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Glucocorticoids are extensively used to treat inflammatory diseases; however, their chronic intake increases the risk for mycobacterial infections. Meanwhile, the effects of glucocorticoids on innate host responses are incompletely understood. In this study, we investigated the direct effects of glucocorticoids on antmycobacterial host defense in primary human macrophages. We found that glucocorticoids triggered the expression of cathelicidin, an antimicrobial critical for antmycobacterial responses, independent of the intracellular vitamin D metabolism. Despite upregulating cathelicidin, glucocorticoids failed to promote macrophage antmycobacterial activity. Gene expression profiles of human macrophages treated with glucocorticoids and/or IFN-γ, which promotes induction of cathelicidin, as well as antmycobacterial activity, were investigated. Using weighted gene coexpression network analysis, we identified a module of highly connected genes that was strongly inversely correlated with glucocorticoid treatment and associated with IFN-γ stimulation. This module was linked to the biological functions autophagy, phagosome maturation, and lytic vacuole/lysosome, and contained the vacuolar H+-ATPase subunit a3, alias TCIRG1, a known antmycobacterial host defense gene, as a top hub gene. We next found that glucocorticoids, in contrast with IFN-γ, failed to trigger expression and phagolysosome recruitment of TCIRG1, as well as to promote lysosome acidification. Finally, we demonstrated that the tyrosine kinase inhibitor imatinib induces lysosome acidification and antmycobacterial activity in glucocorticoid-treated macrophages without reversing the anti-inflammatory effects of glucocorticoids. Taken together, we provide evidence that the induction of cathelicidin by glucocorticoids is not sufficient for macrophage antmycobacterial activity, and identify the vacuolar H+-ATPase as a potential target for host-directed therapy in the context of glucocorticoid therapy.

The Journal of Immunology, 2016, 197: 222–232.
to glucocorticoids strengthened components of the innate response, albeit weakening an acquired immune signature (5).

Human macrophages play a central role in the host defense against mycobacteria, because they are the natural niches for these intracellular pathogens. We have contributed to the understanding of how human macrophages kill mycobacteria by identifying a vitamin D–dependent host defense pathway (6–8). The vitamin D antimicrobial pathway is induced by innate and acquired immune signals, including TLR2/1 ligand, CD40L, and IFN-γ (6–9). Central early events include the induction of IL-32 and IL-15 (10, 11), the CYP27b1 hydroxylase, which converts 25-OH vitamin D (25D) into the active form 1,25-di-OH vitamin D (1,25D) in an autocrine manner, as well as the upregulation of the vitamin D receptor (VDR) (6–9). Subsequently, 1,25D triggers VDR-mediated induction of cathelicidin antimicrobial peptide (12, 13), pivotal for killing mycobacteria in phagolysosomes (14). Furthermore, this pathway involves the vitamin D–dependent induction of autophagy, which is important to overcome the phagosome maturation block (7, 15, 16), an important mycobacterial virulence strategy (17–22). Given the importance of macrophages in combating mycobacteria, we investigated the impact of glucocorticoids on the antimycobacterial response in primary human macrophages.

Materials and Methods

Reagents

Recombinant human M-CSF was purchased from Miltenyi Biotec and recombinant human IFN-γ from Becton Dickinson. M-CSF was used at a concentration of 50 ng/ml and IFN-γ at a concentration of 10 ng/ml. Dexamethasone, hydrocortisone, imatinib mesylate, and mifepristone were from Sigma-Aldrich and used at the following concentrations: dexamethasone 10–1000 nM, hydrocortisone 300 nM, imatinib mesylate 5–10 μM, and mifepristone 1 μM. 25D3 and 1,25D3 were obtained from Discovery (Becton Dickinson and Merck Millipore) and incubated for 21 d at 37˚C. Four to five dilutions of each sample were plated on 7H10 agar plates containing Difco Middlebrook 7H9 broth supplemented with 0.5% glycercol (23). The Journal of Immunology 223

