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Mycobacteria Inhibition of IFN-γ Induced HLA-DR Gene Expression by Up-Regulating Histone Deacetylation at the Promoter Region in Human THP-1 Monocytic Cells[1]

Yue Wang,* Heather M. Curry,* Bruce S. Zwilling,† and William P. Lafuse[2]*

Infection of macrophages with mycobacteria has been shown to inhibit the macrophage response to IFN-γ. In the current study, we examined the effect of *Mycobacteria avium*, *Mycobacteria tuberculosis*, and TLR2 stimulation on IFN-γ-induced gene expression in human PMA-differentiated THP-1 monocytic cells. Mycobacterial infection inhibited IFN-γ-induced expression of HLA-DRα and HLA-DRβ mRNA and partially inhibited CIITA expression but did not affect expression of IFN regulatory factor-1 mRNA. To determine whether inhibition of histone deacetylase (HDAC) activity could rescue HLA-DR gene expression, butyric acid and MS-275, inhibitors of HDAC activity, were added at the time of *M. avium* or *M. tuberculosis* infection or TLR2 stimulation. HDAC inhibition restored the ability of these cells to express HLA-DRα and HLA-DRβ mRNA in response to IFN-γ. Histone acetylation induced by IFN-γ at the HLA-DRα promoter was repressed upon mycobacteria infection or TLR2 stimulation. HDAC gene expression was not affected by mycobacterial infection. However, mycobacterial infection or TLR2 stimulation up-regulated expression of mammalian Sin3A, a corepressor that is required for MHC class II repression by HDAC. Furthermore, we show that the mammalian Sin3A corepressor is associated with the HLA-DRα promoter in *M. avium*-infected THP-1 cells stimulated with IFN-γ. Thus, mycobacterial infection of human THP-1 cells specifically inhibits HLA-DR gene expression by a novel pathway that involves HDAC complex formation at the HLA-DR promoter, resulting in histone deacetylation and gene silencing. *The Journal of Immunology,* 2005, 174: 5687–5694.

Mycobacteria are facultative intracellular pathogens that are able to survive and multiply within macrophages for an extended period of time. *Mycobacterium tuberculosis*, the causative agent of tuberculosis, is responsible for 3 million deaths annually. Infections by *Mycobacterium avium* are common in AIDS patients (1, 2). The activity of macrophages is regulated by IFN-γ. IFN-γ increases expression of MHC class II molecules and other IFN-γ-inducible genes in macrophages (3) and activates anti-mycobacterial activity (4–6). However, macrophages infected with mycobacteria respond poorly to IFN-γ. Studies have shown that cell-mediated immune response of infected macrophages is impaired by decreased IFN-γ-induced expression of MHC class II genes and other IFN-γ-inducible genes (7–10).

IFN-γ-stimulated signal transduction results in the activation of the JAK-STAT pathway in which STAT1 is tyrosine phosphorylated and translocates to the nucleus where it binds to the IFN-γ activation site (GAS)[3] sequence in the promoters of IFN-γ-induced genes (11–13). Studies from this laboratory (7) have investigated the mechanism involved in the inhibition of IFN-γ-induced gene expression in *M. avium*-infected mouse macrophages. We observed that *M. avium* inhibits IFN-γ-inducible genes by interfering with the JAK-STAT1 signal transduction pathway, resulting in reduced phosphorylation of IFN-γRα, JAK1/JAK2, and STAT1. Recognition of *M. avium* by macrophages has been shown to involve TLR2 (14, 15). IFN-γ-induced gene expression is also inhibited by prior treatment with TLR2 agonists (16–18). Although TLR2 signaling does not inhibit STAT1α phosphorylation, we showed that TLR2 stimulation increases expression of the transcriptionally inactive STAT1β by stabilizing the STAT1β mRNA and thereby decreasing IFN-γ-induced gene expression by dominant negative inhibition (18). In contrast, *M. tuberculosis* infection of human macrophages results in more limited inhibition of IFN-γ-induced gene expression with some genes being inhibited such as CD64, HLA-DR, and CIITA, while others were unaffected (7–10). *M. tuberculosis* infection of human macrophages also does not appear to inhibit the JAK-STAT1 pathway.

The purpose of the current study was to compare the effects of *M. tuberculosis*, *M. avium*, and TLR2 stimulation on IFN-γ induction of HLA-DR gene expression in human THP-1 monocytic cells differentiated with PMA. Class II MHC expression in macrophages results from IFN-γ-induced expression of CIITA (19–21). Although CIITA does not bind to DNA directly, it functions as a transcriptional coactivator by interacting with MHC class II promoter-bound transcription factors (22) and coordinating histone acetylation modification at the HLA-DRA promoter (23) by interacting with CREB-binding protein (CBP/p300) (24, 25), which has intrinsic histone acetyltransferase (HAT) activity (26–28). Histone acetylation is reversible and is regulated by HATs, which promote gene activation, and histone deacetylases (HDACs), which promote repression of acetylation-sensitive genes.

We found that induction of HLA-DR expression by IFN-γ was highly inhibited by *M. avium*, *M. tuberculosis*, and TLR2 stimulation, whereas induction of CIITA was partially inhibited. In contrast, IFN regulatory factor (IRF)-1 expression was not altered.

Departments of *Molecular Virology, Immunology, and Medical Genetics and †Microbiology, Ohio State University, Columbus, OH 43210

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[2] Address correspondence and reprint requests to Dr. William P. Lafuse, Department of Molecular Virology, Immunology, and Medical Genetics, Ohio State University, 333 West 10th Avenue, Columbus, OH 43210. E-mail address: lafuse.1@osu.edu

[3] Abbreviations used in this paper: GAS, IFN-γ activation site; IRF-1, IFN regulatory factor-1; HDAC, histone deacetylase; HAT, histone acetyltransferase; IRF, IFN regulatory factor; ChIP, chromatin immunoprecipitation assay; mSin3A, mammalian Sin3A; CBP, CREB-binding protein.

