experiment, the culture tubes were vortexed and left standing at room temperature for 10 min. The upper 6 ml was withdrawn and centrifuged (220 × *g*, 10 min), the supernatants were recentrifuged (900 × *g*, 3 min), and the new pellets were resuspended in RPMI 1640 medium supplemented with 10% fetal calf serum. Clumps of mycobacteria were dispersed by multiple passages through a 10-ml syringe with a 25-gauge needle. The final suspension consisted of separate bacteria which were counted in a hemocytometer chamber. To confirm bacterial counts, serial dilutions of bacteria were cultured (for 4 weeks) on Middlebrook 7H10 agar plates (Adams Scientific Inc., West Warwick, R.I.). Colony counts from these cultures corresponded with *Mycobacteria* counts.

Heat-killed mycobacteria were obtained after incubation of live organisms at 60°C (for 2 h in a water bath). The nonviability of the organisms was confirmed by their failure to grow on Middlebrook 7H10 agar plates after 6 weeks of culture.

Cell culture. THP-1 cells (ATCC TIB 202) were maintained in RPMI 1640 medium with L-glutamine (2 mM) (Whittaker Bioproducts, Walkersville, Md.) and supplemented with 10% fetal calf serum (GIBCO Laboratories, Grand Island, N.Y.). Fresh medium was added to the cells 24 h before each experiment. At appropriate time points, experiments were terminated by incubating the cells (10^6/ml) with EDTA (0.02%, 37°C, 30 min).

Antibodies. Monoclonal antibody (MAb) 84H10 (anti-ICAM-1; mouse immunoglobulin G1 [IgG1]) and MAb 25.3.1 (anti-LFA-1; mouse IgG1k) were obtained from AMAC, Inc. (Westbrook, Maine). RR1/1 was a kind gift from Robert Rothlein (Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, Conn.). The neutralizing MAb for TNF-α (IgG3) was purchased from Boehringer Mannheim; MAb MN41 (anti-CR3; IgG1) (12) and MAb W6/32 (anti-HLA A,B,C; mouse IgG1) (5) were kind gifts from Jill Buyon (New York University Medical Center, New York). Neutralizing goat polyclonal antibody for IL-1α and IL-1β were obtained from R&D Systems (Minneapolis, Minn.). MOPC 21 (mouse IgG1k), FLOPC 21 (mouse IgG3k), and fluorescein isothiocyanate (FITC) conjugate (goat anti-mouse IgG) were obtained from Sigma Chemical Co. (St. Louis, Mo.).

Immunofluorescence flow cytometry. Cells were washed with ice-cold phosphate-buffered saline with 10% fetal calf serum and 0.02% Na azide. Cells (10^6) were then incubated with the specific MAb (30 min, 4°C) at the appropriate concentration. Cells were then washed thrice and incubated with FITC-labeled goat anti-mouse IgG (30 min, 4°C) and then fixed (1% paraformaldehyde, 4°C), and all studies were analyzed within 3 days. Flow cytometry was performed on 10,000 cells per sample in a FACScan (Lysis II; Becton Dickinson).

Reagents. Lipopolysaccharide (LPS) from *Escherichia coli* O55:B5, phorbol myristate acetate, and EDTA were purchased from Sigma. Lipoarabinomannan (LAM) was a kind gift from P. Brennan (Colorado State University, Fort Collins) and was derived from a laboratory-attenuated strain of *Mycobacterium* sp. (15, 27). LAM was isolated via a detoxygel column, and a *Limulus* amebocyte lysate assay (QCL-1000; Whittaker Bioproducts) was used to determine endotoxin contamination of the LAM extract.

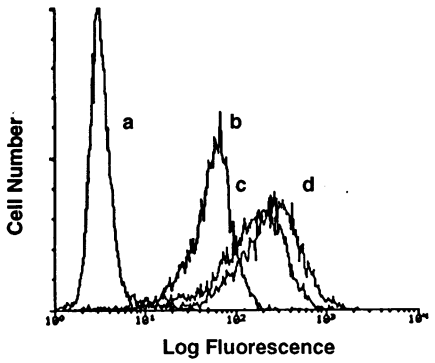

FIG. 1. Expression of ICAM-1 on THP-1 Cells. Indirect immunofluorescence staining was performed on THP-1 cells by using a MAb directed against the first extracellular domain of the ICAM-1 (84H10; IgG1) and revealed by an FITC-conjugated goat anti-mouse antibody. Fluorescence was performed by FACS analysis of 10,000 events by using a Lysis II program. Results are plotted as the $\log_{10}$ of the fluorescence against the cell number. The expression of ICAM-1 was analyzed on THP-1 cells exposed to control buffer and an irrelevant MAb (MOPC 21) (a), control buffer (24 h) (b), phorbol myristate acetate (8 nM, 24 h) (c), or LPS (10 ng/ml, 24 h) (d) and then exposed to an FITC-conjugated goat anti-mouse antibody.