Infection of MDMs, treatment with stimuli, and quantification

Surface staining with IL-15 PE or the appropriate isotype control was performed in PBS containing 20% FCS and 0.1% sodium azide. For intracellular measurement of cathelicidin and TCIIRG1, cells were stained using FlowFix/Cytoperm kit (Becton Dickinson) according to the manufacturer’s recommended instructions. For acidification assays, MDMs or monocytes were incubated with LysoSensor Green (1–2 or 1 μM, respectively) for 30 min and subsequently washed with PBS. Flow cytometry was conducted on a FACScanCepherBio (BD Biosciences), and data were analyzed using FlowJo software (Tree Star). MDM and monocyte gates were set based on forward and sideward scatter.

Infection of MDMs, treatment with stimuli, and quantification of mycobacterial growth

Mycobacterium bovis Bacille Calmette–Guérin strain Pasteur (BCG) was grown in suspension with constant, gentle rotation at 37 C in 7H9 media containing Difco Middlebrook 7H9 broth supplemented with 0.5% glycerol, 0.05% tyloxylosap, and 10% BBL Middlebrook OADC Enrichment.

MDMs were infected for 3–4 h with single-cell suspensions of BCG at a multiplicity of infection of three to five in a 24-well plate. Afterward extracellular bacteria were removed by vigorous washing. For CFU assays, MDMs were treated with dexamethasone or IFN-γ and incubated for 3 d. To determine the number of viable intracellular bacilli, cells were lysed with 0.05% SDS (Serva Electrofrosorhesis). Lysates of infected cells were resuspended vigorously and diluted in 0.05% tyloxylosap (Sigma-Aldrich). Five to dilutions of each sample were plated on 7H10 agar plates (Becton Dickinson and Merck Millipore) and incubated for 21 d at 37 C.

Immunostaining for TCIIRG1 and LAMP1

For intracellular staining of TCIIRG1 and LAMP1, MDMs were grown on eight-well chamber slides (Becton Dickinson) and stimulated with dexamethasone or IFN-γ, or left untreated for 20 h. Cells were fixed with methanol and permeabilized with saponin (0.01%) and Triton X-100.
Dexamethasone (Dexa) promotes an anti-inflammatory cytokine profile in human macrophages. MDMs were stimulated with Dexa (100 nM), IFN-γ, or media alone for 20 h. Gene expression of pro–IL-1β (n = 8), TNF-α (n = 5), and IL-10 (n = 8) was determined by quantitative PCR (mean fold change ± SEM). *p < 0.05.

Dexamethasone induces cathelicidin expression

Anti-inflammatory macrophage phenotypes have a reduced capacity to control intracellular infections. For instance, in mouse macrophages, the expression of inducible NO synthase and the production of NO (25–27), a fundamental murine antimycobacterial molecule, are significantly reduced by glucocorticoids (28, 29). We and others previously reported that in humans, but not in mice, the vitamin D–mediated induction of cathelicidin antimicrobial peptide has a central role in the antimycobacterial activity in macrophages (6, 7, 14, 30, 31). Thus, we compared the ability of dexamethasone and IFN-γ to induce cathelicidin. MDMs were stimulated with dexamethasone, IFN-γ, or media alone for 20 h, and expression of cathelicidin mRNA was measured. Dexamethasone and IFN-γ each induced cathelicidin mRNA expression by 3.1- and 2.9-fold compared with media (both p < 0.001; Fig. 2A). Furthermore, dexamethasone induction of cathelicidin gene expression was observed at 100 and 1000 nM, but not at 10 nM (data not shown). Next, we tested whether cathelicidin induction was specific for dexamethasone or whether a different member of the glucocorticoid family, hydrocortisone, also induces cathelicidin gene expression. We found that hydrocortisone induced cathelicidin expression by 2.3-fold compared with media (p < 0.05; Fig. 2B). Taken together, these data demonstrate that glucocorticoids induce cathelicidin expression.