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Because studies (29, 30) have shown that MHC class II expression can be inhibited by HDAC activity, we investigated the role of HDAC activity in the inhibition of HLA-DR expression. Our studies show that mycobacteria and mycobacterial products inhibit IFN-γ-induced HLA-DR expression in human macrophages through a repressor pathway involving chromatin deacetylation in the HLA-DRα promoter.

Materials and Methods

Reagents

PMA, sodium butyrate, and RNase A were purchased from Sigma-Aldrich. MS-275 was purchased from Alexis Biochemicals. Human recombinant IFN-γ was obtained from Genentech. Pam_3CSK_4 was acquired from EMC Microcollections. [^{32}P]dCTP was obtained from Amersham Biosciences. Proteinase K was purchased from Invitrogen Life Technologies.

Cell culture

The human THP-1 monocyte leukemia cell line, obtained from American Type Culture Collection (ATCC TIB-202), was cultured in RPMI 1640 containing 10% heat-inactivated FBS, 4 g/L glucose, 1.0 mM sodium pyruvate, 10 mM HEPES, 0.05 mM 2-ME, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37°C in 5% CO_2. Before each experiment, THP-1 monocytes were plated in six-well culture plate and treated with 10 ng/ml PMA for 48 h to differentiate the cells into macrophage-like cells.

Mycobacterium infection and IFN-γ stimulation *M. avium* (ATCC 35713) were grown and stored as described previously (7). Bacteria concentration was determined by OD at 600 nm wavelength and confirmed by plate counting. Gamma-irradiated *M. tuberculosis* H37Rv (Colorado State University; National Institutes of Health, National Institute of Allergy and Infectious Diseases Contract N01 AI-75320) was diluted in Middlebrook 7H9 broth (Difco) and centrifuged 800 rpm for 10 min to eliminate clumped bacteria. The protein concentration in the supernatant was determined by the Bradford protein assay (Bio-Rad). The supernatant was stored in aliquots at −80°C. Frozen *M. avium* and *M. tuberculosis* aliquots were thawed and briefly sonicated before use. Differentiated THP-1 cells were treated with *M. avium*, gamma-irradiated *M. tuberculosis*, and Pam_3CSK_4 for 16–20 h. The THP-1 cells were then stimulated with 100 U/ml human IFN-γ for the indicated time in each experiment.

Northern blot hybridization

Total cytoplasmic RNA was isolated by the acid guanidinium isothiocyanate-phenol-chloroform extraction method (31). Fifteen micrograms of RNA were separated in 1% formaldehyde agarose gels and transferred to Hybond-N^+ membranes by capillary blotting. Northern blot hybridization was performed as described previously (32). The cDNA probes to HLA-DRα (ATCC clone 57392), DRβ (ATCC clone 57081), human IRF-1 (Genestorm clone RG001570; Invitrogen Life Technologies), murine β-actin, and murine G3PDH (7) were labeled with [^{32}P]dCTP by the High Prime labeling system (Roche).

RT-PCR

One microgram of total RNA was reverse transcribed with avian myeloblastosis virus reverse transcription system (Promega). PCRs were performed in 1× PCR buffer, 3 mM $MgCl_2$, 0.2 μM dNTP, 0.4 μM of each gene-specific primer, and 2 U of Platinum TaqDNA polymerase (Invitrogen Life Technologies). The amplification was 95°C for 5 min, then 27–35 cycles of 94°C for 45 s, 60°C for 45 s, and 72°C for 1 min.

The following primers were used: human mammalian Sin3A (mSin3A) (617 bp) sense, 5′-TGTTCACCATTCATGCCTACATTGCC-3′, and antisense, 5′-GGCGGTCCTCCGATACATATAGTCC-3′; G3PDH (988 bp), sense, 5′-TGAAGGTCGGTGTGAACGGATTTGGC-3′, and antisense, 5′-CATGTAGGCCATGAGGTCCACCAC-3′; and human β-actin (170 bp) sense, 5′-CCCCGCGAGCACAGA-3′, and antisense, 5′-CAC CGATGGAGGGGAAGAC-3′. PCR products were visualized on 1.6% agarose gels containing ethidium bromide.

Protein extraction and Western blot analysis

Whole cell lysates were prepared by extraction in lysis buffer containing 1% Triton X-100, 20 mM Tris-HCl (pH 8.0), 150 mM NaCl, 3 mM sodium pyrophosphate, 10% glycerol, 2 mM sodium orthovanadate, and protease inhibitors pepstatin (6 μg/ml), aprotinin (6 μg/ml), leupeptin (10 μg/ml), and 4-(2-aminoethyl)-benzenesulforyl fluoride (100 μg/ml). Nuclear extracts were prepared from 10^7 differentiated THP-1 cells as described previously (7). Protein concentration was determined by the Bradford protein assay (Bio-Rad).

Twenty-five to 40 μg of protein were analyzed by SDS-PAGE using 10% Tris-glycine gels (Invitrogen Life Technologies), followed by transfer to Hybond-P membranes (Amersham Biosciences). Membranes were blocked in 5% BLOT-QuickBlocker (Genotech) in TBS containing 0.05% Tween 20 for 1 h and incubated with primary Abs at 4°C overnight. The detection step was performed with HRP-coupled anti-mouse or anti-rabbit IgG Abs (1:2500 and 1:5000; Genotech). Primary Abs were monoclonal mouse anti-tyrosine-phospho-STAT1 Ab (1:4000; Zymed Laboratories), polyclonal rabbit anti-STAT1α p91 Ab (1:1000; Santa Cruz Biotechnology), polyclonal rabbit anti-HDAC1 Ab (1:200; Santa Cruz Biotechnology), polyclonal rabbit anti-HDAC2 Ab (1:200; Santa Cruz Biotechnology), and polyclonal rabbit anti-mSin3A Ab (1:200; Santa Cruz Biotechnology). Blots were developed with the femtoLucent chemiluminescence detection system (Genotech).

