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Pathogenic *Mycobacterium tuberculosis* Evades Apoptosis of Host Macrophages by Release of TNF-R2, Resulting in Inactivation of TNF-α[1]

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Infection by *Mycobacterium tuberculosis* (MTB) induces human alveolar macrophage (AMϕ) apoptosis by a TNF-α-dependent mechanism. The apoptotic response is postulated to be a defense mechanism, limiting the growth of this intracellular pathogen. Consistent with that model, recent studies showed that the virulent MTB strain H37Rv induces substantially less AMϕ apoptosis than the attenuated strain H37Ra. We now report that AMϕ infection with either H37Rv or H37Ra induces comparable levels of TNF-α measured by ELISA but that TNF-α bioactivity is reduced in supernatants of H37Rv-infected AMϕ. Differential release of soluble TNFR2 (sTNFR2), with formation of inactive TNF-α-TNFR2 complexes accounted for the difference in TNF-α bioactivity in these cultures. Release of sTNFR2 by H37Rv-infected AMϕ was IL-10 dependent since it was inhibited by neutralizing anti-IL-10 Ab. Thus, the effect of TNF-α produced by AMϕ following infection can be modulated by virulent MTB, using IL-10 as an upstream mediator. *The Journal of Immunology*, 1998, 161: 2636–2641.

Tuberculosis is the predominant cause of human mortality from infectious agents, with a death rate of 3 million individuals per year worldwide. The number of new cases for the decade 1990 to 2000 is estimated at 90 million (1). Moreover, the severity of this global medical problem has worsened in recent years due to emergence of drug-resistant *Mycobacterium tuberculosis* (MTB)[3] strains (2).

The primary route of infection with MTB is via the lung. In the alveolus, AMϕ are both the primary host cell for MTB and the first line of defense for the infected individual. Following infection of AMϕ by MTB, a variety of cytokines are induced that orchestrate containment of the infection. Among these cytokines, TNF-α is involved in the protective immune response in mice (3). Production of TNF-α is also required for the induction of AMϕ apoptosis in response to inoculation with MTB (4). Comparison in mice of different MTB strains correlated resistance against tuberculosis in vivo with susceptibility of infected Mϕ to apoptosis measured in vitro and in vivo (5). Apoptosis of human monocytes was shown to limit the growth of *Mycobacterium bovis* bacille Calmette-Guérin (BCG) (6) and *Mycobacterium avium* (7) in vitro. The latter studies shed light on the outcomes of host Mϕ apoptosis. First, apoptosis of the infected cells contains the mycobacteria, likely preventing spread of infection in vivo (8). Secondly, the process of host Mϕ apoptosis prevents growth of the mycobacteria (7). Indeed, H37Rv, a well-characterized pathogenic MTB strain, was found to induce significantly less host Mϕ apoptosis than the closely related attenuated strain H37Ra (4), suggesting that abrogation of host Mϕ apoptosis might be a virulence-associated phenotype of MTB strains.

The present study was performed to delineate the mechanism by which virulent MTB interferes with the host Mϕ apoptotic response to infection. We identify inactivation of the cytokine TNF-α as a key event leading to decreased host Mϕ apoptosis. We also describe proximal events: the induction of IL-10 production by virulent MTB leading to release of soluble TNFR2 (sTNFR2) from the AMϕ and inactivation of TNF-α by complex formation with sTNFR2. Together, these events comprise an apparent virulence mechanism operative in virulent and not in attenuated MTB.

Materials and Methods

Alveolar macrophages

AMϕ were obtained from bronchoalveolar lavage fluid of healthy nonsmoking volunteers with their informed consent, at Boston University Medical Center or Beth Israel-Deaconess Medical Center, under human study protocols approved by the respective institutional review boards. Lavage fluids and cells were filtrated through a single layer of sterile gauze, centrifuged ($450 \times g$, 100 min), and the cell pellet was suspended in RPMI 1640 medium (Sigma, St. Louis, MO) with 10% FCS, supplemented with cefotaxime (50 μg/ml, Sigma) or penicillin and streptomycin (100 μg/ml each, Sigma). Cells were plated on 24-well plates (Costar, Cambridge, MA) (5×10^5 cells/well) directly on the bottom of wells or on round glass coverslips for staining purposes. Nonadherent cells were removed by washing at 24 h, and fresh antibiotic-free medium was added. Cell morphology and differential counts were determined with light microscopy following cytocentrifugation and modified Giemsa staining (Dif-Quik, Sigma). Viability of adherent alveolar macrophages was examined in representative samples by trypan blue dye exclusion.