Isolation of RNA and Northern (RNA) blot analysis. THP-1 cells were treated with the indicated stimuli and lysed with 6 M guanidinium-HCl. Total RNA was isolated over a guanidinium-cesium chloride cushion (30). Northern blot analysis was performed on RNA denatured at 56°C and run through 1% agarose gels (30 μg per lane) containing 2.2 M formaldehyde in 10 mM sodium phosphate (pH 7.0). RNA was transferred to nitrocellulose filters (BA 85; Schleicher & Schuell) overnight. The filters were baked (80°C, 2 h), blocked (0.1% Ficoll), and prehybridized (50% formamide, 0.5% sodium dodecyl sulfate [SDS], 10× Denhardt's solution, 0.1 mg of calf thymus DNA per ml, and 4× SSPE [1× SSPE is 0.18 M NaCl, 10 mM $NaPO_4$, and 1 mM EDTA {pH 7.7}]) at 42°C (6 h) and then hybridized (20 h, 42°C) with a ^{32}P random primer-labelled synthetic DNA fragment spanning the cDNA sequence for the human ICAM-1 gene from positions 1388 to 1487 (32). The filter was washed in serial dilutions of SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) and 0.5% SDS (room temperature) with a final wash in 0.1% SSC and 0.5% SDS (65°C, 20 min) and exposed to Fuji RX film at −70°C for 4 days with an intensifying screen. The filter was subsequently stripped and rehybridized with a ^{32}P random primer-labelled synthetic DNA fragment to glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Densitometry was performed (with an LKB Ultroscan XL enhanced laser densitomer) to calculate the relative amounts of ICAM-1 mRNA.

RESULTS

Expression of ICAM-1 in THP-1 cells. As demonstrated in Fig. 1, THP-1 cells exposed to a murine MAb directed against ICAM-1 (84H10 [AMAC] or RR1/1) and then to an FITC-conjugated goat anti-mouse IgG displayed increased fluorescence compared with an IgG1 isotype control (MOPC 21) when analyzed by immunofluorescence flow cytometry. Phor-

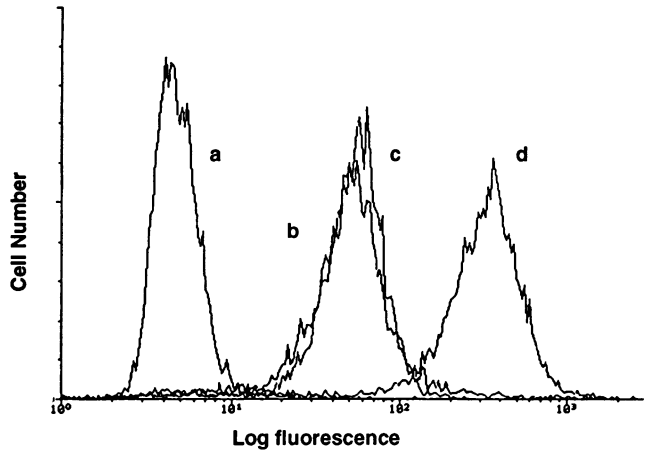

FIG. 2. *M. tuberculosis* alters the surface expression of ICAM-1 on THP-1 cells. THP-1 cells were incubated with *M. tuberculosis* H37Ra at a 1:1 ratio, and the expression of ICAM-1 was evaluated by FACS analysis for cells exposed to an irrelevant MAb (a), control buffer (24 h) (b), *M. tuberculosis* for 4 h (c), or *M. tuberculosis* for 24 h (d).

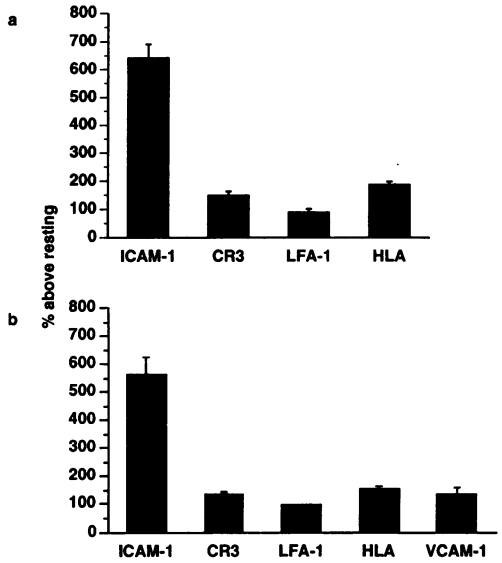

FIG. 3. Effect of *M. tuberculosis* or LAM on expression of adhesion molecules. (a) THP-1 cells were exposed to *M. tuberculosis* (1:1 bacterium/cell ratio) for 24 h, and expression of ICAM-1, CR3, LFA-1, and HLA A,B,C was determined by FACS analysis after indirect immunofluorescence staining of cells with the appropriate primary MAb and then with an FITC-conjugated goat anti-mouse antibody. Data are presented as percentages above resting levels (means ± standard errors of the means; $n = 3$). (b) Effect of LAM on expression of adhesion molecules. THP-1 cells were stimulated with LAM (250 ng/ml, 24 h), and expression of ICAM-1, CR3, LFA-1, and HLA A,B,C was determined by indirect immunofluorescence staining and then by FACS analysis. Cells stained for expression of VCAM-1 were exposed to LAM (100 ng/ml).

