Tumor Necrosis Factor Blockers Influence Macrophage Responses to *Mycobacterium tuberculosis*

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Tumor necrosis factor (TNF)–α is a proinflammatory cytokine that mediates inflammation in response to various pathogens, including *Mycobacterium tuberculosis,* but is also a key factor in the pathogenesis of rheumatoid arthritis and other autoimmune diseases. Three TNF-α–suppressing drugs have been approved to treat selected autoimmune diseases; 2 are monoclonal antibodies against TNF-α (adalimumab and infliximab), and the other is a soluble TNF receptor/Fc fusion protein (etanercept). TNF blockers have been shown to increase the risk of reactivation of latent tuberculosis, and this risk is higher in patients treated with the monoclonal antibodies. We studied the effects of TNF-α blockers on the maturation of mycobacteria-containing phagosomes in human macrophages. All 3 drugs had an inhibitory effect on IFN-γ–induced phagosome maturation in phorbolmyristate acetate–differentiated human THP-1 cells. Adalimumab and infliximab, but not etanercept, suppressed phagosome maturation in primary human peripheral blood monocyte-derived macrophages in the presence or absence of IFN-γ. Treatment of macrophages with TNF-α led to increased maturation of phagosomes containing *Mycobacterium bovis* bacillus Calmette-Guérin or *M. tuberculosis* H37Rv. These results suggest a role for TNF-α in activating phagosome maturation and highlight a mechanism through which TNF-α blockade can affect the host response to mycobacteria.

Tumor necrosis factor (TNF)–α mediates early inflammatory responses to a variety of physical, environmental, and immunological stimuli. Produced by macrophages, lymphocytes, neutrophils mast cells, and some endothelial cells, TNF-α coordinates the inflammatory response through the induction of other cytokines (e.g., interleukin [IL]–1 and IL-6) and by recruitment of immune and inflammatory cells through the induction of chemokine release and up-regulation of adhesion molecules [1, 2]. Experimental models have shown that

TNF-α plays a major role not only in the host response against *Mycobacterium tuberculosis* but also in the immunopathology of tuberculosis (TB) [3].

Infection with mycobacteria or treatment with specific mycobacterial molecules has been shown to induce TNF-α secretion by macrophages [4–6]. Studies in vitro have demonstrated that TNF-α increases the ability of macrophages to phagocytose and kill mycobacteria [7, 8] and stimulates macrophage apoptosis, depriving the bacilli of their niche cells and leading to increased killing and presentation of mycobacterial antigens by dendritic cells [9, 10]. In vivo, TNF-α is required for formation and maintenance of granulomas. Mice deficient in TNF-α or TNF receptor 1 are more susceptible to mycobacterial infection and display impaired granuloma formation [11, 12]. Neutralization of TNF-α with specific monoclonal antibodies, pentoxifylline, or gene targeting in mice chronically infected with *M. tuberculosis* disrupts the integrity of granulomas, exacerbates infection, and increases mortality [11, 13, 14]. TNF-α is synthesized as a 26-kDa transmembrane monomer (tmTNF) that is cleaved by the TNF-α–converting enzyme to yield a soluble TNF-α trimer [15, 16]. Both soluble TNF-α and tmTNF-α can exert biological and metabolic effects [17].

TNF-α–blocking agents are now routinely and effectively used in the treatment of chronic autoimmune inflammatory diseases, including rheumatoid arthritis, psoriasis, ankylosing spondylitis, and Crohn disease [18]. Three TNF-α–inhibiting drugs are currently approved for treatment of autoimmune diseases: adalimumab (Humira), infliximab (Remicade), and etanercept (Enbrel). All 3 are 150-kDa proteins comprising 2 TNF-binding domains linked to human IgG1 Fc. Adalimumab is a humanized monoclonal antibody, infliximab is a chimeric monoclonal antibody with a human Fc region and murine variable region, and etanercept is made up of 2 human p75 TNF receptors bound to human IgG1 Fc. The monoclonal antibodies recognize both monomeric and trimeric TNF-α; etanercept binds to trimeric TNF-α only. Given the importance of TNF-α in host responses and granuloma formation and maintenance, it is perhaps not surprising that anti–TNF-α therapy has been linked with increased susceptibility to infection with numerous pathogens, including *M. tuberculosis* [19–21]. Reactivation of latent TB is increased in patients receiving TNF-α blockers, and this effect appears to be more pronounced in patients treated with infliximab than in those treated with etanercept [20–22]. The mechanisms underlying these observations have yet to be elucidated.

The aim of the present study was to determine the effects of adalimumab, infliximab, and etanercept on the responses of macrophages to infection with mycobacteria in vitro. In particular, phagosome acidification and fusion with lysosomes (phagosome maturation) was studied, because this process is essential for the presentation of mycobacterial antigens to T cells and the initiation of adaptive immune responses.