Mycobacterium tuberculosis

Stock cultures of MTB H37Ra and H37Rv (ATCC 25177 and ATCC 25618; American Type Culture Collection (ATCC), Manassas, VA) were stored frozen in 1-ml aliquots. Before inoculation of AMϕ, the thawed mycobacteria were vortexed, sonicated for 15 s at 500 W, and allowed to stand for 10 min. The upper 500 μl of the solution were used for experiments. Dilution of these dispersed mycobacterial preparations were made to yield a multiplicity of infection 1 to 5 organisms/cell. For each experiment, the adequacy of dispersion and the multiplicity of infection were

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[3] Abbreviations used in this paper: MTB, *Mycobacterium tuberculosis*; AMϕ, alveolar macrophages; sTNFR, soluble TNFR; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP-nick end labeling.

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checked by acid-fast staining of the washed cells 4 h after inoculation. The rate of internalization and intracellular growth during 5 days after infection was similar for H37Ra and H37Rv as determined by electron microscopy, plate counting, and BACTEC (9).

Reagents, Abs, and cytokines

Human rIL-10, polyclonal anti-human IL-10, and polyclonal anti-human TNF-α Abs were obtained from Endogen (Cambridge, MA). Human rTNF-α was purchased from Genzyme (Cambridge, MA). Human rsTNFR2 and monoclonal anti-human sTNFR2 Ab were purchased from R&D Systems (Minneapolis, MN).

Measurement of TNF-α, IL-10, sTNFR, and TNF-α-sTNFR2 complexes

AMϕ were cultured and challenged with MTB as described above, in triplicate cultures. Supernatants were harvested at the times indicated and passed through 0.22-μm pore-size filter (Costar). The level of immunoreactive TNF-α and IL-10 was determined by ELISA using matched Ab pairs and cytokine standards (Genzyme and Endogen, respectively). The amount of sTNFR was estimated using commercial ELISA kits (R&D Systems) according to manufacturer's instructions.

TNF bioactivity was assessed by measuring cytotoxicity on L929 cells (10). In brief, L929 cell monolayers, cultured for 48 h on flat-bottom 96-well plates (0.2×10^5 cells/well), were overlaid with twofold serial dilutions of AMϕ supernatants in RPMI 1640 + 10% FCS supplemented with actinomycin D to a final concentration of 1 μg/ml.

After incubation at 37°C for 24 h the cells were washed with saline, stained with 0.05% crystal violet in 20% ethanol, and allowed to dry overnight. The stain was eluted from the cells with 100% methanol and read with a microtiter plate reader at an absorbance of 570 nm. Estimates of the concentrations of bioactive TNF in the supernatants were obtained by comparison with calibration curves established with a rTNF-α standard. TNF bioactivity in selected AMϕ supernatant samples was inhibited by anti-TNF-α Ab, but not by control rabbit IgG, indicating that the cytotoxic activity in AMϕ supernatants represents TNF-α.

TNF-α bound to TNFR2 was detected in supernatants of MTB-infected AMϕ by a mixed Ab ELISA assay. The supernatants were added to 96-well polystyrene microtiter plates coated with a murine monoclonal Ab against sTNFR2 (part of TNFR2 ELISA; R&D Systems), and, after 2 h at room temperature, the plates were washed and incubated for 1 h at room temperature with biotinylated rabbit anti-human TNF-α Ab (0.2 μg/ml, Genzyme). After washing, peroxidase-labeled streptavidin (Genzyme) was added. Peroxidase activity was determined by the addition of 3,3′,5,5′-tetramethylbenzidine (Vector Laboratories, Burlingame, CA), and photospectrometry (450 nm) was performed using a microplate reader. Since the stability of TNF-α-sTNFR2 complexes strongly depends on the experimental conditions (11), calibrated dilutions of rTNF-α captured by immobilized anti-TNF-α Ab were used as an internal standard for the comparative measurements of TNFR2-bound TNF-α.