bol myristate acetate (8 nM) as well as LPS (10 ng/ml) elicited a time-dependent increase in the surface expression of ICAM-1 in THP-1 cells. Little upregulation was noted at 4 h (data not shown); by 24 h there was a significant increase in the expression of ICAM-1 (437.95% ± 38% and 681% ± 47% [means ± standard errors of the means] above basal levels, respectively; $n = 4$). Thus, ICAM-1 was constitutively expressed on THP-1 cells, and its expression was modulated by known stimuli for ICAM-1.

To determine the effect of exposure to *M. tuberculosis* on the expression of ICAM-1 on THP-1 cells, these cells were incubated with *M. tuberculosis* H37Ra. A 1:1 ratio (bacillus/cell) was chosen for experiments since this ratio induced phagocytosis (light microscopy) and minimal toxicity (≥90% cell viability as determined by trypan blue staining). Bacterial counts were confirmed by colony counts after a 6-week culture of serial dilutions. As shown in the representative experiment in Fig. 2, incubation of THP-1 cells with *M. tuberculosis* (1:1) resulted in a time-dependent increase in the surface expression of ICAM-1. A significant increase in the expression of ICAM-1 was noted after 24 h of incubation with mycobacteria (660.06% ± 39% above resting levels; $n = 9$). The expression of ICAM-1 continued to increase for at least 72 h (1632.7% ± 52% above resting levels; $n = 3$).

To determine whether the effect on ICAM-1 expression was specific for *M. tuberculosis*, THP-1 cells were also exposed to the attenuated bovine tuberculosis strain *Mycobacterium bovis* BCG. Exposure of THP-1 cells to BCG elicited an increase in the expression of ICAM-1 that was evident by 24 h (567.8% ± 191% above control levels) and continued to increase for 72 h (1,152.33% ± 458% above resting levels; $n = 3$). Heat-killed *M. tuberculosis* (1:1 bacterium/cell ratio) also enhanced the expression of ICAM-1 (552.9% above resting levels; $n = 2$).

Effect of *M. tuberculosis* and LAM from *Mycobacterium* sp. on other adhesion molecules. Since ICAM-1 functions as the ligand for the integrins LFA-1 (CD11a/CD18) and CR3 (CD11b/CD18), we examined the effect of *M. tuberculosis* on the expression of LFA-1 and CR3 on THP-1 cells. As demonstrated in Fig. 3a, the expression of LFA-1 was not increased at 24 h. The expression of CR3 and the surface protein HLA A,B,C was minimally increased by 24 h (148.4% ± 15% and 187.0% ± 11%, respectively, above resting levels; $P < 0.01$). None of these surface proteins were increased when shorter incubation times were used (4 h; data not shown). Thus, LFA-1 and CR3 are constitutively expressed in THP-1 cells but are not upregulated to the same degree or with the same kinetics as ICAM-1. LAM has been characterized and is a highly antigenic component of the cell wall of a rapidly growing *Mycobacterium* sp. (14, 27). It consists of a multiglycosylated (mannose core) region on a phosphatidylinositol backbone (14, 26). The major acyl groups are palmitate and tuberculostearate (10-methyloctadecanoate). LAM demonstrated a functional profile identical to that of *M. tuberculosis* on the expression of ICAM-1 and the integrins CR3 and LFA-1. LAM (250 ng/ml) elicited a minimal increase in CR3 expression (136.0% ± 8%; $P < 0.01$) and failed to stimulate LFA-1 (96.0% ± 2%). In addition, although there was constitutive expression of vascular cell adhesion molecule-1 (VCAM-1) in THP-1 cells, neither LAM (Fig. 3b) nor *M. tuberculosis* (data not shown) elicited upregulation of VCAM. These data suggest that the mechanism of activation of ICAM-1 expression is similar for both LAM and the intact mycobacterium. Thus, at the time points analyzed, *M. tuberculosis* and a *Mycobacterium* sp. cell wall component selectively induced an increase in the expression of ICAM-1.