Dexamethasone induction of cathelicidin is independent of the macrophage vitamin D defense pathway

The induction of cathelicidin by TLR2/1 ligand, IFN-γ, or CD40L is dependent on the bioavailability of 25D in the sera added to the cultures, limiting the intracellular vitamin D metabolism in human macrophages (6–8). Thus, we compared the ability of dexamethasone or media alone for 20 h. We found that dexamethasone treatment significantly inhibited the mRNA expression of pro–IL-1β and TNF-α by 95 and 30%, respectively, when compared with the media control (both p < 0.05; Fig. 1), whereas IFN-γ induced pro–IL-1β expression by 3.9-fold and TNF-α expression by 21-fold (both p < 0.05; Fig. 1). Moreover, dexamethasone induced IL-10 by 3.4-fold (p < 0.05; Fig. 1). IFN-γ had no significant effect on IL-10 gene expression. Taken together, these data confirm that dexamethasone promotes an anti-inflammatory macrophage cytokine profile.

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Results

Dexamethasone promotes an anti-inflammatory macrophage cytokine profile

Because the therapeutic benefits of glucocorticoids are fundamentally linked to glucocorticoid-mediated anti-inflammatory effects, we tested whether glucocorticoid treatment of primary human macrophages results in a shift from a proinflammatory toward an anti-inflammatory profile. Therefore, we compared mRNA expression of key proinflammatory and anti-inflammatory cytokines, specifically pro–IL-1β, TNF-α, and IL-10, in MDMs cultured with dexamethasone, the inflammatory stimulus IFN-γ, or media alone for 20 h. We found that dexamethasone treatment significantly inhibited the mRNA expression of pro–IL-1β and TNF-α by 95 and 30%, respectively, when compared with the media control (both p < 0.05; Fig. 1), whereas IFN-γ induced pro–IL-1β expression by 3.9-fold and TNF-α expression by 21-fold (both p < 0.05; Fig. 1). Moreover, dexamethasone induced IL-10 by 3.4-fold (p < 0.05; Fig. 1). IFN-γ had no significant effect on IL-10 gene expression. Taken together, these data confirm that dexamethasone promotes an anti-inflammatory macrophage cytokine profile.

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and IFN-γ to regulate key components of the vitamin D defense pathway in macrophages. Dexamethasone had no effect on IL-15 mRNA (data not shown) and surface expression (Fig. 3A, 3B), nor on IL-32 mRNA expression (Fig. 3C), early events of the vitamin D defense pathway. In addition, dexamethasone significantly suppressed expression of the CYP27B1-hydroxylase by 56% and did not induce VDR mRNA expression (p < 0.001 and p < 0.01; Fig. 3D). As expected, IFN-γ induced IL-15 mRNA (data not shown) and surface expression when compared with media control (Δ mean fluorescence intensity [ΔMFI] = 11.4 versus 5.4, p < 0.01; Fig. 3A, 3B), as well as IL-32 (50-fold, p < 0.05; Fig. 3C), CYP27B1 (17.0-fold, p < 0.001; Fig. 3D), and VDR (2.5-fold, p < 0.001; Fig. 3D) mRNA expression. These findings indicate that the glucocorticoid-mediated induction of cathelicidin is not linked to the previously described induction of the intracellular vitamin D defense pathway in human macrophages. Thus, we hypothesized that, in contrast with the 25D-dependent IFN-γ induction of cathelicidin, dexamethasone triggers cathelicidin expression independently of serum 25D. Therefore, we stimulated MDMs with dexamethasone, IFN-γ, or media alone in vitamin D–deficient human serum (25D serum level = 31 nmol/l). The active form of vitamin D, 1,25D, was used as positive control, 1,25D-induced and dexamethasone both induced cathelicidin (both p < 0.05; Fig. 4A) as compared with media control, yet, as expected, IFN-γ failed to induce cathelicidin mRNA expression in 25D–deficient culture conditions (Fig. 4A). CYP24, a classical genomic target of vitamin D used as a control readout, was induced by neither dexamethasone nor IFN-γ in 25D–deficient conditions (Fig. 4A). Given that the vitamin D–deficient serum used in these assays still contains 31 nmol/l 25D, we next analyzed dexamethasone induction of cathelicidin in SFM, which does not contain detectable amounts of 25D. Again, IFN-γ failed to induce, whereas dexamethasone and 1,25D were able to trigger, cathelicidin mRNA expression by 5.3- and 21-fold, respectively (both p < 0.05; Fig. 4B). CYP24 was induced by neither dexamethasone nor IFN-γ under these conditions.