In situ DNA fragmentation analysis

DNA fragmentation was determined by the in situ TUNEL technique (12). The method is based on the specific binding of terminal deoxynucleotidyl transferase to 3′-OH ends of DNA. AMϕ adherent to glass coverslips were fixed with 4% formaldehyde in PBS, rinsed with double distilled water, immersed in 0.5 U/μl of terminal transferase and 1 mM Biotin-16-dUTP in reaction buffer (Boehringer Mannheim, Indianapolis, IN) and incubated in a humid atmosphere at 37°C for 60 min. The reaction was terminated by transferring the coverslips to a solution of 300 mM NaCl, 30 mM sodium citrate, pH 7.2, for 15 min at room temperature. The coverslips were then rinsed with water, blocked with 2% BSA in PBS for 10 min, rinsed again with water, immersed in PBS for 5 min at room temperature, and immersed in avidin peroxidase (1:10) for 30 min at 37°C. The cells were then stained with 1% 3-amino, 9-ethyl carbazole (Sigma) in dimethyl formamide (Sigma) diluted 1:20 with 50 mM acetate buffer, pH 4.5, containing 0.018% hydrogen peroxide for 20 min. Slides of experimental and control cells subjected to in situ TUNEL were analyzed by light microscopy in a blinded fashion. A total of 600 cells per slide were evaluated for the presence of red-brown nuclear staining.

Statistical analysis

Results are expressed as mean ± SD. Statistical analysis was performed employing SigmaStat Statistical Software (Jandel Scientific, San Rafael, CA), using the ANOVA with correction for multiple comparisons for analyses for multiple comparisons and paired *t* test for analysis of the data on IL-10 production by AMϕ from individual donors.

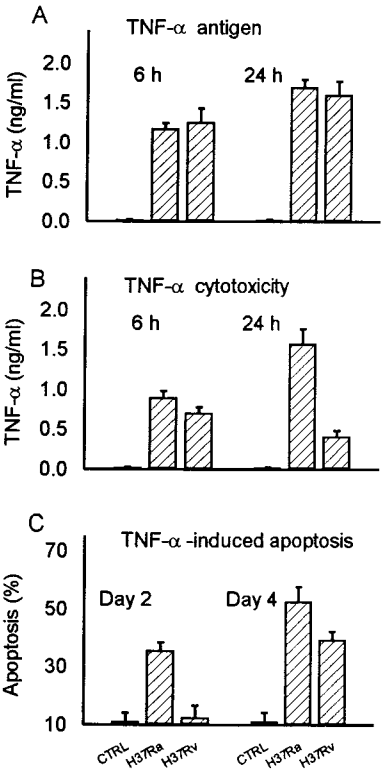

FIGURE 1. Quantitation of TFN-α Ag (*A*), TFN-α bioactivity (*B*), and apoptosis (*C*) in cultures of AMϕ infected with virulent and attenuated MTB. Shown are mean ± SD of three representative experiments. Bars labeled "CTRL" represent uninfected AMϕ. *A*, AMϕ were infected with H37Rv or H37Ra, and culture supernatants were assayed at 6 and 24 h for TNF-α Ag by ELISA ($p > 0.4$ for 6 and 24 h). *B*, Culture supernatants at 6 and 24 h after infection were assayed for TNF-α bioactivity by measuring cytotoxicity for L929 mouse fibroblasts. Dilutions of rTNF-α were used as internal standard. The differences in TNF-α cytotoxicity between H37Ra- and H37Rv-infected cultures were statistically significant at 6 h and 24 h after infection ($p < 0.05$). *C*, Apoptosis of host AMϕ at 2 and 4 days after infection measured by TUNEL assay and shown as percent of apoptotic cells. The differences in % apoptosis between H37Ra- and H37Rv-infected cultures were statistically significant at day 2 and day 4 ($p < 0.05$).