EMSA

EMSA was performed in 20-μl binding reactions containing 5 μg of nuclear extracts, 10 mM Tris-HCl (pH 7.5), 50 mM NaCl, 50 mM DTT, 5 mM $MgCl_2$, 10% glycerol, 0.2% Nonidet P-40, 1 μg of poly(dI:dC), and 70,000 cpm of [^{32}P]dCTP-labeled, double-stranded GAS oligonucleotide radiolabeled by fill-in reaction with Klenow DNA polymerase. The sequence of the GAS oligonucleotide (5′-AGCCATTTCCAGGAATC GAAA-3′) contains the optimum GAS sequence (5′-TTCCSGGAA-3′) for STAT1 DNA binding (33). The samples were electrophoresed in 5% polyacrylamide gels in 0.5× Tris-borate EDTA. The gels were then dried and analyzed by autoradiography.

Chromatin immunoprecipitation assay (ChIP)

Chromatin immunoprecipitations were done using the Acetyl-Histone H4 ChIP assay kit (Upstate Biotechnology). A total of 4×10^6 cells was fixed in 1% formaldehyde at 37°C for 10 min, then washed with ice-cold PBS containing protease inhibitors, 1 mM PMSF, 1 μg/ml aprotinin, 10 μg/ml 4-(2-aminoethyl)-benzenesulforyl fluoride, and 1 μg/ml pepstatin A. Cells were incubated in 400 μl of lysis buffer containing 1% SDS, 10 mM EDTA, 50 mM Tris-HCl (pH 8.1), and protease inhibitors for 10 min on ice. The chromatin was sheared on ice by sonication to lengths between 300 and 800 bp by six 10-s bursts with a Branson 350 sonifier (Branson Precise Processing Group). Two-hundred microliters of sheared chromatin were diluted 1/10 with ChIP dilution buffer containing 0.01% SDS, 1.1% Triton X-100, 1.2 mM EDTA, 16.7 mM Tris-HCl (pH 8.1), 167 mM NaCl, and protease inhibitors. Chromatin was precleared with 80 μl of salmon sperm DNA-blocked protein A agarose beads for 30 min at 4°C, then incubated with 5 μl of rabbit anti-acetyl-histone H4 Ab (Upstate Biotechnology) at 4°C overnight. In a separate set of experiments, chromatin was incubated with 25 μl of rabbit anti-mSin3A, and rabbit anti-CREB-binding protein (CBP) (Santa Cruz Biotechnology). A no-Ab control reaction was set up as well. The immune complexes were precipitated with 60 μl of salmon sperm DNA-blocked protein A agarose beads for 30 min at 4°C, followed by washing with low salt wash buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl (pH 8.1), and 150 mM NaCl), high salt wash buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl (pH 8.1), and 500 mM NaCl), LiCl wash buffer (0.25 M LiCl, 1% Nonidet P-40, 1% deoxycholate, 1 mM EDTA, and 10 mM Tris-HCl (pH 8.1)), and TE buffer (1 mM EDTA and 10 mM Tris-HCl (pH 8)). The immune complexes were eluted with 500 μl of 1% SDS, 0.1 M $NaHCO_3$ and incubated in the presence of 200 mM NaCl at 65°C for 4 h to reverse the histone-DNA cross-links. The samples were then digested with 40 μg/ml proteinase K, 10 mM EDTA, and 40 mM Tris-HCl (pH 6.5) at 45°C for 1 h. The DNA was purified by phenol-chloroform extraction, followed by ethanol precipitation. The recovered DNA was dissolved in 20 μl of molecular grade water. Two microliters of DNA were used for real-time PCR as described below.

Real-time PCR

Total RNA was extracted with RNeasy Mini kit (Qiagen) following manufacturer's instructions. One microgram of RNA was reverse transcribed with avian myeloblastosis virus reverse transcription system (Promega) and dissolved in 50 μl of molecular grade water. Two microliters of cDNA were analyzed by real-time PCR using LightCycler-FastStart DNA Master SYBR Green I (Roche), according to manufacturer's directions. Reactions were run at 95°C for 10 min and 40 cycles of 95°C for 15 s, 60°C for 5 s, and 72°C for 15 s on a RotorGene 2000 Real-Time Quantitative Thermal Cycler System (Corbett Research, Pyrosequencing). The following primers were used for detecting HDAC and CIITA gene expression: HDAC1 217-773 (5′-GAAATCTATCGCCCTCACAAAGCCAATGC-3′, 5′-TAG GACTCGTCATCAATCCCGTCTCGG-3′); HDAC2 487-995 (5′-TTTA

ATGTTGGAGAAGATTGTCCAGCG-3′, 5′-GCACCACACTGTAATA CCACAGCACTAGG-3′); HDAC3 144-795 (5′-CAGACCCATAGCTG GTCCTGCATTACG-3′, 5′-AGAAGTCCACTACCTGGTTGATAACC GGC-3′); and CIITA 3222-3713 (5′-TGACCTGGGTGCCTACAAACTC-3′, 5′-GCAAGATGTGGTTCATTCCGC-3′). Gene expression was normalized with G3PDH primers 66-291, (5′-GAAGGTGAAGGTCG GAGTC-3′, 5′-GAAGATGGTGATGGGATTTC-3′).