Next, we analyzed whether dexamethasone induces expression of cathelicidin protein. MDMs were stimulated with dexamethasone in SFM for 20 h, and cathelicidin expression was measured by intracellular FACS. Dexamethasone triggered protein expression of cathelicidin in SFM (38.9 versus 5.1% positive cells in the media control; Fig. 4C). Finally, cathelicidin induction by glucocorticoids was dependent on the glucocorticoid receptor (GR), because mifepristone, a GR antagonist, completely blocked dexamethasone, but not 1,25D-induced cathelicidin gene expression (Fig. 4D). Taken together, these data showed that dexamethasone triggers cathelicidin expression by a mechanism that is different from the previously described IFN-γ–induced vitamin D–dependent pathway. Glucocorticoid induction of cathelicidin was observed at concentrations of dexamethasone (100 and 1000 nM), as well as of hydrocortisone (300 nM), that are within the range of the serum levels in individuals taking moderate-to-high therapeutic doses of glucocorticoids (32).

**Dexamethasone fails to induce antimycobacterial activity**

Cathelicidin is pivotal to the antimycobacterial response in human macrophages and kills bacteria by directly binding bacterial membranes (6, 7, 14–16). To investigate whether dexamethasone-induced cathelicidin targets intracellular mycobacteria, we infected MDMs with BCG (pMV261::dsRed) expressing dsRed2 fluorescent protein. Afterward, infected cells were stimulated with either dexamethasone or IFN-γ for 20 h and stained with anti-cathelicidin mAb. Confocal microscopy analysis showed colocalization of dexamethasone-induced and IFN-γ–induced cathelicidin with BCG (Supplemental Fig. 1A). Because dexamethasone induced cathelicidin expression and dexamethasone-induced cathelicidin colocalized with intracellular BCG, we asked whether dexamethasone promotes antimycobacterial activity in human macrophages. To test this, we infected human MDMs from three individual donors with BCG and/or dexamethasone (100 and 1000 nM), as well as hydrocortisone and/or IFN-γ, or media alone. We measured between 50 and 73% bacterial growth restriction in the IFN-γ–treated MDMs as compared with media control (Fig. 5). In contrast, we observed no reduction in intracellular bacterial load in the dexamethasone-treated MDMs (Fig. 5). Moreover, we measured a similar bacterial growth pattern in the IFN-γ plus dexamethasone-treated cells as in the IFN-γ–treated cells (Fig. 5). IFN-γ plus dexamethasone also resulted in a comparable
induction of cathelicidin gene expression as IFN-\(\gamma\) alone (Supplemental Fig. 1). Taken together, our data show that dexamethasone induces cathelicidin antimicrobial peptide expression but fails to promote antimicrobial activity against intracellular mycobacteria in human macrophages.

Identification of a macrophage host defense network associated with IFN-\(\gamma\) and inversely correlated with dexamethasone stimulation