METHODS

Antibodies and reagents. Recombinant human TNF-α and IFN-γ (R&D Systems) were used at 5 ng/mL and 200 U/mL, respectively. Adalimumab, infliximab, and etanercept were all used at a concentration of 10 μg/mL, on the basis of findings of other studies [23, 24]. Human IgG1 from patients with myelomas producing IgG1 (Calbiochem) was used as an antibody control at 10 μg/mL. Mouse monoclonal antibody against CD63 (LAMP-3; Santa Cruz Biotechnology) was used at 1 μg/mL. Mouse monoclonal antibody against cathepsin D (Calbiochem) was used at 10 μg/mL. Fluorescein isothiocyanate (FITC)–conjugated mouse anti–human HLA-DR monoclonal antibody (BD) and corresponding FITC-conjugated isotype control antibody (BD) were used in accordance with the manufacturer's instructions. Phosphospecific monoclonal antibody against signal transducer and activator of transcription (STAT) 1 (pY701; BD) and mouse IgG1 isotype control (Serotec) were used at 5 μg/mL. For secondary staining, Alexa Fluor 488– or 568–labeled goat anti–mouse IgG (Invitrogen) were used at 4 μg/mL. LysoTracker Red DND-99 (Invitrogen) was used at 100 nmol/L.

Cells. THP-1 cells were cultured in RPMI 1640 (Invitrogen) with 10% fetal bovine serum (FBS; Gibco). Cells were differentiated into macrophage-like cells by treatment with phorbol myristate acetate (100 nmol/L) for 24 h and then cultured in normal medium for 3 days. Human monocyte-derived macrophages (MDMs) were isolated and cultured as described elsewhere [25]. Briefly, peripheral blood mononuclear cells were isolated from buffy coats (Irish Blood Transfusion Service) or blood from healthy donors by density gradient centrifugation on Histopaque 1077 (Sigma). Monocytes were isolated by adherence to gelatin-coated culture dishes and cultured overnight in RPMI 1640 with 5% human AB serum (Sigma). Adherent cells were removed with 10 mmol/L EDTA in PBS and grown on coverslips in 12-well plates for 10 days.

Mycobacteria. Green fluorescent protein–labeled *Mycobacterium bovis* bacillus Calmette-Guérin (GFP-BCG; provided by V. Deretic, University of New Mexico Health Sciences Center) and *M. tuberculosis* H37Ra and H37Rv were grown in Middlebrook 7H9 broth with 0.5% Tween, 0.2% glycerol, and 10% albumin-dextrose-catalase supplement (BD). Mycobacteria were grown to log phase before use and were resuspended in RPMI 1640 with 10% FBS before infection. *M. tuberculosis* strain H37Ra was fluorescently labeled with PKH67 (Sigma), in accordance with the manufacturer's protocol. *M. tuberculosis* strain H37Rv was labeled with FITC (1 mg/mL; Sigma), in accordance with the manufacturer's protocol. The MOI was recorded microscopically 15 min after infection of macrophages by acid-fast bacilli staining. Cells were infected at an MOI of 1–5 bacilli in ~70% of cells.

Phagosome maturation. Macrophage phagosome maturation was analyzed microscopically, as described elsewhere [26]. THP-1 cells or MDMs were grown on coverslips at a concentration of 2×10^5 cells/well. Unless otherwise stated, TNF-α blockers (10 μg/mL) and IFN-γ (200 U/mL) were added to cells for 24 h before infection. Cells were infected with *M. bovis* GFP-BCG, PKH67-labeled *M. tuberculosis* H37Ra, or FITC-labeled *M. tuberculosis* H37Rv for 15 min, washed 3 times with PBS to remove unbound mycobacteria, and incubated for 2 h. Cells were fixed in 2% paraformaldehyde for 20 min at room temperature (for H37Rv, 4% paraformaldehyde overnight), permeabilized with 0.1% Triton X-100 in PBS, and blocked with 1% bovine serum albumin and 1% goat serum in PBS for 30 min at room temperature. Cells were incubated with primary antibody for 1 h followed by secondary antibody for 1 h, both at room temperature. Alternatively, before fixation, cells were incubated with LysoTracker Red DND-99 (Invitrogen) for the final 60 min of incubation with mycobacteria. Coverslips were mounted onto glass slides with fluorescent mounting medium (Dako), and images were recorded on an Olympus FluoView 1000 and a Zeiss LSM 510 laser scanning confocal microscope. Images were analyzed and prepared using the appropriate proprietary software and Adobe Photoshop.
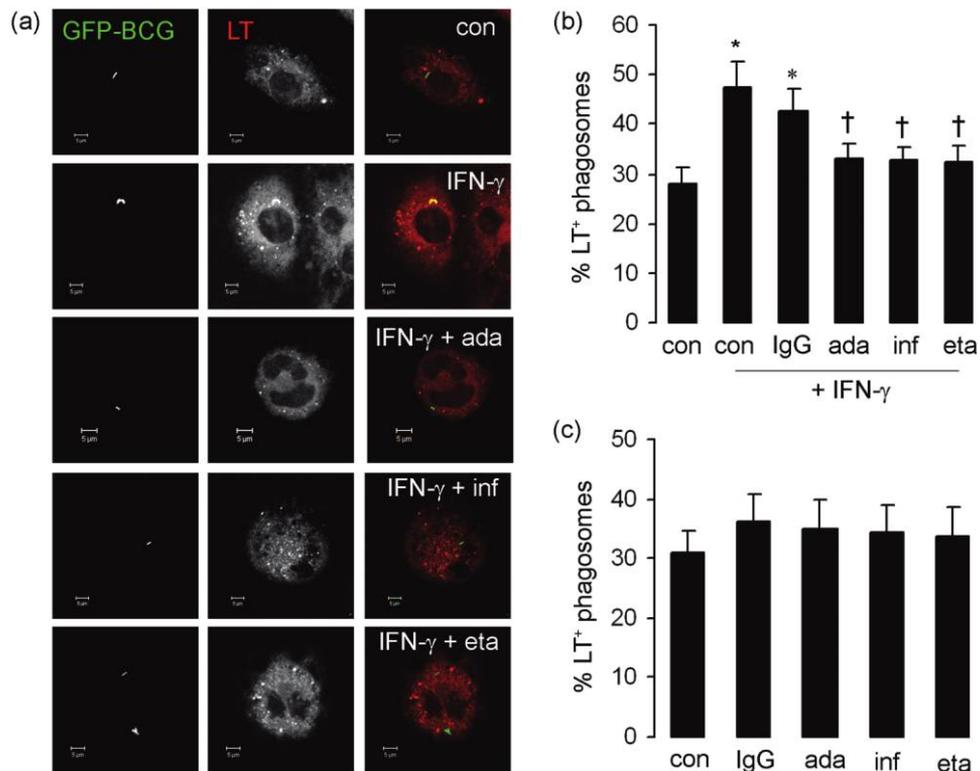
Figure 1. Effects of tumor necrosis factor (TNF) blockers on the acidification of mycobacterial phagosomes in PMA-differentiated THP-1 cells. *a,* Representative confocal images of cells infected with green fluorescent protein–labeled *Mycobacterium bovis* bacillus Calmette-Guérin (GFP-BCG) for 2 h and stained with LysoTracker Red (LT). Cells were treated with medium only (control [con]), interferon (IFN)–γ (pretreated for 24 h; 200 U/mL), or IFN-γ plus adalimumab (ada), infliximab (inf), or etanercept (eta) (all 10 μg/mL). *b,* Quantitative analysis of colocalization between GFP-BCG and LT in IFN-γ–treated cells. *c,* Quantitative analysis of colocalization between GFP-BCG and LT in the absence of IFN-γ. Data are means ± SEs from 6 *(b)* or 7 *(c)* separate experiments. IgG, human myeloma IgG control antibody. *$P < .05$ (significant difference from control); †$P < .05$ (significant inhibition compared with corresponding IFN-γ–treated cells).