Double-strand DNA purified from the ChIP assay was also subject to real-time PCR with previously described HLA-DRA promoter primers (5′-GATTTGTTGTTGTTGTCCTGTTC-3′, 5′-CCCAATTACTCTTTG GCCAATCAGAAAAATATTTTG-3′) (20). The relative values was calculated by the comparative C_T method (34). Statistical analysis was determined using one-way ANOVA and Tukey's test. Differences were considered to be significant if $p < 0.05$.

Results

M. avium *inhibits IFN-γ-induced HLA-DR and CIITA gene expression but not IRF-1 gene expression in human THP-1 monocytic cells*

To investigate whether *M. avium* inhibits IFN-γ-inducible gene expression in human THP-1 cells, we infected PMA-differentiated human THP-1 cells with *M. avium* at 5:1, 10:1, 20:1, and 40:1 bacteria to cell ratios for 16 h, followed by stimulated with human rIFN-γ at 100 U/ml for 20 h. The expression of HLA-DR and IRF-1 was investigated by Northern blot analysis and expression of CIITA by real-time RT-PCR. Expression of HLA-DRα and HLA-DRβ mRNA was highly induced by IFN-γ. The IFN-γ induction of the MHC class II genes was inhibited by *M. avium* infection, and the degree of inhibition correlated with the dosage of *M. avium* (Fig. 1*A*). Expression of IRF-1 mRNA was also highly induced by IFN-γ treatment. However, the expression remained unaffected by *M. avium* infection in human THP-1 cells (Fig. 1*B*). Similar induction of CIITA gene expression by IFN-γ was also observed in CIITA gene expression (Fig. 1*C*). *M. avium* infection also decreased the induction of CIITA expression by IFN-γ. However, at bacteria:cell ratios of 20:1 and higher, where IFN-γ induction of HLA-DRα is inhibited completely, substantial induction of CIITA was still observed ~20-fold.

M. avium *infection does not inhibit STAT1 activation and tyrosine phosphorylation in human THP-1 monocytic cells*

To determine the effect of *M. avium* infection of THP-1 cells on STAT1 activation, we examined by EMSA the binding of STAT1 to a consensus GAS element and by Western blotting STAT1 tyrosine phosphorylation. Nuclear extracts prepared from *M. avium*-infected THP-1 cells stimulated with IFN-γ showed no reduction of STAT1 binding to the GAS element (Fig. 2*A*). There was also no reduction in STAT1 tyrosine phosphorylation by Western blot analysis (Fig. 2*B*). We did observe a slight increase of total STAT1 protein expression in infected cells, suggesting that *M. avium* infection increases STAT1 gene expression in THP-1 cells (Fig. 2*B*). These results are in contrast to our previous studies of *M. avium*-infected mouse macrophages in which we observed inhibition of the JAK-STAT1 pathway (7) but in agreement with the recent observations in *M. tuberculosis*-infected human THP-1 cells (10). Taken together, these results indicate that in human THP-1 cells, inhibition of HLA-DR gene expression by *M. avium* infection is independent of the JAK-STAT1 signal transduction pathway.

IFN-γ-induced HLA-DR gene expression is also inhibited by M. tuberculosis *infection and TLR2 agonist stimulation of human THP-1 monocytic cells*

We also investigated whether *M. tuberculosis* affects IFN-γ-induced gene expression through a similar mechanism as *M. avium*. We infected PMA-differentiated THP-1 cells with gamma-irradiated *M. tuberculosis* for 16 h and stimulated the THP-1 cells with IFN-γ for 20 h. Gene expression of HLA-DRα, HLA-DRβ, and IRF-1 was examined by Northern blot analysis. *M. tuberculosis* also inhibited HLA-DRα and HLA-DRβ gene expression in a dose-dependent manner. In contrast, IRF-1 gene expression was not affected following *M. tuberculosis* infection (Fig. 3*A*).

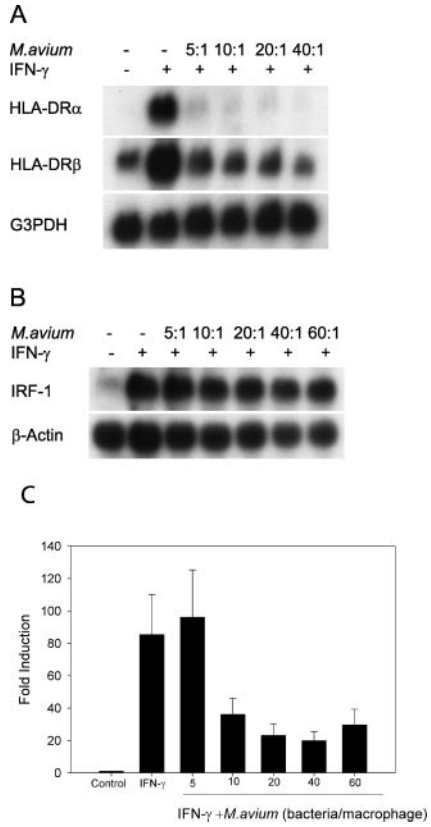

FIGURE 1. Infection of THP-1 cells with *M. avium* results in inhibition of IFN-γ-induced HLA-DRα and HLA-DRβ gene expression and CIITA gene expression but no inhibition of IRF-1. THP-1 cells, differentiated by treatment with 10 ng/ml PMA for 48 h, were infected with live *M. avium* at the indicated bacteria-to-macrophage ratios for 20 h and then stimulated with 100 U/ml human rIFN-γ. Total RNA was extracted after 20 h of IFN-γ stimulation, and gene expression was determined by Northern blot hybridization. *A*, HLA-DRα and HLA-DRβ; *B*, IRF-1. Blots were stripped and reprobed with G3PDH or β-actin probes. These experiments are representative of three independent experiments. *C*, *M. avium*-infected THP-1 cells were stimulated with human IFN-γ for 12 h. Real-time RT-PCR was performed with human CIITA and G3PDH cDNA primers. Results with CIITA primers were normalized to G3PDH. These experiments are representative of five independent experiments.