The finding that dexamethasone triggers cathelicidin expression, yet fails to induce antimicrobial activity, suggested that dexamethasone does not induce or dexamethasone suppresses other critical factors involved in the macrophage antimycobacterial host response. To further investigate dexamethasone-regulated macrophage programs, we performed whole genome microarrays. Therefore, MDMs were cultured with dexamethasone and/or IFN-\(\gamma\), or media alone. To uncover networks of highly interconnected genes, we subjected the microarray data to WGCNA, a systems biology approach. In brief, this analysis uses pairwise gene correlations, which are weighted to favor more robust coexpression values, to group genes into modules. MEs were calculated that represented a theoretical average gene for each module. Enrichment for particular modules in each treatment group was calculated by correlating MEs to experimental conditions (Fig. 6A). This approach identified a green module of 620 genes, which was significantly associated with IFN-\(\gamma\) stimulation and the most inversely correlated module to dexamethasone treatment. ClueGO functional enrichment analyses showed that the green module was significantly linked to key functional pathways in antimicrobial host defense, including lytic vacuole/lysosome, latent infection (Mycobacterium tuberculosis), phagosome maturation (early endosomal stage), and defense response (Fig. 6B, 6C). Among the genes in this module are several v-ATPase subunits, such as TCIRG1 alias v-ATPase V0 subunit a3, ATP6V1C1, ATP6V1H, and ATP6V0E1, which are involved in acidification of lysosomes; DRAM1, which promotes autophagic defense against mycobacteria (33); and VPS18, linked to autophagolysosome fusion.

FIGURE 4. Dexamethasone (Dexa) induces cathelicidin independent of serum 25D in human macrophages. (A and B) MDMs were stimulated for 20 h either in (A) serum with 10% 25D-deficient human serum (25D serum level = 31 nmol/l) or (B) SFM with Dexa (100 nM), IFN-\(\gamma\), 1,25D (10 nM), or media alone. Gene expression of cathelicidin and CYP24A1 was assessed by quantitative PCR [mean fold change ± SEM; (A), \(n=5\), (B), \(n=6\)]. (C) MDMs were stimulated for 20 h in SFM with either mifepristone (Mife; 1 \(\mu\)M), Dexa (1000 nM), 1,25D (10 nM), or media alone as indicated. Cathelicidin gene expression was determined by quantitative PCR (mean fold change ± SEM, \(n=5\)). *\(p<0.05\).

FIGURE 5. Dexamethasone (Dexa) fails to induce antimycobacterial activity in human macrophages. MDMs were infected with BCG for 3–4 h before stimulation with Dexa (100 nM), IFN-\(\gamma\), both in combination, or media alone for 3 d. Viable bacteria were quantified by CFU assay on day 3. Percentage antimicrobial activity relative to media (left panel) and CFU counts (right panel) are shown. Each colored row represents data obtained with MDMs from one individual donor (\(n=3\)).
Several genes (CYBB, IFI6, PRKCD, SOCS3, SPI1) for IFN-γ signaling and TNF were also connected to this module. Taken together, our analyses revealed a network of host defense genes connected to phagosome maturation and the lysosome, fundamental aspects of the antimycobacterial host defense, which is associated with IFN-γ but not glucocorticoid treatment.  

**IFN-γ, yet not dexamethasone, induces TCIRG1 expression and phagolysosome recruitment, as well as lysosome acidification**