Measurement of TNF-α. THP-1 cells were prepared as described above and infected with BCG or *M. tuberculosis* H37Ra, with or without IFN-γ pretreatment. Levels of immunoreactive TNF-α in supernatants were measured using a commercial ELISA kit (R&D Systems), in accordance with the manufacturer's instructions.

Statistical analysis. Data were analyzed using Student's *t* test. For human MDM experiments, data were analyzed using Student's paired *t* test. Differences with $P < .05$ were considered significant.

RESULTS

Inhibition of phagosome maturation in macrophages by TNF-α blockers. Mycobacteria are able to inhibit fusion of phagosomes with lysosomes, preventing acidification and recruitment of lysosomal hydrolases [27]. This phagosome maturation block can be overcome by pretreatment of macrophages with IFN-γ [26, 28, 29]. To determine the effects of TNF-α blockers on phagosome-lysosome fusion, we infected THP-1 cells with GFP-BCG or PKH67-labeled *M. tuberculosis* H37Ra and analyzed phagosome-lysosome fusion by confocal micros-

copy, using LysoTracker Red as a marker for acidified phagosomes and CD63 and cathepsin D as phagolysosomal markers. In the absence of IFN-γ, none of the TNF-α blockers had any effect on phagosome acidification in BCG-infected THP-1 cells (figure 1*c*). However, in THP-1 cells pretreated with IFN-γ for 24 h in the presence or absence of the blockers, adalimumab, infliximab, and etanercept all inhibited IFN-γ–induced phagosome maturation, as determined by colocalization of GFP-BCG with LysoTracker Red (figure 1*a* and 1*b*), CD63, and cathepsin D (figure 2*a* and 2*b*). Similarly, in THP-1 cells infected with PKH67-labeled *M. tuberculosis* H37Ra, all 3 TNF-α blockers inhibited IFN-γ–induced phagosome acidification (figure 3*a* and 3*b*).