Activation of the macrophage antimicrobial response involves stimulation of TLRs. *M. avium* activation of macrophages involves TLR2 (14, 15), whereas *M. tuberculosis* activation of macrophages involves both TLR2 and TLR4 (35, 36). As both *M. tuberculosis* and *M. avium* can activate macrophages through TLR2, we investigated whether TLR2 stimulation of THP-1 cells could also affect IFN-γ-inducible HLA-DR gene expression. PMA-differentiated THP-1 cells were treated with a TLR2 agonist, synthetic bacterial lipopeptide Pam_3CSK_4 (37), for 16 h followed by IFN-γ for 20 h. We found that IFN-γ-induced HLA-DRα and HLA-DRβ gene expression was also inhibited in differentiated THP-1 cells treated with Pam_3CSK_4. IRF-1 gene expression was not inhibited by TLR2 agonist treatment (Fig. 3*B*). Taken together, the results indicate that *M. avium* and *M. tuberculosis*, acting through TLR2, inhibit HLA-DR gene expression at an unknown step that is independent of JAK-STAT1 activation.

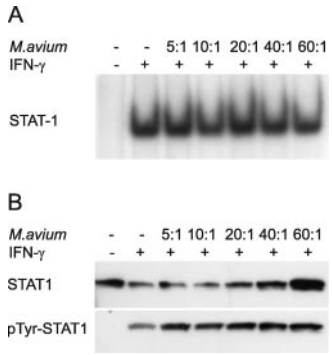

FIGURE 2. *M. avium* infection of THP-1 cells does not inhibit STAT1 signaling. PMA-differentiated THP-1 macrophages were infected for 20 h with live *M. avium* at the indicated bacteria-to-macrophage ratios and then were stimulated with human IFN-γ (100 U/ml) for 45 min. *A*, Nuclear extracts were prepared and STAT1 binding analyzed by EMSA with a ^{32}P-labeled GAS sequence. *B*, Western blots prepared from whole cell lysates probed with polyclonal anti-total STAT1 Ab and monoclonal anti-phospho-STAT1 (Y701) Ab. These results are representative of three experiments.

HDAC inhibitor rescues IFN-γ-induced HLA-DR gene expression in M. avium*, M. tuberculosis, and TLR2 agonist infected THP-1 cells*

Studies have shown that interactions of HAT and HDAC activity are important in regulating gene expression at nucleosomes (38, 39). Acetylation of lysine residues in the N-terminal tails of the core histone proteins results in the uncoiling of the chromatin, allowing increased accessibility for transcription factors, while tightly bound DNA around a nucleosome core suppresses gene transcription by decreasing the accessibility of transcription factors to the gene promoter (40). Gene-specific repression is associated with the recruitment of multicomponent HDAC complexes to promoters (40). Suppression of HDAC activity in human tumor cell lines, which fail to express HLA-DR expression upon IFN-γ stimulation, has been shown to rescue IFN-γ-inducible HLA-DR gene expression (29). Therefore, we were interested in determining whether modulation of chromatin conformation via enhanced HDAC activity was a possible mechanism involved in the HLA-DR gene repression by mycobacteria in THP-1 cells. To test this hypothesis, we treated PMA-differentiated THP-1 cells with a HDAC inhibitor, sodium butyrate, at the time of mycobacteria infection. A butyrate dose-response experiment was done on HLA-DRα and HLA-DRβ expression with *M. avium*-infected THP-1 cells. Treatment with butyrate resulted in a recovery of HLA-DRα and HLA-DRβ gene expression in *M. avium*-infected THP-1 cells in a dose-dependent manner (Fig. 4*A*). Similar results were observed in *M. tuberculosis*-infected and Pam_3CSK_4-treated THP-1 cells (Fig. 4*B*). The IRF-1 gene expression was not affected by butyrate (Fig. 4*B*). Butyrate alone was not an inducer of HLA-DRα and HLA-DRβ gene expression in THP-1 cells. It also did

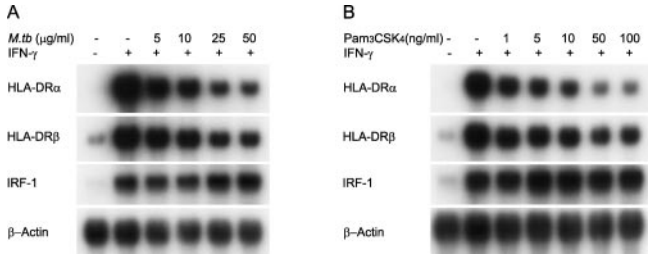

FIGURE 3. Irradiated *M. tuberculosis* and TLR2 stimulation with Pam_3CSK_4 also inhibits MHC class II expression but not IRF1. *A*, PMA-differentiated THP-1 cells were infected for 20 h with gamma-irradiated *M. tuberculosis* at the indicated protein concentrations (5–50 μg/ml) and then were stimulated with IFN-γ (100 U/ml) for 20 h. RNA was isolated, and Northern blot was hybridized with radiolabeled probes for HLA-DRα, HLA-DRβ, IRF-1, and β-actin. *B*, PMA-differentiated THP-1 cells were treated with the TLR2 agonist Pam_3CSK_4 for 20 h at the indicated concentrations (1–100 ng/ml) and then stimulated with IFN-γ (100 U/ml) for 20 h. RNA was isolated and gene expression determined by Northern blot hybridization. These results are experiments are representative of three independent experiments.