Results

Inactivation of TNF-α in AMϕ cultures infected with virulent MTB

Using AMϕ from healthy donors, we measured TNF-α levels by ELISA in culture supernatants after infection with the virulent MTB strain H37Rv or the attenuated strain H37Ra at 6 and 24 h. The immunoreactive TNF-α levels increased over time in both infections and were not significantly different (Fig. 1*A*). In contrast, TNF-α bioactivity measured at the same time by assessing L929 fibroblast cytotoxicity was lower in H37Rv-infected AMϕ cultures than in H37Ra-infected AMϕ cultures (Fig. 1*B*). Twenty-four hours after infection, the level of TNF-α bioactivity was 412 ± 75 pg/ml in supernatants from H37Rv-infected AMϕ vs 1573 ± 189 pg/ml in supernatants from H37Ra-infected cells ($n = 3$, $p < 0.05$). Control experiments confirmed that the measurements of bioactivity reflected TNF-α activity since the addition of anti-TNF-α Ab (5 μg/ml) completely inhibited L929 fibroblast cytotoxicity in both cultures, whereas irrelevant Ab had no effect (data not shown). Consistent with the previous report that apoptosis in MTB-infected AMϕ resulted from TNF-α signaling (4), the level of AMϕ apoptosis mirrored the pattern of TNF-α bioactivity

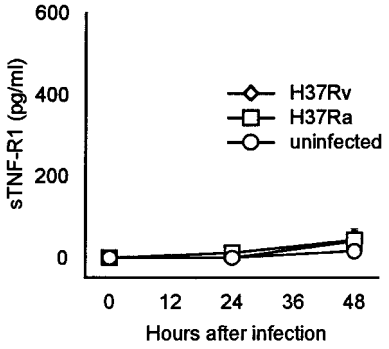

FIGURE 2. Levels of soluble TNFR1 in supernatants from AMϕ inoculated with H37Rv, H37Ra, or uninfected AMϕ and harvested at the indicated times. Shown are mean ± SD of triplicate values from one representative experiment of three.

(Fig. 1*C*). In contrast to the measurement of TNF-α by ELISA or by using a cytotoxicity assay, apoptosis was determined at day 2 and 4 because optimal manifestation of apoptosis as measured by the TUNEL technique (12) was found to occur several days after inoculation of the Mϕ with mycobacteria (13). Together, these data suggest that the reduced apoptotic response of AMϕ to infection by the virulent MTB strain H37Rv was due to the coinduction of a TNF-α-neutralizing activity.

Inactivation of TNF-α in AMϕ infected with virulent MTB correlates with release of sTNFR2

To further dissect the events leading to attenuation of host Mϕ apoptosis, we tested whether release of sTNFR occurred following MTB infection as has been described in other systems (14–17). Although TNFR1 has been implicated as dominant in TNF-α-induced apoptosis (18), levels of sTNFR1 measured by ELISA were not significantly increased following MTB infection, and the levels did not differ with infection by the attenuated or virulent strain (at 48 h: $n = 7$, $p = 0.75$, Fig. 2). On the other hand, at 24 h the increase in sTNFR2 concentration in supernatants of H37Rv-infected AMϕ was six times higher than in culture supernatants of AMϕ infected with H37Ra (Fig. 3*B*). The difference in the TNFR2 levels was statistically significant at 6, 8, 24, 32, 48, and 96 h after infection ($n = 3$, $p < 0.05$ for all time points).

sTNFR2 inactivates TNF-α

To establish a causal relationship between levels of sTNFR2 and TNF-α bioactivity, we added recombinant sTNFR2 to cultures of AMϕ infected with the attenuated MTB strain H37Ra and measured the bioactivity of TNF-α. Bioactivity measured by the L929 cell cytotoxicity assay was reduced by addition of exogenous sTNFR2 in a dose-dependent manner, and the reduction of cytotoxicity was statistically significant in cultures treated with 10 and 50 ng/ml of rTNFR2 compared with untreated controls ($n = 3$, $p < 0.05$; a representative experiment is shown in Fig. 4*A*). Apoptosis of host AMϕ measured in the same cultures was also inhibited by sTNFR2 (Fig. 4*B*), and the amount of apoptosis was comparable to the level of TNF-α bioactivity. Addition of an irrelevant protein (bovine albumin) had no effect on either bioactivity.