The host defense genes in the green module identified by the ClueGO functional group analyses were next ranked according to their kME values (Fig. 6C), revealing TCIRG1 (kME = 0.98) as the top hub gene, which is the most connected gene. We and others previously showed that induction and lysosomal recruitment of TCIRG1 is linked to lysosome acidification, a hallmark of phagolysosome maturation, and subsequent antimycobacterial activity in human macrophages (23, 34), prompting us to study TCIRG1 in more detail. First, we measured TCIRG1 mRNA expression using quantitative PCR, demonstrating that dexamethasone suppressed TCIRG1 mRNA expression by 33% compared with media in MDMs after 20 h (p < 0.01; Fig. 7A). In contrast, IFN-γ triggered TCIRG1 mRNA expression by 2.1-fold when compared with media (p < 0.01; Fig. 7A). Similar results were observed in BCG-infected
FIGURE 7. IFN-γ triggers but dexamethasone (Dexa) fails to induce TCIRG1 expression, phagolysosome recruitment, and lysosome acidification in human macrophages. (A–C) MDMs were stimulated with Dexa (100 nM), IFN-γ, or media alone for 20 h. (A) Gene expression of TCIRG1 was assessed by quantitative PCR (mean fold change ± SEM, n = 12). (B) Intracellular TCIRG1 protein expression was determined by FACS analyses (% TCIRG1bright cells). (C) MDMs were fixed and immunostained with anti-TCIRG1 and anti-LAMP1. Original magnification ×40. (D) Quantification of (C). Left panel, Percentage of TCIRG1bright cells. Right panel, Manders overlap coefficient M2 (fraction of LAMP1 overlapping TCIRG1). (E) MDMs were infected with BCG (pMV261::dsRed) for 3 h and stimulated with Dexa (100 nM), IFN-γ, or media alone for 20 h, fixed, immunostained with anti-TCIRG1, and analyzed by confocal microscopy. Image brightness was enhanced equally across the entire image per journal policy. (F) Quantification of (E). Left panel, Percentage of BCG/BCG clusters with TCIRG1 recruitment. Right panel, Manders overlap coefficient M2 (fraction of BCG overlapping TCIRG1). (G) MDMs were stimulated with Dexa (100 nM), IFN-γ, IFN-γ and Bafilomycin A1 in combination, or media alone for 20 h and stained with LysoSensor Green. Acidification was determined by FACS analyses (% LysoSensor-positive cells). *p < 0.01.
dexamethasone inhibited DRAM1 mRNA expression by 58% (p < 0.05; Supplemental Fig. 3C), whereas IFN-γ triggered DRAM1 mRNA expression by 4.3-fold (p < 0.05; Supplemental Fig. 3C) as measured by quantitative PCR. The induction of DRAM1 mRNA expression and the association of the green module to autophagy supported the known role of autophagy in the IFN-γ-mediated phagosome maturation and lysosome acidification (7, 8, 35, 36), which we further investigated. Macrophages were activated with IFN-γ in the absence or presence of two inhibitors of autophagy, specifically 3-MA and Wortmannin, both of which blocked induction of lysosome acidification by IFN-γ (Supplemental Fig. 3D), supporting the pivotal role of autophagy in promoting lysosome acidification (7, 8, 35, 36).

**Imatinib induces antimicrobial activity but has no effect on inflammatory cytokine patterns in dexamethasone-treated macrophages**

The tyrosine kinase inhibitor imatinib has emerged as a host-directed therapy for TB and other infections (23, 39, 40). In human macrophages imatinib was shown to induce the v-ATPase, leading to lysosome acidification and antimicrobial activity against *M. tuberculosis* (23). This prompted us to investigate whether imatinib can trigger lysosome acidification in dexamethasone-treated human macrophages. We found that imatinib alone and in combination with dexamethasone promoted lysosome acidification (10.8 and 15.5% LysoSensor-positive cells versus 1.4% in the media control, both p < 0.05; Fig. 8A, 8B). The ability to promote lysosome acidification was next tested in monocytes from patients on long-term glucocorticoid therapy. We decided to use monocytes directly isolated from blood, instead of MDMs, for this experiment to avoid decline of glucocorticoid effects during the in vitro differentiation period. Of relevance for this model we showed that IFN-γ induced, yet dexamethasone failed to trigger TCIRG1 and DRAM1 mRNA expression, as well as lysosome acidification in monocytes from healthy donors (Supplemental Fig. 4A, 4B). We cultured blood-derived monocytes of four patients receiving long-term glucocorticoid therapy for pyoderma gangrenosum, polymyalgia rheumatica, psoriatic arthritis, and pyoderma gangrenosum, respectively, with imatinib or media alone for 20 h. Imatinib stimulation increased the number of LysoSensor-positive cells when compared with media as measured in patient monocytes by FACS (30.1% LysoSensor-positive cells versus 6.0% in the media control, p < 0.01; Fig. 8C, Supplemental Fig. 4C).