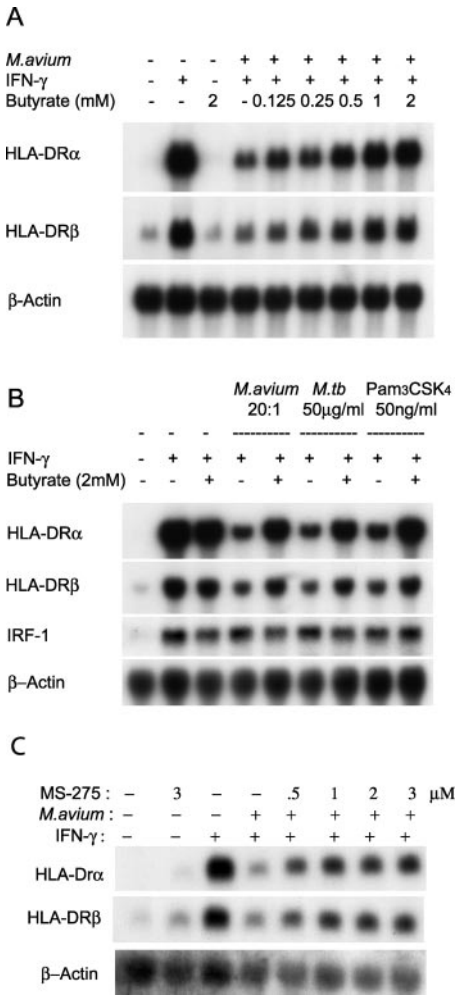

FIGURE 4. Inhibition of IFN-γ-induced HLA-DRα and HLA-DRβ gene expression by *M. avium*, *M. tuberculosis*, and TLR2 stimulation in human THP-1 cells was rescued by inhibition of HDAC activity with butyrate and MS-275. *A*, PMA-differentiated THP-1 cells were infected for 20 h with live *M. avium* at 10:1 in the presence of sodium butyrate at the indicated concentrations (0.125–2 mM) and then stimulated with IFN-γ (100 U/ml) for 20 h. RNA was isolated, and gene expression was determined by Northern blot hybridization. *B*, PMA-differentiated THP-1 cells were treated with *M. avium* (20:1 bacteria-to-macrophage ratio), irradiated *M. tuberculosis* (50 μg/ml), and Pam_3CSK_4 (50 ng/ml) in the presence of 2 mM butyrate for 20 h and then stimulated with IFN-γ (100 U/ml) for 20 h. RNA was isolated and gene expression analyzed by Northern blot hybridization. *C*, PMA-differentiated THP-1 cells were infected for 20 h with live *M. avium* at 10:1 in the presence of the HDAC inhibitor MS-275 at the indicated concentrations (0.5–3 μM) and then stimulated with IFN-γ (100 U/ml) for 20 h. RNA was isolated, and gene expression was determined by Northern blot hybridization. These results are representative of three independent experiments.

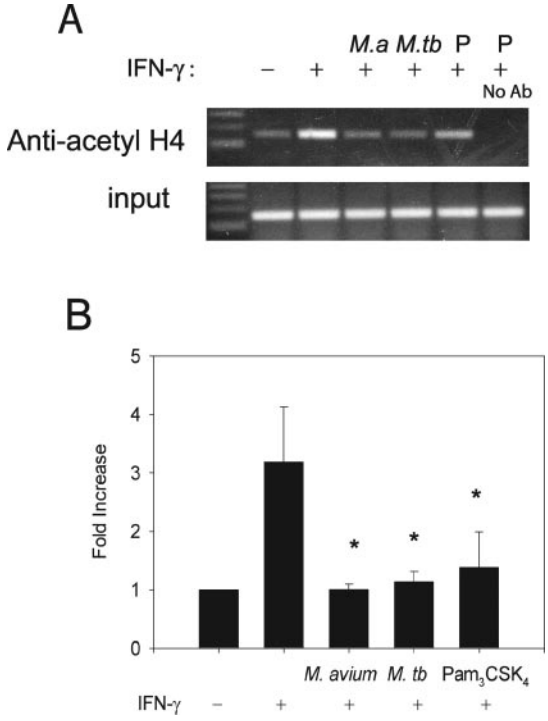

FIGURE 5. Mycobacteria infection and TLR2 stimulation inhibits histone acetylation at the HLA-DRα promoter in THP-1 cells. PMA-differentiated THP-1 cells were infected for 16 h with *M. avium* (20:1 bacteria-to-macrophage ratio), irradiated *M. tuberculosis* (50 μg/ml), or treated with Pam_3CSK_4 (50 ng/ml) and then stimulated with IFN-γ (100 U/ml) for 24 h. *A*, Chromatin was harvested from 1% formaldehyde-fixed cells and immunoprecipitated with anti-acetylated histone H4. DNA was purified from chromatin immunoprecipitates and amplified by PCR with primers to the HLA-DRα gene promoter. Input DNA purified from aliquots of chromatin representing 1% of the total amount of chromatin was also amplified by PCR. PCR products were fractionated on an agarose gel followed by ethidium bromide staining. *B*, Analysis by real-time PCR. Results are the mean $\pm$ SD of three separate experiments. $*$, $p < 0.05$, one-way ANOVA and Tukey's test.