The presence of TNF-α bound to sTNFR2 in supernatants of MTB-infected AMϕ cultures was examined by a mixed ELISA assay. The TNF-α-sTNFR2 complexes were captured by immobilized Abs to sTNFR2 and quantified using Abs to TNF-α. Detectable levels of TNF-α-sTNFR2 complexes were found in cultures of AMϕ infected with both MTB strains and were not found in uninfected AMϕ. The levels of complexes in supernatants of AMϕ infected with virulent H37Rv were about three times higher than in Mϕ infected with the attenuated strain H37Ra (Fig. 5).

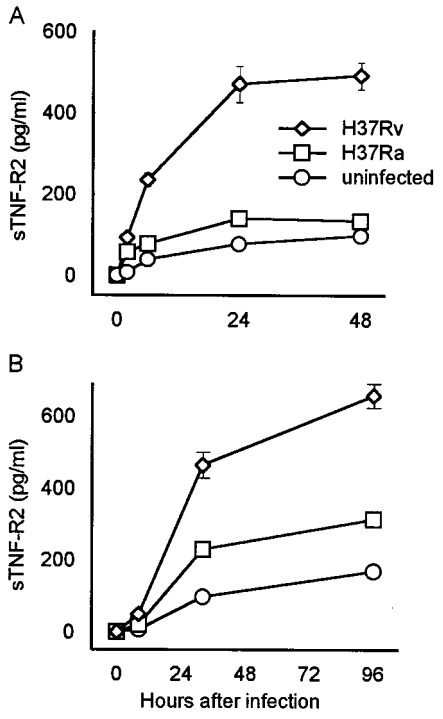

FIGURE 3. Levels of soluble TNFR2 in supernatants from AMϕ inoculated with H37Rv, H37Ra, or uninfected AMϕ and harvested at the indicated times. The experiment shown in *A* displays various early time points. The experiment shown in *B* covers a wider time range including a 96-h time point. For *A* and *B*, shown are mean + SD of triplicate values from one representative experiment of three. The differences in TNFR2 release by AMϕ infected with H37Ra and H37Rv were statistically significant at 8, 32, and 96 h (*A*) and at 6, 24, and 48 h ($p < 0.05$).

IL-10 down-regulates AMϕ apoptosis

Previous studies indicate that IL-10 has an anti-apoptotic function (19–21) and induces the release of sTNFR2 from several cell types (22, 23). To identify mechanisms regulating TNFR shedding from MTB-infected AMϕ, we tested the effects of exogenous IL-10 and of neutralization of endogenous IL-10. Addition of anti-IL-10 Ab (10 μg/ml) to AMϕ cultures infected with the virulent MTB strain H37Rv significantly reduced the release of sTNFR2 ($n = 3$, $p < 0.05$; Fig. 6*A*) and increased Mϕ apoptosis ($n = 6$, $p < 0.05$, Fig. 6*B*). Conversely, addition of exogenous rIL-10 to AMϕ cultures infected with the attenuated H37Ra decreased apoptosis of the host Mϕ in a dose-dependent manner (Fig. 6*C*). Reduction of apoptosis was statistically significant after addition of 5, 10, and 20 ng/ml IL-10 ($n = 6$, $p < 0.05$) to the infected cultures. Treatment of uninfected AMϕ with IL-10 (10 ng/ml) was also found to increase sTNFR2 shedding from 56 ± 8 pg/ml to 160 ± 26 pg/ml at 6 h, and from 121 ± 38 pg/ml to 248 ± 45 pg/ml at 48 h of culture, and this difference was statistically significant ($n = 3$, $p < 0.05$). These findings indicate that the modulation of MTB-induced Mϕ apoptosis by sTNFR2 release may be regulated by IL-10 and suggest that the virulent strain H37Rv and the attenuated strain H37Ra differ in the induction of endogenous IL-10 secretion.