To test whether TNF blockers influence responses to virulent mycobacteria, PMA-treated THP-1 cells were infected with *M. tuberculosis* H37Rv, and phagosome acidification was recorded in response to treatment with IFN-γ, with or without TNF-α blockers, using LysoTracker Red (figure 3*c* and 3*d*). Pretreatment of cells with IFN-γ increased acidification, and this effect was inhibited by adalimumab, infliximab, and etanercept (figure 3*c* and 3*d*). Treatment of infected THP-1 cells with TNF-α (5 ng/mL) also enhanced phagosome acidification (figure 3*c* and 3*d*).
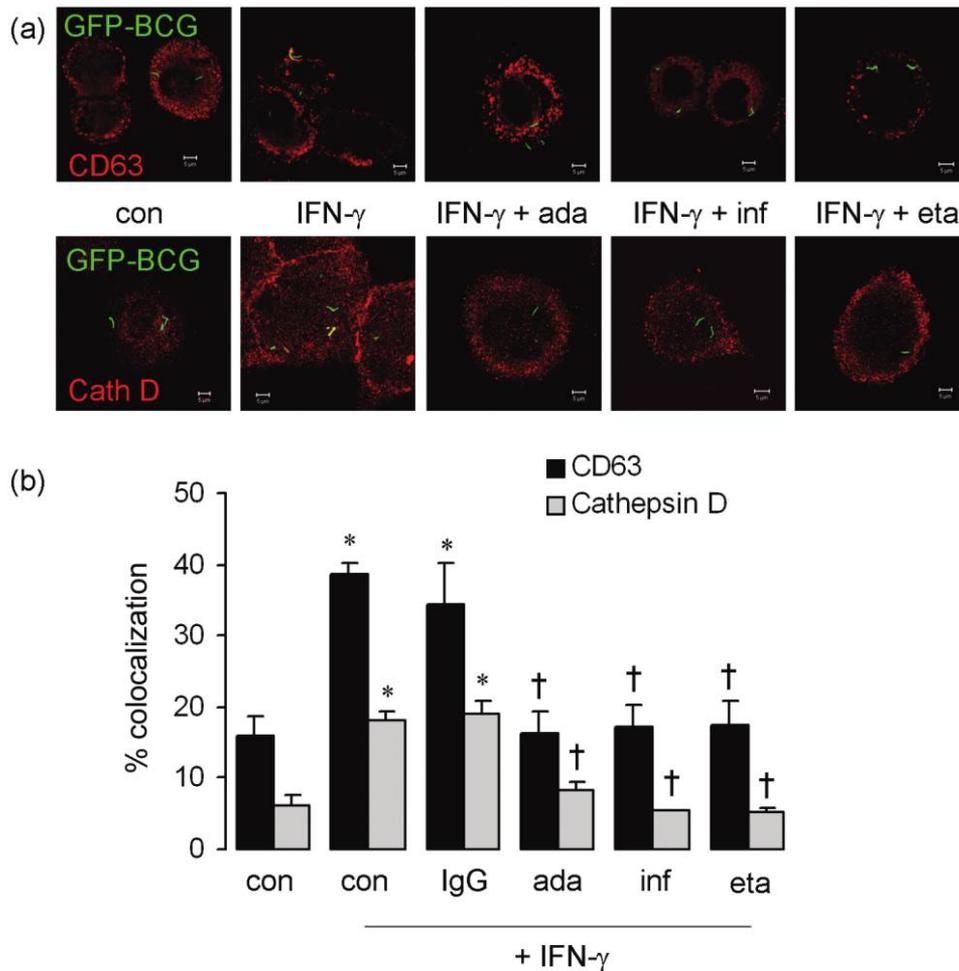
Figure 2. Effects of tumor necrosis factor (TNF) blockers on the fusion of mycobacterial phagosomes with lysosomes in PMA-differentiated THP-1 cells. *a,* Representative confocal images of cells infected with green fluorescent protein–labeled *Mycobacterium bovis* bacillus Calmette-Guérin (GFP-BCG) for 2 h, fixed, and stained with antibody against CD63 (LAMP3) or cathepsin D. Cells were treated with medium only (control [con]), interferon (IFN)–γ (pretreated for 24 h; 200 U/mL), or IFN-γ plus adalimumab (ada), infliximab (inf), or etanercept (eta) (all 10 μg/mL). *b,* Quantitative analysis of colocalization between GFP-BCG and CD63 and cathepsin D in IFN-γ–treated cells. Data are means ± SEs from 3 separate experiments. IgG, human myeloma IgG control antibody. *$P < .05$ (significant difference from control); †$P < .05$ (significant inhibition compared with corresponding IFN-γ–treated cells).

In primary human peripheral blood MDMs, adalimumab and infliximab inhibited phagosome acidification, in both untreated and IFN-γ–pretreated cells, whereas etanercept had no significant effect on phagosome maturation (figure 4*a*–4*c*). Control human IgG had no effect on phagosome maturation in any of the experiments (figures 1*b*, 1*c*, 1*e*, 2, and 4*d*). Neither IFN-γ nor any of the TNF-α blockers had any effect on uptake of mycobacteria by macrophages (data not shown).

Inducement of phagosome acidification by TNF-α. In IFN-γ–treated THP-1 cells, both BCG and *M. tuberculosis* H37Ra significantly increased secretion of TNF-α (figure 5*a*). This response to BCG and H37Ra was significantly greater in IFN-γ–treated cells. Treatment with IFN-γ alone also significantly increased TNF-α secretion (figure 5*a*). To determine whether the inhibition of IFN-γ–induced phagosome acidification by TNF-α blockers was dependent on effects before infec-

tion, THP-1 cells were treated with IFN-γ for 24 h and then infected with GFP-BCG in combination with the drugs. Adalimumab, infliximab, and etanercept all inhibited IFN-γ–induced phagosome acidification, demonstrating that the effects of these blockers occur after infection (figure 5*b*).

Finally, to determine whether TNF-α can itself induce phagosome acidification, THP-1 cells and MDMs infected with GFP-BCG were treated with TNF-α for 2 h and stained with Lyso-Tracker Red. Treatment of cells with TNF-α directly stimulated phagosome acidification in both THP-1 cells and primary human MDMs (figure 5*c* and 5*d*).