Expression of ICAM-1 mRNA after *M. tuberculosis*. To determine whether the enhanced expression of ICAM-1 on the surface of THP-1 cells was associated with an increase in the expression of ICAM-1 mRNA, we measured steady-state ICAM-1 mRNA. As demonstrated in Fig. 4, total RNA extraction, followed by Northern blot analysis using a specific

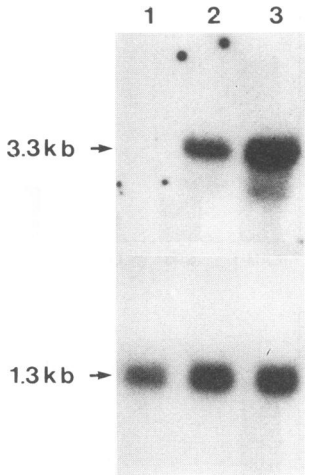

FIG. 4. Northern blot analysis of THP-1 cells exposed to *M. tuberculosis*. A nitrocellulose filter containing total RNA extracted from unstimulated THP-1 cells (lane 1), cells exposed to LPS (10 ng/ml, 4 h) (lane 2), or *M. tuberculosis* (1:1 ratio, 4 h) (lane 3) was consecutively hybridized with a synthetic probe to ICAM-1 and a synthetic probe to GAPDH. The films were exposed for 4 days (ICAM-1) and 15 h (GAPDH) with intensifying screens. Positions of the mRNAs detected (sizes, 3.3 kb for ICAM-1 and 1.3 kb for GAPDH) are indicated.

probe for ICAM-1, revealed a unique hybrid of the expected size (3.3 kb) for ICAM-1 mRNA (35). Unstimulated THP-1 cells displayed little mRNA for ICAM-1. As anticipated, LPS (10 ng/ml) elicited significant expression of ICAM-1 mRNA (4 h). *M. tuberculosis* (1:1) also elicited marked expression of ICAM-1 mRNA (4 h). These findings were confirmed by densitometric comparison of mRNA levels for ICAM-1 with those for the constitutively expressed GAPDH (1).

Role of TNF-α and IL-1β in expression of ICAM-1 induced by *M. tuberculosis*. THP-1 cells synthesize and secrete abundant TNF-α and IL-1β and little IL-1α. In addition, THP-1 cells have also been demonstrated to secrete TNF-α and IL-1β in response to *M. tuberculosis* and its cell wall components (41, 42). Thus, we examined whether the effect of *M. tuberculosis* on expression of ICAM-1 was mediated via a paracrine or autocrine effect by the production of these cytokines. THP-1 cells were preincubated in the presence of neutralizing antibody against TNF-α, IL-1α, or IL-1β and then exposed to *M. tuberculosis* (1:1 ratio, 24 h) or LAM. Direct immunofluorescence staining and flow cytometry were then performed. As can be seen in Fig. 5a, neutralizing antibodies directed against TNF-α significantly reduced the expression of ICAM-1 in cells stimulated with *M. tuberculosis*. Because of the similarity in functional profile between *M. tuberculosis* and LAM, neutralizing MAbs were also used to examine the effect of LAM-stimulated ICAM-1 expression. As seen in Fig. 5b, neutralizing MAbs directed against TNF-α significantly reduced the expression of ICAM-1 in cells stimulated with LAM. In contrast, neutralizing antibodies directed against IL-1α or IL-1β failed to inhibit the response. The combination of antibodies to IL-1α or IL-1β also failed to induce inhibition (data not shown). The neutralizing ability of the antibodies was confirmed by examining their effect on ICAM-1 expression stimulated by IL-1α or IL-1β. These data suggest that the expression of ICAM-1 in response to *M. tuberculosis* is regulated in part by the autocrine or paracrine expression of TNF-α but not IL-1.