To demonstrate a role of IL-10 in modulation and induction of AMϕ apoptosis by virulent and attenuated MTB strains, we measured IL-10 levels by ELISA in supernatants of AMϕ cultures

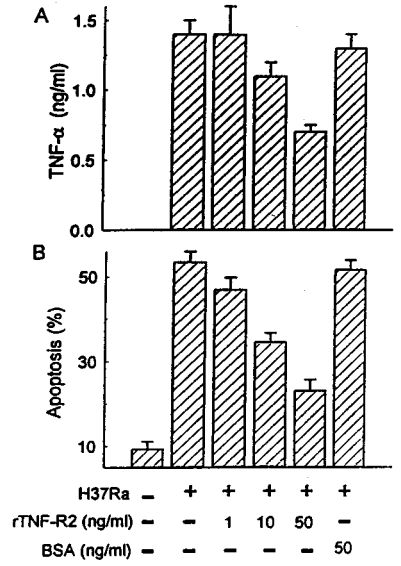

FIGURE 4. Addition of srTNFR2 to AMϕ cultures inoculated with attenuated MTB decreases cytotoxic activity of the supernatants (*A*) and apoptosis of host AMϕ (*B*) in a dose-dependent fashion. One, 10, and 50 μg/ml of srTNFR2 were added to AMϕ cultures at the time of infection with H37Ra. Results from triplicate values of a typical experiment are shown. *A*, Cytotoxicity expressed as ng/ml TNF-α was measured at 24 h by assaying supernatants with L929 target cells. The reduction of cytotoxicity was statistically significant in cultures treated with 10 and 50 ng/ml rTNFR2 ($p < 0.05$). *B*, Apoptosis of host AMϕ was measured at day 4 by the TUNEL assay. The reduction of % apoptosis was statistically significant in cultures treated with 1, 10, and 50 ng/ml rTNFR2 ($p < 0.05$).

inoculated with H37Rv and H37Ra. Uninfected Mϕ from all donors produced negligible levels of IL-10 (3 ± 1 pg/ml, $n = 12$). In contrast, H37Ra induced 58 ± 11 pg/ml, and H37Rv 103 ± 18 pg/ml IL-10 ($n = 12$, $p = 0.0092$, Fig. 7). Thus, the decreased induction of AMϕ apoptosis by the virulent MTB strain H37Rv correlates directly with increased induction of IL-10.

Discussion

Infection with MTB is acquired via the lung. AMϕ are the first line of defense against this infection, but mycobacteria can persist and

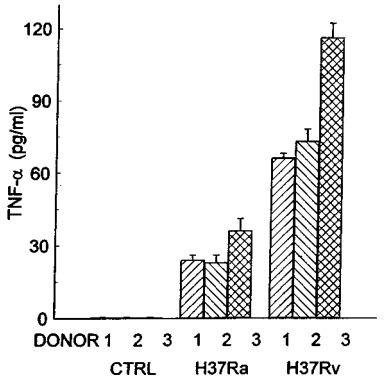

FIGURE 5. Concentration of TNF-α bound to sTNFR2 (representative for TNF-α-sTNFR2 complexes) in cultures of uninfected AMϕ (CTRL) and AMϕ infected with H37Ra or H37Rv. TNF-α-sTNFR2 complexes were assayed by mixed Ab ELISA (see *Materials and Methods*) of supernatants harvested 24 h after infection. The differences in the levels of TNF-α bound to TNFR2 in H37Ra- and H37Rv-infected cultures were statistically significant in all three experiments ($p < 0.05$ for all experiments).