DISCUSSION

Although anti–TNF-α therapies offer effective treatment against a number of inflammatory and autoimmune diseases, their use
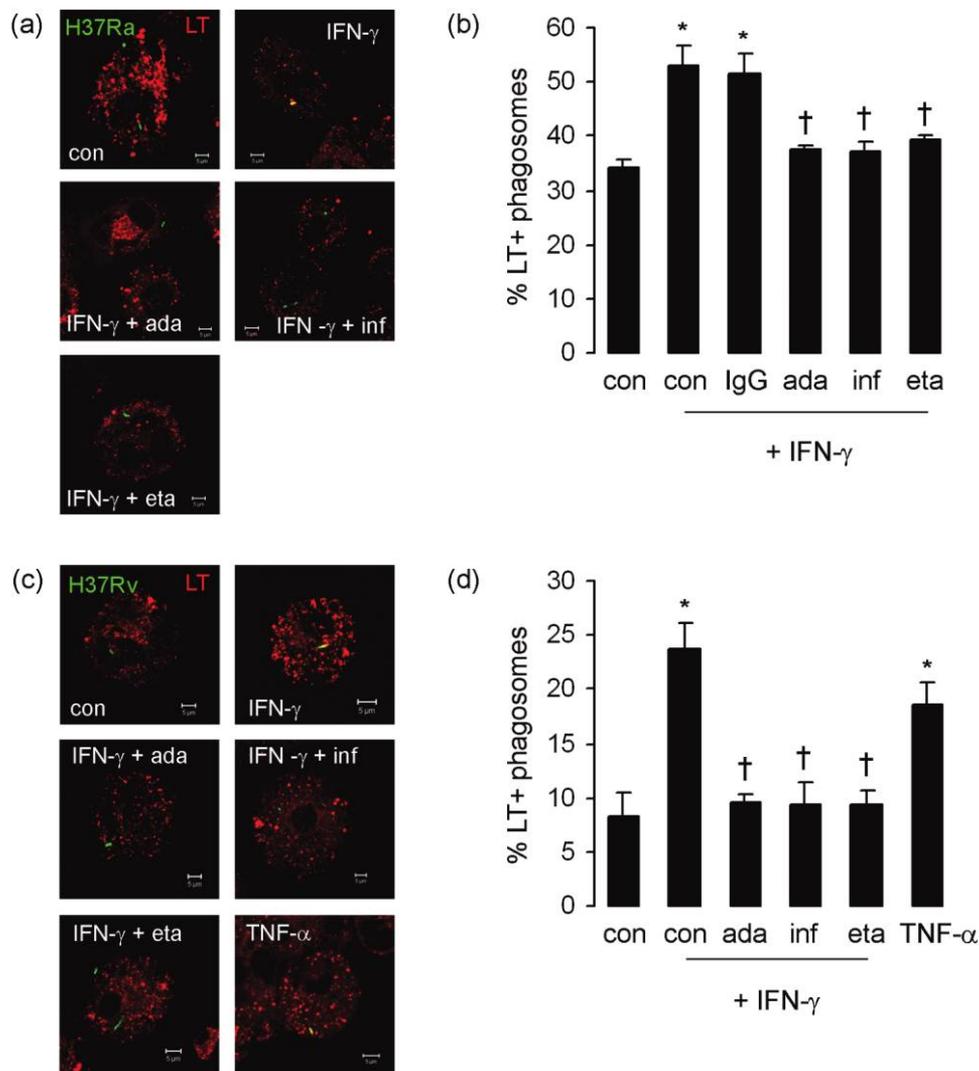
Figure 3. Effects of tumor necrosis factor (TNF) blockers on the acidification of *Mycobacterium tuberculosis*–containing phagosomes in PMA-differentiated THP-1 cells. *a,* Representative confocal images of cells infected with PKH67-labeled *M. tuberculosis* H37Ra and stained with LysoTracker Red (LT). *b,* Quantitative analysis of colocalization between H37Ra and LT in interferon (IFN)–γ–treated cells (200 U/mL for 24 h). *c,* Representative confocal images of cells infected with fluorescein isothiocyanate–labeled *M. tuberculosis* H37Rv and stained with LT. *d,* Quantitative analysis of colocalization between H37Rv and LT cells treated with IFN-γ with or without TNF blockers (all 10 μg/mL) or with TNF-α (5 ng/mL during infection). Data are means ± SEs from 4 (*a* and *b*) or 3 (*c* and *d*) separate experiments. ada, adalimumab; eta, etanercept; IgG, human myeloma IgG control antibody; inf, infliximab. $*P < .05$ (significant difference from control); $†P < .05$ (significant inhibition from corresponding IFN-γ–treated cells).

is also linked with increased susceptibility to a number of opportunistic pathogens, including *Coccidioides immitis, Pneumocystis jiroveci* (previously *Pneumocystis carinii*), *Histoplasma capsulatum, Aspergillus fumigatus, Listeria monocytogenes,* and *Salmonella typhimurium* [21, 30–32]. In addition, TNF-α blockers have been strongly linked to reactivation of latent TB [33], and it has been suggested that this phenomenon is less common in patients treated with etanercept than in those treated with adalimumab or infliximab [34].

Here we have uncovered differences between primary human MDMs and THP-1 cells in the response to TNF-α blockers. In the absence of IFN-γ stimulation, none of the drugs had any effect on acidification of mycobacteria-containing phagosomes

in THP-1 cells, whereas adalimumab and infliximab, but not etanercept, inhibited phagosome acidification in the absence of IFN-γ in MDMs. Moreover, although all 3 TNF blockers inhibited IFN-γ–induced phagosome-lysosome fusion and acidification in THP-1 cells, adalimumab and infliximab inhibited phagosome acidification in MDMs, but etanercept had no effect. It is not clear why the 2 cell types respond differently, although they are undoubtedly differently activated; THP-1 cells are stimulated with PMA and cultured in medium with FBS for 5 days, and MDMs are differentiated in the presence of human serum over a longer time (10 days) without any specific stimulation.