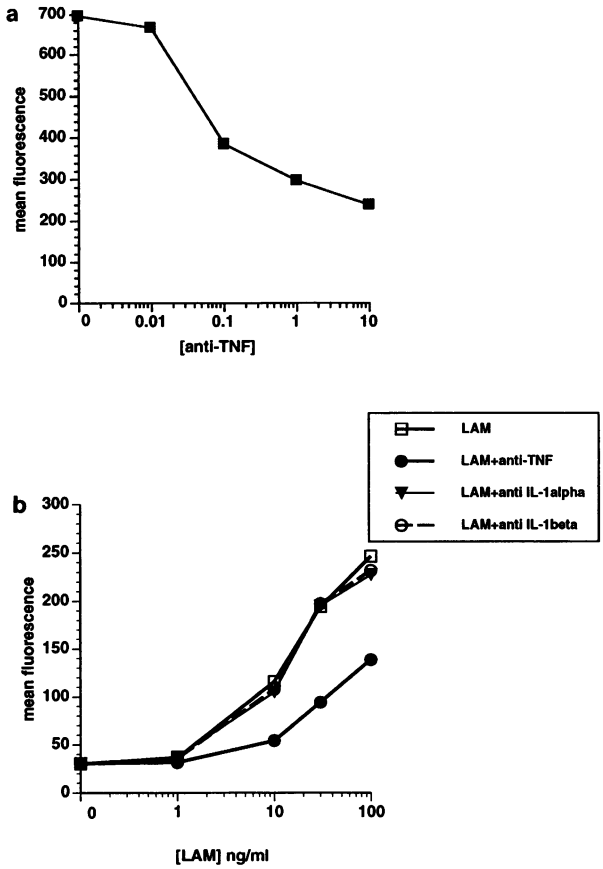

FIG. 5. Effect of neutralizing antibodies to TNF-α or IL-1 on expression of ICAM-1. (a) THP-1 cells were exposed to increasing concentrations of a neutralizing MAb directed against TNF-α, followed by *M. tuberculosis* (1:1 bacterium/cell ratio, 24 h). ICAM-1 expression was determined by direct immunofluorescence and FACS analysis. A representative experiment is shown. (b) THP-1 cells were exposed to a neutralizing MAb to TNF-α (5 μg/ml) or neutralizing polyclonal antibody to IL-1α or IL-1β, followed by stimulation with LAM. Direct immunofluorescence staining with FITC-conjugated MAb directed against ICAM-1, followed by FACS analysis, was performed. A representative experiment is shown.

DISCUSSION

Cell-mediated immunity which requires the recruitment and activation of monocytes/macrophages and T cells is critical for the host response to *M. tuberculosis*; humans and mice that lack $CD4^+$ T cells fail to contain the spread of mycobacteria (20). Since antigen presentation to $CD4^+$ T cells requires antigen-independent as well as -dependent processes, we hypothesized that cell adhesion molecules such as ICAM-1 (CD54) would participate in the response to infection with *M. tuberculosis*.

We have now begun to elucidate the mechanisms by which *M. tuberculosis* elicits an immune response. We have demonstrated that *M. tuberculosis* H37Ra enhanced expression of ICAM-1 in a human monocytic cell line. The kinetics of expression of ICAM-1 were similar to those described in most cells in which maximal expression of ICAM-1 is achieved after 24 h of cytokine induction (11, 34). In response to LPS stimulation, expression of ICAM-1 returns to basal levels in 2 to 5 days. In contrast, ICAM-1 expression in response to *M. tuberculosis* or BCG continued to increase at 72 h. The sustained increase in the expression of ICAM-1 after *M.*

tuberculosis infection may reflect continuous exposure of cells to viable mycobacteria. This prolonged increase in ICAM-1 expression may facilitate the continuous recruitment and fusion of mononuclear cells needed to maintain the structure of a granuloma or facilitate the activation of T cells. Indeed, microorganisms which can be completely degraded by macrophages in culture evoke only transient acute inflammatory responses in vivo, and only those that are resistant to degradation induce granulomas (1, 2, 33). Therefore, the sustained expression of ICAM-1 on monocytic cells after exposure to *M. tuberculosis* may play a role in the subsequent evolution of the inflammatory response in vivo. Indeed, expression of ICAM-1 is found across the spectrum of leprosy dermal lesions and correlates with the outcome of the host response to the infection, i.e., higher expression of ICAM-1 on the epidermis of tuberculoid lesions is associated with well-developed granulomas (36). In contrast, chronically infected macrophages (i.e., for 2 weeks) fail to present mycobacterial antigens to $CD4^+$ T cells (24). Although the mechanism for this inadequate presentation remains to be defined, the defect in antigen presentation may be due to altered expression of adhesive molecules. We have not extended our observations to this period of time to determine whether there is deficient ICAM-1 expression after chronic infection.

The counterreceptors for ICAM-1, CR3, and LFA-1 are also expressed on THP-1 cells. These counterreceptors mediate adhesion of inflammatory cells in part via their upregulation (25, 34, 38). CR3 also participates in the adhesion and phagocytosis of *M. tuberculosis* (31). In THP-1 cells, CR3 expression is enhanced by $1,25(OH)_2D_3$ (39). Despite an increase in ICAM-1 expression in response to *M. tuberculosis*, no increase in CR3 was detected at 4 h, and only a minimal increase was noted by 24 h LFA-1 failed to be upregulated. VCAM-1 is a transmembrane glycoprotein that is also a member of the immunoglobulin gene superfamily (23, 28). Although initially described as an inducible protein on endothelial cells, it has also been demonstrated in cells of dendritic morphology in follicular centers, in interfollicular zones of peripheral lymph nodes and tonsils, and in splenic and thymic macrophages (9). In contrast to ICAM-1, VCAM-1 interacts with a member of the β1 family of integrins, i.e., very late antigen 4 (VLA-4; α4 β1; CD49d/CD29). Like ICAM-1, expression of VCAM-1 is controlled by a variety of cytokines, including TNF-α, IFN-γ, and IL-1. Expression of VCAM-1 was not enhanced in THP-1 cells exposed to mycobacteria. Thus, the increased expression of ICAM-1 on THP-1 cells was relatively selective for ICAM-1 at these kinetics (4 and 24 h).