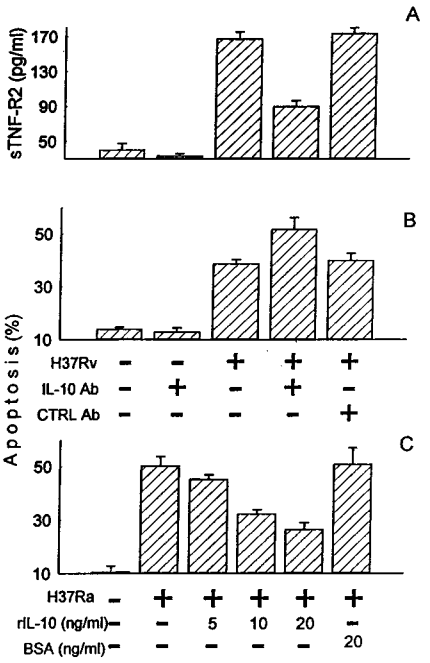

FIGURE 6. Modulation of TNFR2 release (*A*) and apoptosis of host AMϕ (*B, C*) in cell cultures infected with the virulent strain H37Rv after addition of anti-IL-10 Ab (10 μg/ml) (*A, B*) and in cultures infected with attenuated H37Ra after addition of varying amounts of rIL-10 (*C*). TNFR2 was assayed at 24 h and AMϕ apoptosis was assessed at 4 days. Shown are mean ± SD of triplicate values from one representative experiment. The reduction of TNFR2 release (*A*) and the increase of % apoptosis (*B*) after addition of anti-IL-10 Ab is statistically significant ($p < 0.05$). Reduction of % apoptosis after addition of 5, 10, and 20 ng/ml IL-10 is also statistically significant ($p < 0.05$).

replicate within infected Mϕ. Mϕ respond to MTB by producing TNF-α, a pleiotropic cytokine critical for the protective immune response to tuberculosis (3), which elicits a wide spectrum of cellular responses including fever, tissue injury, and Mϕ activation (24). Recent studies showed that TNF-α induces also apoptosis (25). Host cell apoptosis is a defense strategy to limit the growth of certain intracellular pathogens, including viruses (26, 27). In the case of mycobacterial infection, this response has been shown to

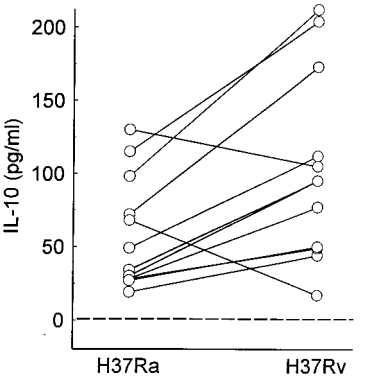

FIGURE 7. IL-10 levels in supernatants of AMϕ from 12 healthy individuals infected with H37Ra or H37Rv. IL-10 was measured by ELISA in supernatants harvested after 24 h. Values for cultures from individual donors are connected with a line. The mean of IL-10 concentration for all donors in supernatants from uninfected AMϕ was 3 ± 1 pg/ml IL-10; this value is shown by the dashed line.

sequester bacilli within apoptotic cells and to restrict their replication directly (6), and by activation of uninfected Mϕ (7).

To investigate the possibility that virulent MTB might suppress host Mϕ apoptosis, we performed in vitro infection of normal human AMϕ with a virulent and an attenuated strain of MTB. Our data show that inactivation of TNF-α and subsequent attenuation of apoptosis by the virulent strain H37Rv occurs by a unique mechanism. Compared with the attenuated strain H37Ra, H37Rv induces a quantitatively greater release of IL-10 from infected Mϕ. The enhanced IL-10 response causes increased accumulation of sTNFR2 in the supernatants, resulting in the inactivation of TNF-α by soluble receptor-ligand complex formation. sTNFR2 was found to inactivate TNF-α, since we showed that TNF-α-dependent cytotoxicity and apoptosis are significantly reduced by addition of rTNFR2 to H37Ra-infected AMϕ cultures (Fig. 4). We have also attempted to demonstrate neutralization of endogenous sTNFR2 with anti-TNFR2 Abs and subsequent enhancement of apoptosis. In these experiments 10 μg/ml anti-TNFR2 Abs were added to AMϕ cultures at the time of inoculation with H37Rv. H37Rv-infected AMϕ cultures to which no anti-TNFR2 Abs were added showed 39 $\pm$ 5% apoptosis at day 4 of culture in comparison with uninfected cultures (9 $\pm$ 1% apoptosis, $p < 0.05$). In contrast, in the presence of anti-TNFR2 Abs, 49 $\pm$ 3% apoptosis ($n = 3$, $p < 0.05$) was observed. These experiments suggest that endogenous sTNFR2 is able to neutralize TNF-α, but do not rule out a stimulatory effect of these Abs on cell-associated TNFR2 and are therefore difficult to interpret. Furthermore, another way to prove involvement of sTNFR2 in TNF-α neutralization, specific blocking of TNFR2 release by metalloproteinase inhibitors, cannot be performed, because such inhibitors also affect processing of TNF-α (28).