Inhibition of IFN-γ–induced phagosome acidification by the drugs in THP-1 cells was dependent on effects after infection, be-
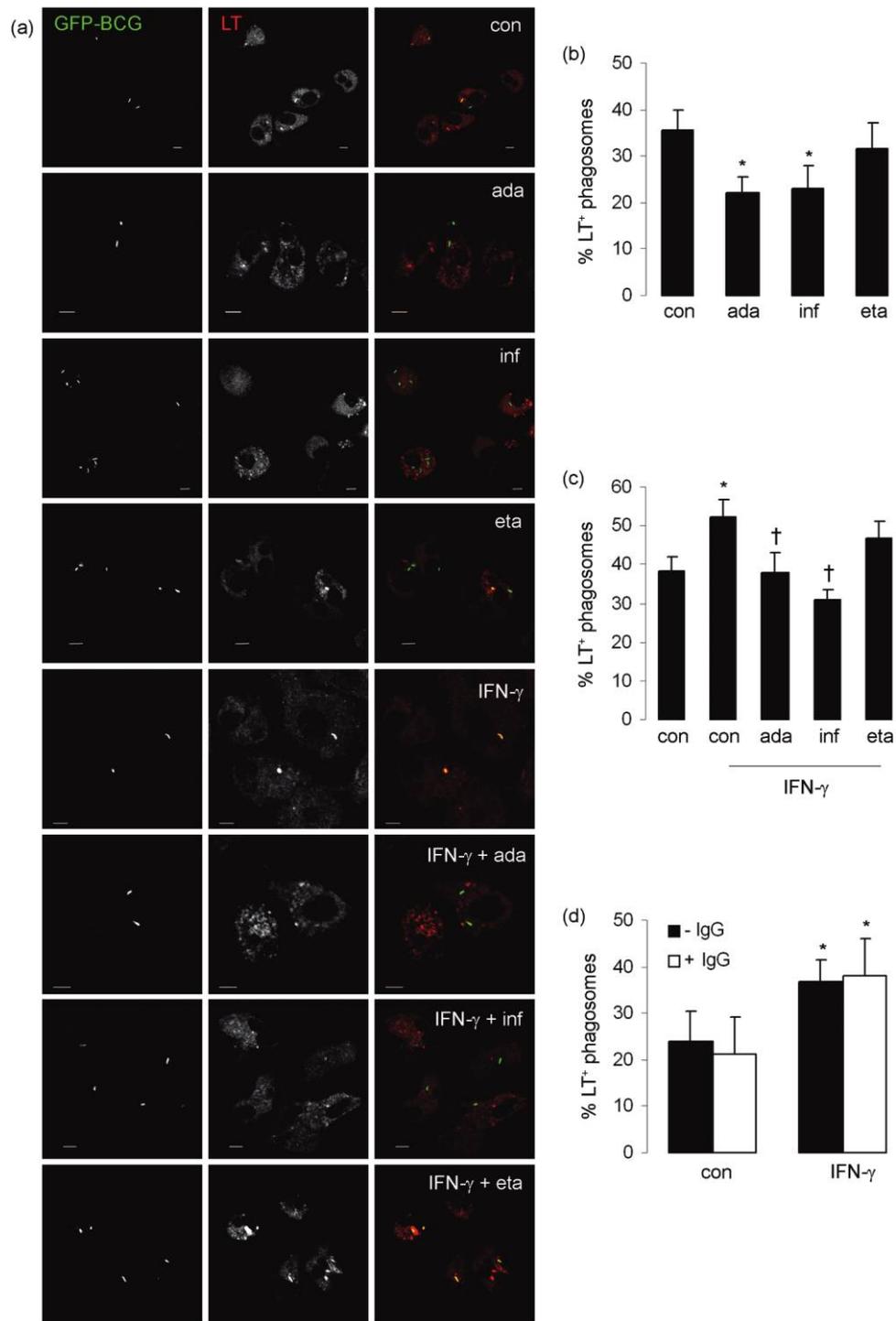
Figure 4. Effects of tumor necrosis factor (TNF) blockers on acidification of mycobacterial phagosomes in human monocyte-derived macrophages. *a,* Representative confocal images of cells infected with green fluorescent protein–labeled *Mycobacterium bovis* bacillus Calmette-Guérin (GFP-BCG) for 2 h and stained with LysoTracker Red (LT). Cells were treated with medium only (control [con]) or with adalimumab (ada), infliximab (inf), or etanercept (eta) (all 10 μg/mL), in the presence or absence of interferon (IFN)–γ (pretreated for 24 h at 200 U/mL). *b,* Quantitative analysis of colocalization between GFP-BCG and LT in the absence of IFN-γ. *c,* Quantitative analysis of colocalization between GFP-BCG and LT in the presence of IFN-γ. *d,* Quantitative analysis of colocalization between GFP-BCG and LT in cells treated with human myeloma IgG control antibody (IgG), in the presence or absence of IFN-γ. Data are means ± SEs from 5 (*b* and *c*) or 4 *(d)* separate donors. *$P <$ .05 (significant difference from corresponding control); †$P <$ .05 (significant inhibition compared with corresponding IFN-γ–treated cells).
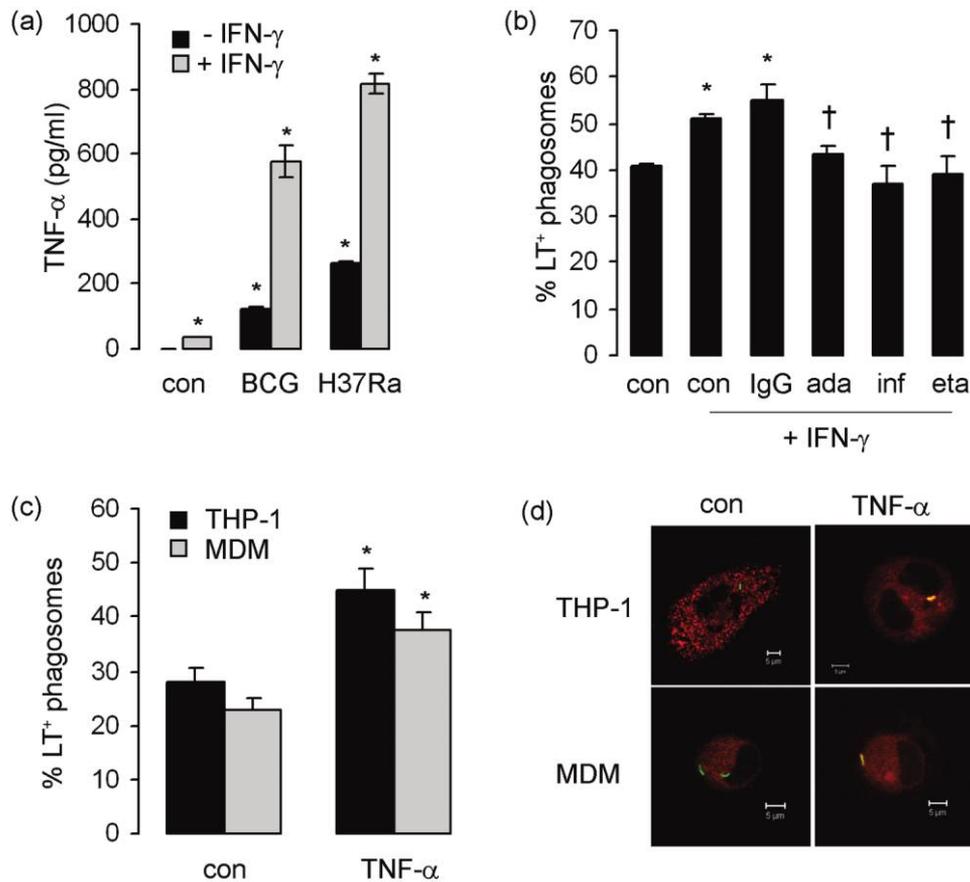
Figure 5. Role of tumor necrosis factor (TNF)–α in phagosome acidification. *a,* Secretion of TNF-α by PMA-differentiated THP-1 cells treated with or without interferon (IFN)–γ (24 h at 200 U/mL) and infected with *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) or *Mycobacterium tuberculosis* H37Ra for 2 h. *b,* Quantitative analysis of colocalization between green fluorescent protein–labeled BCG (GFP-BCG) and LysoTracker Red (LT) in THP-1 cells treated with or without IFN-γ (24 h at 200 U/mL), infected, and incubated with TNF blockers (10 μg/mL) for 2 h after infection. *c,* Quantitative analysis of colocalization between GFP-BCG and LT in THP-1 cells and human monocyte-derived macrophages (MDMs) incubated with or without TNF-α (5 ng/mL) for 2 h. *d,* Representative confocal images of THP-1 cells and MDMs infected with GFP-BCG, with or without TNF-α. Data are means $\pm$ SEs from at least 3 separate experiments. ada, adalimumab; con, control (medium only); eta, etanercept; IgG, human myeloma IgG control antibody; inf, infliximab. *$P < .05$ (significant difference from corresponding control); †$P < .05$ (significant inhibition compared with corresponding IFN-γ–treated cells).