Next, we investigated whether imatinib promotes antimycobacterial activity in dexamethasone-treated MDMs and found that imatinib alone and in combination with dexamethasone induced mycobacterial growth restriction by 69 and 64%, respectively, as compared with media control (both p < 0.05; Fig. 8D), whereas dexamethasone alone failed to induce antimicrobial activity (Fig. 8D). Imatinib had no effect on the dexamethasone induction of cathelicidin (Fig. 8E).

Given that the therapeutic benefits of glucocorticoids are fundamentally linked to glucocorticoid-mediated anti-inflammatory effects, we tested whether imatinib modifies the dexamethasone-induced anti-inflammatory cytokine profile in human macrophages. We found that imatinib treatment did not reverse dexamethasone-mediated suppression of baseline pro-IL-1β and TNF-α expression, as well as induction of IL-10 expression as compared with the media control (Fig. 8F). Taken together, these data showed that imatinib promotes lysosome acidification and antimicrobial activity in glucocorticoid-treated macrophages, without reversing the anti-inflammatory properties of glucocorticoids.

**Discussion**

In this study, we provide evidence that glucocorticoids induce cathelicidin in human macrophages. However, glucocorticoid induction of cathelicidin occurs independently of autophagy, phagosome maturation, and lysosome acidification, known to be crucial for host defense (6–8, 35, 36). Thus, although critical (6, 7, 14–16), the induction of cathelicidin is not sufficient to promote antimycobacterial host defense. Nevertheless, we found that imatinib promotes lysosome acidification and antimycobacterial activity in glucocorticoid-stimulated macrophages. Strikingly, imatinib did not reverse the anti-inflammatory effects of glucocorticoids, thereby suggesting a preventive and therapeutic potential of imatinib in the context of glucocorticoid therapy.

A key finding of our study was the discovery of a glucocorticoid-repressed and IFN-γ–induced host defense network linked to phagosome maturation and lytic vacuole/lysosome, which we identified the v-ATPase subunit a3 TCIRG1 as a top hub gene. A central role for the v-ATPase V0 in the host response to mycobacterial infections was suggested by Russell and colleagues (41), who demonstrated that the v-ATPase V0 is excluded from the nascent mycobacteria phagosome. In this study we found that IFN-γ induces TCIRG1 expression (42), its recruitment to bacterial-loaded vacuoles, and promoted lysosome acidification (23, 43–45). We previously reported that TCIRG1, in concert with other subunits of the v-ATPase V0, was induced and recruited to the mycobacterial phagolysosome by pharmacological activation with imatinib, resulting in phagosome maturation and lysosome acidification, and subsequent antimicrobial activity against *M. tuberculosis* in human macrophages (23). The ability of imatinib to trigger lysosome acidification in glucocorticoid-treated macrophages identifies a strategy for augmenting antimicrobial responses in the context of glucocorticoid treatment.

We observed that in dexamethasone-treated macrophages cathelicidin colocalizes with intracellular mycobacteria. Therefore, the crucial role of phagolysosome maturation and acidification in the antimicrobial response could mean that cathelicidin-mediated killing is pH dependent. Another, yet not mutually exclusive explanation is that dexamethasone-induced cathelicidin is not efficiently processed from its inactive precursor hCAP18 into the active peptide LL-37. Despite the pivotal role of cathelicidin in the macrophage host response, only few mechanisms involved in regulating its expression are known. The human cathelicidin promoter contains three vitamin D response elements, and 1,25D is a direct inducer of cathelicidin (12, 13). Because physiological 1,25D serum levels are not sufficient to trigger the VDR-mediated induction of cathelicidin, macrophages rely on the intracellular conversion of 25D to 1,25D by the CYP27B1-hydroxylase. In fact, described immune mechanisms resulting in upregulation of cathelicidin gene expression to date, including activation by TLR2/1 ligand and IFN-γ, converge on the induction of CYP27B1 and the conversion of 25D to 1,25D (6, 7). However, glucocorticoid induction of cathelicidin was not linked to CYP27B1 induction, nor was it dependent on serum 25D levels. Thus, glucocorticoid induction of cathelicidin does not involve the previously described vitamin D pathway. The detailed molecular mechanisms by which glucocorticoids induce cathelicidin remain to be investigated. One mechanism, by which glucocorticoids induce transcription, is via GR binding to GR response elements on target genes. GR response elements are found within the promoter, introns, or exons of target genes (1). However, the human cathelicidin gene does not contain GR binding sites based on the DNA consensus.
sequences 5′-GGTACAnnnTGTCT-3′ (46) and 5′-AGAA-
CAnnnTGTCT-3′ (1). Notably, besides the vitamin D–mediated induction, which is amplified by increased histone acetylation (47), little is known about pathways activating cathelicidin. In keratinocytes, but not myeloid cells, endoplasmic reticulum stress or resveratrol were shown to trigger induction of sphingosine-1-phosphate, which in turn activates NF-κB–mediated activation of C/EBP leading to cathelicidin production (48, 49). In human macrophages activated with dexamethasone, we observed repression of cathelicidin mRNA expression was asessed by quantitative PCR (mean fold change ± SEM, n = 6). (E) MDMs were stimulated as in (A). Cathelicidin mRNA expression was assessed by quantitative PCR (mean fold change ± SEM). (F) MDMs were treated with glucocorticoids (59).