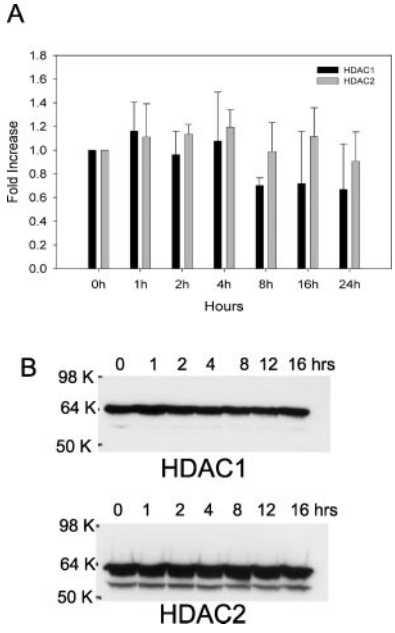

FIGURE 6. HDAC gene expression was not affected by mycobacteria infection. PMA-differentiated THP-1 cells were infected with *M. avium* at a bacteria-to-macrophage ratio of 20:1 for 0–24 h. *A*, HDAC1 and HDAC2 gene expression was detected by quantitative real-time RT-PCR. All results were normalized to G3PDH. The results represent mean $\pm$ SD of three separate experiments. *B*, Western blot analysis of HDAC1 and HDAC2 protein expression. Differentiated THP-1 cells were infected with *M. avium* at 20:1 for 0–16 h. Nuclear extracts were prepared, and HDAC protein expression was determined by Western blotting with Ab specific for HDAC1 and HDAC2. Results are representative of two experiments.

not alter IFN-γ-induced HLA-DR expression in THP-1 cells that were not treated with mycobacteria; it only rescued mycobacterial suppressed HLA-DR gene expression. Butyrate is a weak inhibitor of HDAC activity, and the high concentrations of butyrate required to inhibit HDAC activity raises concerns that butyrate may have other effects on cells. As an alternative to butyrate, experiments were also done using MS-275, which is a novel HDAC isoform selective inhibitor that preferentially inhibits HDAC1 over HDAC3 and HDAC8 (41). As shown in Fig. 4*C*, MS-275 also rescued *M. avium* inhibition of HLA-DR gene expression. Thus, these results using two different HDAC inhibitors suggest HDAC activity at the HLA-DR gene is involved in repression by *M. avium* and *M. tuberculosis* infection, and this effect may be regulated by TLR2 signaling in THP-1 cells.

Histone acetylation at the promoter region of the HLA-DRα gene is impaired by mycobacteria infection in vivo

HLA-DR gene expression is controlled by a complex promoter region containing W/S, X1, X2, Y box, and octamer elements (42, 43). To determine whether mycobacteria infection alters DNA-binding proteins interacting with the promoter region of HLA-DR genes, we examined by EMSA the DNA-binding capacity of nuclear extracts, isolated from *M. avium*-infected THP-1 cells, to X1, X2, Y, and octamer elements in the promoter and the YY1-binding element in the first exon of the HLA-DRα gene. We did not observe any changes in the DNA-binding capacity of the nuclear proteins from mycobacteria-infected THP-1 cells (data not shown). We next investigated whether chromatin remodeling of the promoter of the HLA-DRα gene by histone acetylation is affected by mycobacteria infection in vivo. PMA-differentiated THP-1 cells were infected with *M. avium* at 20:1 bacteria to macrophage ratio or gamma-irradiated *M. tuberculosis* at 50 μg/ml or Pam_3CSK_4 at 50 ng/ml for 16 h, followed by stimulation with human rIFN-γ at 100 U/ml for 24 h. ChIPs were performed using Abs against acetylated-histone H4. PCR specific for the HLA-DR promoter was done with the DNA purified from the chromatin immunoprecipitates (Fig. 5*A*). To ensure that the amount of input chromatin was equal, DNA from 1% of chromatin was also amplified by PCR. To compare differences in level of acetylated-histone H4, real-time quantitative PCR was performed (Fig. 5*B*). We found that the baseline amount of acetylated HLA-DR promoter was low without treatment, whereas IFN-γ stimulation increased the histone H4 acetylation at the HLA-DR promoter region to more than three times the baseline level. This effect was impaired significantly by *M. avium*, *M. tuberculosis* infection, and Pam_3CSK_4 treatment (Fig. 5*A*). These results indicate that mycobacteria infection inhibits IFN-γ-induced HLA-DR gene expression by histone deacetylation at the HLA-DR promoter through a mechanism that involves a TLR2 signal transduction pathway.

Gene expression of the HDAC corepressor mSin3A is highly up-regulated following mycobacteria infection

The decrease in the amount of acetylated histone at HLA-DRα promoter suggests that HDAC activity is elevated at the promoter region by mycobacteria infection. One possibility is an increase in

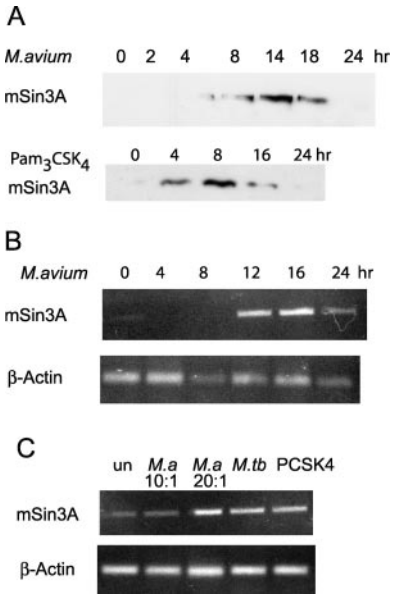

FIGURE 7. HDAC corepressor mSin3A expression was up-regulated by mycobacteria infection and TLR2 stimulation. *A*, Western blot analysis of mSin3A expression. PMA-differentiated THP-1 cells were treated with *M. avium* at a bacteria-to-macrophage ratio of 20:1 and 50 ng/ml Pam_3CSK_4 for the indicated time periods. Forty micrograms of nuclear extracts were subjected to immunoblotting with rabbit anti-mSin3A antibody. These experiments are representative of three repeats. *B*, RT-PCR analysis of mSin3A mRNA expression. PMA-differentiated THP-1 cells were treated with *M. avium* at 20:1 for the indicated times. RNA was extracted, and mRNA expression of mSin3A and β-actin was determined by RT-PCR. These results are representative of two experiments. *C*, Differentiated THP-1 cells were treated with *M. avium* at 10:1 and 20:1 bacteria:macrophage, irradiated *M. tuberculosis* at 50 μg/ml, and Pam_3CSK_4 at 25 ng/ml. After 16 h, RNA was isolated and mSin3A and β-actin mRNA expression determined by RT-PCR. These results are representative of two experiments.