Locally produced cytokines such as IFN-γ, IL-1α, IL-1β, and TNF-α are clearly implicated in the development and persistence of granulomatous inflammation (7, 16, 22). TNF-α is abundantly secreted by alveolar macrophages of patients with tuberculosis and is highly expressed in granulomatous lesions in tuberculosis (16, 17, 29). These cytokines also induce expression of ICAM-1. The continuous production of TNF-α and other cytokines released locally by stimulated macrophages and T cells may account for the sustained expression of ICAM-1 on infected macrophages. Peripheral blood mononuclear cells elicit mRNA transcription for TNF-α, IL-1α, and IL-1β but not IFN-γ in response to antigens from *M. tuberculosis* (4, 41). In contrast, THP-1 cells secrete little IL-1α in response to IFN-γ or $1,25(OH)_2D_3$ (39). Moreover, mRNA levels of IFN-γ and TNF-α have been correlated with the degree of epidermal expression of ICAM-1 in leprotic lesions (33). Thus, we hypothesized that the upregulation of ICAM-1 by *M. tuberculosis* was mediated in a paracrine or autocrine manner by some of these cytokines. Since previous studies have failed to demonstrate production of IL-1α and IFN-γ by THP-1 cells, we suspected that predominantly TNF-α or IL-1β would participate in the increase in ICAM-1 (39). In accordance with this hypothesis, neutralizing antibodies to TNF-α were demonstrated to significantly inhibit the stimulated expression of ICAM-1 by *M. tuberculosis* or LAM. Neutralizing antibodies to IL-1 (anti IL-1α, anti-IL-1β, or both) failed to alter the expression of ICAM-1. Thus, ICAM-1 expression is a paracrine or autocrine response to the production of TNF-α elicited by *M. tuberculosis* or its cell surface component. This selective role of TNF-α is of interest since THP-1 cells are capable of expressing ICAM-1 in response to both IL-1β and IL-1α (data not shown) and suggest that an additional factor may be required.

Expression of ICAM-1 is controlled at both transcriptional and posttranscriptional levels. Regulatory elements for the ICAM-1 gene include a consensus sequence recognized by NF-κB as well as sites homologous with the AP-1/TRE, AP-2, and AP-3 sequences (40). Two transcription initiation sites are utilized differentially in cell lines, and regulation of the ICAM-1 gene involves upstream elements which differ depending on the stimulus (40). In addition, whereas TNF-α stimulates ICAM-1 gene transcription, ICAM-1 expression can also be regulated by increasing mRNA stability, the mechanism used in response to phorbol myristate acetate (8). In response to *M. tuberculosis*, THP-1 cells clearly increase mRNA for ICAM-1, suggesting that, in part, the enhanced surface expression of ICAM-1 is elicited at the transcriptional level. Since the expression of ICAM-1 is dependent on the production of TNF-α, we suspect that the increase in mRNA is most likely due to increased transcription, although posttranscriptional regulation of ICAM-1 remains a possible mechanism as well.

These data begin to define mechanisms whereby *M. tuberculosis* elicits a host response and modulates the properties of APCs via an effect on the display of adhesion molecules. Understanding this cascade of events will allow for future clinical interventions.

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REFERENCES

1. **Adams, D. O.** 1975. The structure of mononuclear phagocytes differentiating *In Vivo*. Am. J. Pathol. **80:**101–113.
2. **Adams, D. O.** 1976. The granulomatous inflammatory response. Am. J. Pathol. **84:**164–191.
3. **Altmann, D. M., N. Hogg, J. Trowdale, and D. Wilkinson.** 1989. Cotransfection of ICAM-1 and HLA-DR reconstitutes human antigen-presenting cell function in mouse L cells. Nature (London) **338:**512–514.
4. **Barnes, P. F., D. Chatterjee, J. S. Abrams, S. Lu, E. Wang, M. Yamamura, P. Brennan, and R. L. Modlin.** 1992. Cytokine production induced by *Mycobacterium tuberculosis* lipoarabinomannan. J. Immunol. **149:**541–547.
5. **Barnstable, C. J., W. F. Bodmer, G. Brown, G. Galfre, C. Milstein, A. F. Williams, and A. Ziegler.** 1978. Production of monoclonal antibodies to group A erythrocytes, HLA and other human cell surface antigens—new tools for genetic analysis. Cell **14:**9.
6. **Bloom, B. R., and C. J. L. Murray.** 1993. Tuberculosis: commentary on a reemergent killer. Science **257:**1055–1064.