The release of TNFR2 from the AMϕ, which differs significantly in H37Rv- and H37Ra-infected AMϕ, comprises an apparent virulence mechanism operative in pathogenic MTB.

This study indicates that a virulent MTB strain can modulate TNF-α activity by IL-10-dependent TNFR2 shedding. IL-10 has also been reported to prevent apoptosis of Mϕ during *Salmonella* infection (19), to prevent apoptosis of T cells in infectious mononucleosis (21), and to prevent apoptosis of germinal center B cells (20). In addition to attenuating apoptosis by increasing the release of sTNFR2, IL-10 might also down-regulate apoptosis by inhibiting IFN-γ and TNF-α production (29), and by down-regulating surface TNFR expression (23).

sTNFR2 release from the AMϕ caused by IL-10 has been found to depend on the action of metalloproteinases (28). IL-10 has been found to down-regulate (30), to activate (31), and to have no effect (32) on the activity of metalloproteinases. In future studies we will examine the role of proteolytic enzymes in the IL-10-dependent release of TNFR2 from AMϕ.

The cytokine responses of primary human AMϕ to a MTB challenge typically demonstrate a high degree of individual variation, and a high IL-10 response might represent a host susceptibility factor to tuberculosis. On the other hand, the two individuals (of 12 individuals) whose AMϕ did not respond to H37Rv inoculation with increased IL-10 levels (Fig. 7) might be part of a population with innate resistance to MTB. We are currently investigating this possibility.

AMϕ apoptosis is induced by the action of TNF-α. TNF-α elicits cellular responses through its interaction with two distinct receptors, the 55-kDa TNFR1 and the 75-kDa TNFR2 (33). A distinctive difference between these two TNFR, which are both members of the TNF receptor superfamily including CD30, CD40, CD27, and Fas (34), is the presence of a death domain (DED) in TNFR1. Stimulation of TNFR1 results in activation of the apoptotic protease cascade (35, 36) leading to apoptosis. Inactivation of TNF-α by formation of a complex with sTNFR2 abrogates the induction of apoptosis upstream of the death-signaling cascade by elimination of the inducing cytokine signal. Related strategies are used by human herpes virus 4, which encodes a viral IL-10 homologue (37), parainfluenza virus, which induces IL-10 (38), and pox virus, which encodes a sTNFR1-like molecule (39).

sTNFR are naturally occurring inhibitors of TNF-α, which are normally present in the blood and urine (14–17). Serum concentrations of sTNFR increase significantly in a variety of disease states including tuberculosis and HIV-1 infection (40, 41). sTNFR2 were reported to be more prone to being released from AMϕ than TNFR1 (42, 43), which is in agreement with our finding that MTB infection induced shedding of TNFR2, but not TNFR1, from AMϕ. sTNFR2 are also more abundant than TNFR1 in the serum of patients with cancer and in the serum and synovial fluid of patients with rheumatoid arthritis (44, 45). This evidence suggests that sTNFR2 may be an important regulator of TNF-α activity in vivo. Our present investigations indicate that a virulent MTB strain can modulate TNF-α activity by IL-10-dependent shedding of sTNFR2.

The mycobacterial determinants that effect the unique capacity of virulent MTB to manipulate host TNF-α responses have yet to be defined. Identifying the basis of this multistep mechanism should provide further insight to the pathogenesis of human tuberculosis and may suggest novel approaches to therapy.

Note. A study describing reduction of TNF-α biological activity in supernatants from murine Mϕ infected with virulent *M. avium* strains with a possible role for sTNFR2 has recently come to our attention (46).

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