cause they exerted the same effects regardless of whether they were added to the cells with IFN-γ (24 h before infection) or during infection. Moreover, the effect of these blockers on acidification and maturation of mycobacteria-containing phagosomes is consistent for *M. bovis* BCG and *M. tuberculosis* strains H37Ra and H37Rv. It is notable that the basal level of acidification was significantly lower for the virulent H37Rv strain (8%) than for the avirulent H37Ra strain (34%) and BCG (28%–41%). These differences may be attributable, at least in part, to differences in stimulation of TNF-α release by the different strains.

Another study has demonstrated that lipoarabinomannan (LAM) from H37Ra induced TNF-α secretion by infected murine bone marrow–derived macrophages but that LAM from the virulent Erdman strain did not [6]. However, other studies using whole bacteria have suggested little or no difference in TNF-α secretion by human and murine macrophages infected with avirulent and virulent *M. tuberculosis* [4, 5]. Although we observed lower TNF-α secretion by THP-1 cells infected with

H37Rv, compared with that for H37Ra, IFN-γ did have a stimulatory effect on secretion (data not shown). However, differences between the assays used make direct comparison between strains difficult in this study. Our data suggest that, despite differences in the extent of the phagosome maturation block imposed by virulent and nonvirulent strains of mycobacteria, BCG and H37Ra can still be useful models for studying the effects of TNF-α blockers and IFN-γ on phagosome maturation and acidification in mycobacteria-infected macrophages.