7. **Chensue, S. W., I. G. Otterness, G. I. Higashi, C. S. Forsch, and S. L. Kunkel.** 1989. Monokine production by hypersensitivity (*Schistosoma mansoni* egg) and foreign body (sephadex bead)-type granuloma macrophages. J. Immunol. **142:**1281–1286.
8. **Cybulsky, M., J. W. Fries, A. J. Williams, P. Sultan, R. Eddy, M. Byers, T. Shows, M. A. Cimbrone, and T. Collins.** 1991. Gene structure, chromosomal location, and basis for alternative mRNA splicing of the human VCAM1 gene. Proc. Natl. Acad. Sci. USA **88:**7859–7863.
9. **Cybulsky, M. I., J. W. Fries, A. J. Williams, P. Sultan, V. M. Davis, M. A. Gimbrone, and T. Collins.** 1991. Alternative splicing of human VCAM-1 in activated vascular endothelium. Am. J. Pathol. **138:**815–820.
10. **Devergne, O., D. Emilie, M. Peuchmaur, M. C. Crevon, M. F. D'Agay, and P. Galanaud.** 1992. Production of cytokines in sarcoid lymph nodes: preferential expression of interleukin-1 beta and interferon-gamma genes. Hum. Pathol. **23:**317–323.
11. **Dustin, M. L., R. Rothlein, A. K. Bhan, C. A. Dinarello, and T. A. Springer.** 1986. Induction by IL-1 and interferon-gamma: tissue distribution, biochemistry, and function of a natural adherence molecule (ICAM-1). J. Immunol. **137:**245–254.
12. **Eddy, A., S. L. Newman, F. Cosio, T. LeBien, and A. Michael.** 1984. The distribution of the CR3 receptor on human cells and tissue as revealed by a monoclonal antibody. Clin. Immunol. Immunopathol. **31:**371–389.
13. **Hance, A. J., S. Douches, R. J. Winchester, V. J. Ferrans, and R. G. Crystal.** 1985. Characterization of mononuclear phagocyte subpopulations in the human lung by using monoclonal antibodies: changes in alveolar macrophage phenotype associated with pulmonary sarcoidosis. J. Immunol. **134:**284–292.
14. **Hunter, S. W., and P. J. Brennan.** 1990. Evidence for the presence of a phosphatidylinositol anchor on the lipoarabinomannan and the lipomannan of *Mycobacterium tuberculosis*. J. Biol. Chem. **265:**9272–9279.
15. **Hunter, S. W., H. Gaylord, and P. J. Brennan.** 1986. Structure and antigenicity of the phosphorylated lipopolysaccharide antigens from the leprosy and tubercle bacilli. J. Biol. Chem. **261:**12345–12351.
16. **Kindler, V., A.-P. Sappino, G. E. Grau, P.-F. Piguet, and P. Vassalli.** 1989. The inducing role of tumor necrosis factor in the development of bactericidal granulomas during BCG infection. Cell **56:**731–740.
17. **Kunkel, S. L., S. W. Chensue, R. M. Strieter, J. P. Lynch, and D. G. Remick.** 1989. Cellular and molecular aspects of granulomatous inflammation. Am. J. Respir. Cell Mol. Biol. **1:**439–447.
18. **Marlin, S. D., and T. A. Springer.** 1987. Purified intercellular adhesion molecule-1 (ICAM-1) is a ligand for lymphocyte function-associated antigen 1 (LFA-1). Cell **51:**813–819.
19. **Mellis, M., M. Gjomarkaj, E. Pace, G. Malizia, and M. Spatafora.** 1991. Increased expression of leukocyte function associated antigen-1 (LFA-1) and intercellular adhesion molecule-1 (ICAM-1) by alveolar macrophages of patients with pulmonary sarcoidosis. Chest **100:**910–916.
20. **Moreno, C., A. Mehlert, and J. Lamb.** 1988. The inhibitory effects of mycobacterial lipoarabinomannan and polysaccharides upon polyclonal and monoclonal human T cell proliferation. Clin. Exp. Immunol. **74:**206–210.
21. **Most, J., H. P. Neumayer, and M. P. Dierich.** 1990. Cytokine-induced generation of multinucleated giant cells *in vitro* requires interferon-γ and expression of LFA-1. Eur. J. Immunol. **20:**1661–1667.
22. **Murray, H. W., K. E. Squires, C. D. Miralles, M. Y. Stoeckle, A. M. Granger, A. Granelli-Piperno, and C. Bogdan.** 1992. Acquired resistance and granuloma formation in experimental visceral leishmaniasis. J. Immunol. **148:**1858–1863.