The induction of cathelicidin by glucocorticoids seems not to be macrophage specific, because microarray analyses showed that dexamethasone also triggered cathelicidin gene expression in the human lung A549 cell line (50).

An important mechanism of phagolysosome maturation and acidification is the induction of autophagy (7, 8, 35, 36). Consistently, IFN-γ, but not glucocorticoids, promoted induction of DRAM1. Recently, DRAM1 was identified as a key host defense gene in antimycobacterial host defense in zebrafish and human macrophages (33). DRAM1 was found to trigger selective autophagy downstream of TLR signaling promoting mycobacterial phagosome maturation, phagolysosome fusion, and antimycobacterial activity (33). Knockdown of DRAM1 inhibits the v-ATPase and acidification of lysosomes (51). Thus, it is tempting to speculate that DRAM1 links IFN-γ induction of autophagy, TCIRG1, and acidification of lysosomes.

There is a great interest in the biomedical community regarding the development of host-directed therapies to treat infections. In TB, for instance, the induction of cathelicidin, autophagy, and phagosome maturation are potential targets (52). In our study the ability of imatinib to trigger an antibacterial response was performed using an avirulent mycobacterium, such that future studies are required to examine the role of imatinib against virulent mycobacteria. Nevertheless, imatinib has been demonstrated effective as a host-directed therapy in a mouse model of TB infection (39, 40) and is currently being developed for a TB clinical trial (53). Future studies in animal models and clinical trials should investigate the effectiveness of imatinib in combination with glucocorticoids in the host-directed treatment of mycobacterial infections. This may be particularly useful in the management of inflammatory reactions (54–58), including immune reconstitution inflammatory syndrome, which occurs in HIV/TB-coinfected individuals at the initiation of chemotherapy and which is treated with glucocorticoids (59).
Although we demonstrate the role of glucocorticoids in modulating the innate macrophage antimicrobial response, there is evidence for immunosuppressive effects on innate instruction of the adaptive T cell response as well as a direct effect on T cells (60). In this context, glucocorticoid therapy increases the likelihood of false-negative or indeterminate results of the IFN-γ release assay used to diagnose TB in humans (61, 62). Although our data indicate that imatinib can overcome the deleterious effects of glucocorticoids on innate antimicrobial responses, imatinib is also known to suppress adaptive T cell responses (23). Nevertheless, there are only sporadic reports of infections in imatinib-treated patients (23). In this regard, our study, by showing that imatinib promotes lysosome acidification and antimicrobial activity in glucocorticoid-stimulated macrophages without reversing glucocorticoid-mediated anti-inflammatory effects, suggests one promising strategy to maintain innate antimicrobial activity in patients receiving glucocorticoids for treatment of chronic inflammatory diseases.

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