Because we have shown that TNF-α induces phagosome acidification in THP-1 cells and MDMs infected with BCG and that pretreatment of THP-1 cells with IFN-γ augments TNF-α secretion by infected macrophages, it seems likely that the drugs act through blockade of soluble TNF-α or interaction with tmTNF-α rather than through effects on IFN-γ signaling. Indeed, the drugs had no effect on IFN-γ–dependent STAT1 signaling or on IFN-γ–induced up-regulation of major histocompatibility complex class II on the plasma membrane (data not

shown). Our data suggest that TNF-α, secreted by macrophages in response to infection, can act in an autocrine manner to stimulate phagosome maturation and that this effect is increased by activation of cells with IFN-γ. Other studies have demonstrated that TNF monoclonal antibodies, but not etanercept, inhibit IFN-γ release by lymphocytes [23, 24, 35]. Thus, treatment with these drugs may have a dual effect on macrophage responses to *M. tuberculosis* and other infections: inhibition of both IFN-γ– and TNF-α–dependent activation of macrophages.

The difference in the response of THP-1 cells and MDMs to etanercept is particularly intriguing. In another study, Saliu et al. [24] found that adalimumab and infliximab, but not etanercept, inhibited activation of CD4+ T cells in whole blood, as measured by CD69 expression, in response to *M. tuberculosis* culture filtrate. The production of IFN-γ in whole blood stimulated with *M. tuberculosis* culture filtrate was inhibited by adalimumab and infliximab. Treatment with etanercept did not affect IFN-γ production [24]. This difference could not be attributed to an increase in IL-10 production, which inhibits IFN-γ expression, because all 3 drugs inhibited IL-10 production equally [24]. However, the balance between IL-10 and TNF-α secretion by macrophages could be significant, and loss of TNF activity after treatment with blockers could favor an IL-10–directed response. In a separate study, all 3 drugs were found to inhibit CD4+ T cell proliferation and tmTNF-α expression in vitro, but this effect was stronger with adalimumab and infliximab than with etanercept [23]. Similarly, another study demonstrated reduced T cell proliferation and IFN-γ production in response to mycobacterial antigens in cells treated with infliximab and adalimumab, but not in those treated with etanercept [36].

The mechanisms behind these differences are not clear. Infliximab binds to TNF-α with greater avidity and for longer than etanercept, which releases >90% of the bound cytokine after 2–3 h [37]. In addition, although infliximab binds to soluble TNF-α and tmTNF-α irreversibly, etanercept binds to soluble TNF-α strongly but has a lower avidity for tmTNF-α and may leave binding sites free [38]. In this context, it is interesting that mice engineered to express only tmTNF display efficient cell-mediated immunity to BCG, acute *M. tuberculosis,* and *Listeria* infections [3, 39, 40]. Thus, the differential effects of the drugs observed here could reflect differences in disassociation rates and/or be dependent on tmTNF-α. Similarly, the differences observed between THP-1 cells and MDMs in response to etanercept treatment may reflect differences in the expression and/or function of tmTNF-α.

It is likely that primary human macrophages represent a better model than THP-1 cells for extrapolating the effects of TNF-α blockers in vivo. In this context, our findings, combined with those of other studies, may offer some explanation for the reported differences in the reactivation of latent TB in patients receiving different anti–TNF-α therapies. Decreased T cell activation and IFN-γ production in patients treated with infliximab or adalimumab could in turn inhibit IFN-γ–dependent macrophage activation and secretion of TNF-α. Numerous studies have shown that IFN-γ activates antimycobacterial responses in macrophages [28, 29, 41–44]. The inhibition of IFN-γ release alone may not account for the differential effects of these drugs in vivo, because at least one study has demonstrated that infliximab and etanercept affect IFN-γ release to a similar extent in patients with inflammatory diseases [23]. However, we have shown that the drugs act directly on macrophages, possibly through inhibition of autocrine TNF-α signaling, to inhibit phagosome maturation. This in turn will likely affect intracellular killing and processing of mycobacterial antigens for presentation to T cells.

Studies in mouse models have suggested that anti–TNF-α monoclonal antibody penetrates more easily into the granuloma and may remain at higher levels than receptor fusion molecules [45]. In addition, mathematical modeling studies have indicated that the relative bioavailabilty of TNF-α after treatment with different TNF-α blockers is responsible for the differences in reactivation of latent TB, suggesting that the timing and dose of anti–TNF-α therapy could be crucial [46]. Thus, the differences in reactivation of latent disease observed are likely due to differential effects on monocytes/macrophages and T cells (and possibly other immune cells), combined with differences in drug delivery, penetration, and pharmacodynamics.

In conclusion, we have shown that TNF-α blockade inhibits maturation of mycobacteria-containing phagosomes in human macrophages in vitro. In MDMs, the anti–TNF-α monoclonal antibodies adalimumab and infliximab inhibited phagosome acidification, but the TNF receptor fusion protein etanercept had no significant effect. Moreover, treatment of macrophages with TNF-α enhanced phagosome maturation. These data provide insight into the differential effects of these drugs on reactivation of latent TB in patients receiving anti–TNF-α therapy.

Acknowledgments

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