23. **Osborn, L., C. Hession, R. Tizard, C. Vassalio, S. Luhowskyj, G. Chi-Rossi, and R. Lobb.** 1989. Direct expression cloning of vascular cell adhesion molecule 1, a cytokine-induced endothelial protein that binds lymphocytes. Cell **59:**1203–1211.
24. **Pancholi, P., A. Mirza, N. Bhardwaj, and R. M. Steinman.** 1993. Sequestration from immune $CD4^+$ T cells of mycobacteria growing in human macrophages. Science **260:**984–986.
25. **Philips, M. R., J. P. Buyon, R. Winchester, G. Weissmann, and S. B. Abramson.** 1988. Up-regulation of the iC3b receptor (CR3) is neither necessary nor sufficient to promote neutrophil aggregation. J. Clin. Invest. **82:**495–501.
26. **Postlethwaite, A. E., B. K. Jackson, E. H. Beachey, and A. H. Kang.** 1982. Formation of multinucleated giant cells from human monocyte precursors. J. Exp. Med. **155:**168–178.
27. **Prinzis, S., D. Chatterjee, and P. J. Brennan.** 1993. Structure and antigenicity of lipoarabinomannan from *Mycobacterium bovis* BCG. J. Gen. Microbiol. **139:**2649–2658.
28. **Rice, G. E., J. M. Munro, C. Corless, and M. P. Bevilacqua.** 1990. Inducible cell adhesion molecule 110 is an endothelial receptor for lymphocytes. A CD11/CD18-independent adhesion mechanism. J. Exp. Med. **171:**1369–1374.
29. **Rook, G. A. W., J. Taverne, C. Leveton, and J. Steele.** 1987. The role of gamma-interferon, vitamin D_3 metabolites and tumour necrosis factor in the pathogenesis of tuberculosis. Immunology **62:**229–234.
30. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
31. **Schlesinger, L. S., D. G. Bellinger-Kawahara, N. R. Payne, and M. A. Horwitz.** 1990. Phagocytosis of *Mycobacterium tuberculosis* is mediated by human monocyte complement receptors and complement component C3. J. Immunol. **144:**2771–2780.
32. **Simmons, D., M. W. Makgoba, and B. Seed.** 1988. ICAM, an adhesion ligand of LFA-1, is homologous to the neural cell adhesion molecule NCAM. Nature (London) **331:**624–627.
33. **Spector, W. G., N. Reichhold, and G. B. Ryan.** 1970. Degradation of granuloma-inducing micro-organisms by macrophages. J. Pathol. **101:**339–354.
34. **Springer, T. A.** 1990. Adhesion receptors of the immune system. Nature (London) **346:**425–434.
35. **Staunton, D. E., S. D. Marlin, C. Stratowa, M. L. Dustin, and T. A. Springer.** 1988. Primary structure of ICAM-1 demonstrates interaction between members of the immunoglobulin and integrin supergene families. Cell **52:**925–933.
36. **Sullivan, L., S. Sano, C. Pirmez, P. Salgame, C. Mueller, F. Hofman, K. Uyemura, T. H. Rea, B. R. Bloom, and R. L. Modlin.** 1991. Expression of adhesion molecules in leprosy lesions. Infect. Immun. **59:**4154–4160.
37. **Van Seventer, G. A., Y. Shimizu, K. J. Horgan, and S. Shaw.** 1990. The LFA-1 ligand ICAM-1 provides an important costimulatory signal for T cell receptor-mediated activation of resting T cells. J. Immunol. **144:**4579–4586.
38. **Vedder, N. B., and J. M. Harlan.** 1988. Increased surface expression of CD11/CD18 (Mac-1) is not required for stimulated neutrophil adherence to cultured endothelium. J. Clin. Invest. **81:**676–682.
39. **Vey, E., J.-H. Zhang, and J.-M. Dayer.** 1992. IFN-g and $1,25(OH)_2D_3$ induce on THP-1 cells distinct patterns of cell surface antigen expression, cytokine production, and responsiveness to contact with activated T cells. J. Immunol. **149:**2040–2046.
40. **Voraberger, G., R. Schafer, and C. Stratowa.** 1991. Cloning of the human gene for intercellular adhesion molecule 1 and analysis of its 5′-regulatory region. J. Immunol. **147:**2777–2786.
41. **Zhang, Y., M. Doerfler, T. C. Lee, B. Guillemin, and W. N. Rom.** 1993. Mechanisms of stimulation of interleukin-1β and tumor necrosis factor-α by *Mycobacterium tuberculosis* components. J. Clin. Invest. **91:**2076–2083.
42. **Zhang, Y., and W. N. Rom.** 1993. Regulation of the interleukin-1β gene by mycobacterial components and lipopolysaccharade is mediated by two NF-IL6 modifs. Mol. Cell. Biol. **13:**3831–3837.