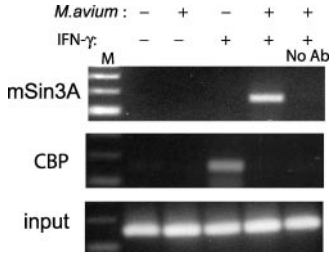

FIGURE 8. mSin3A associates with the HLA-DRα promoter in *M. avium*-infected THP-1 cells stimulated with IFN-γ. Association of mSin3A and CBP was analyzed by chromatin immunoprecipitation. PMA-differentiated THP-1 cells were infected for 16 h with *M. avium* (20:1 bacteria-to-macrophage ratio) and then stimulated with IFN-γ (100 U/ml) for 24 h. Chromatin was harvested from 1% formaldehyde-fixed cells and immunoprecipitated with anti-mSin3A and anti-CBP. DNA was purified from chromatin immunoprecipitates and amplified by PCR with primers to the HLA-DRα gene promoter. Input DNA purified from aliquots of chromatin representing 1% of the total amount of chromatin was also amplified by PCR. PCR products were fractionated on an agarose gel followed by ethidium bromide staining. Results are representative of two independent experiments.

HDAC gene expression. To determine whether mycobacteria infection is able to up-regulate HDAC gene expression in THP-1 cells, we used real-time RT-PCR to examine the HDAC1, HDAC2, and HDAC3 mRNA expression following infection. HDACs were expressed constitutively in PMA-differentiated THP-1 cells, whereas no significant increase in mRNA expression was detected after *M. avium* infection. Fig. 6*A* shows the results for HDAC1 and HDAC2. Identical results were obtained for HDAC3 (data not shown). These results were confirmed by Western blot analysis with Abs to HDAC1 and HDAC2 (Fig. 6*B*).

mSin3A, which is associated physically with HDAC1 and HDAC2 in multicomponent complexes, has been recognized as an important corepressor protein in HDAC-regulated transcriptional repression (44–46). Zika et al. (30) have reported that mSin3A is required for MHC class II repression. Therefore, we hypothesized that repression of the IFN-γ-induced HLA-DR gene expression was related to the regulation of the corepressor mSin3A. PMA-differentiated THP-1 cells were infected in time course experiments with *M. avium*, *M. tuberculosis*, or Pam_3CSK_4. By Western blot analysis, the expression of mSin3A was not detectable in the nuclear extracts from untreated THP-1 cells. However, a dramatic increase in mSin3A protein expression was observed following mycobacteria infection and TLR2 stimulation. This increase in protein expression peaked at 8–14 h and dropped to baseline by 24 h after *M. avium* infection or TLR2 stimulation (Fig. 7*A*). These results were confirmed by RT-PCR (Fig. 7*B*). mSin3A mRNA levels were increased following infection by mycobacteria and TLR2 stimulation (Fig. 7*C*).

The corepressor mSin3A but not the coactivator CBP is associated with the HLA-DRα promoter in M. avium*-infected THP-1 cells stimulated with IFN-γ*

The presence of mSin3A and CBP at the HLA-DRα promoter was studied by chromatin immunoprecipitation (Fig. 8). IFN-γ stimulation of PMA-differentiated THP-1 cells induced the association of the coactivator CBP with the HLA-DRα promoter. This association was absent in *M. avium*-infected cells stimulated with IFN-γ. Instead, in these cells, the corepressor mSin3A was present at the HLA-DRα promoter. The association of the corepressor with the HLA-DRα promoter required *M. avium* infection and IFN-γ because *M. avium* infection or IFN-γ alone did not induce association of mSin3A with the promoter. Thus, our results identify a novel mechanism for inhibition of IFN-γ-induced HLA-DRα gene expression by mycobacteria through increased expression of the corepressor mSin3A and association with the HLA-DRα promoter, resulting in histone deacetylation and transcriptional repression.

Discussion

Intracellular pathogens have been shown to compromise the host immune response by affecting the IFN-γ response of infected macrophages. Compromised MHC class II expression in response to IFN-γ in infected macrophages contributes largely to the inhibition of Ag presentation by intracellular pathogens. Our previous studies with murine macrophages have shown that *M. avium* inhibits IFN-γ-inducible gene expression by down-regulating IFN-γR expression and IFN-γRα, JAK1, JAK2, and STAT1 phosphorylation (7, 47). Similar research with other pathogens, including *Toxoplasma gondii* (48), *Leshmania donovani* (49), and *Erlichia chaffeensis* (50), has also shown inhibition of IFN-γ-induced JAK-STAT1 signaling. However, studies have shown that IFN-γ-induction of the JAK-STAT1 pathway is not inhibited by *M. tuberculosis* infection in human macrophages (9, 10). Our observations in this article with *M. avium* show that *M. avium* infection also does not inhibit the JAK-STAT1 pathway in human THP-1 monocytic cells. We do show by Northern blot hybridization that infection with *M. avium* and *M. tuberculosis* or treatment with a TLR2 agonist profoundly attenuates IFN-γ-induced HLA-DR mRNA expression.

IRF-1 and CIITA expression are essential for MHC class II expression. Several studies have shown that CIITA expression is reduced significantly in macrophages infected with mycobacteria or treated with mycobacteria lipoprotein (7, 10, 51). We also observed a reduction in IFN-γ-induced CIITA mRNA expression. However, at *M. avium* concentrations that are sufficient to completely repress HLA-DR gene expression in human THP-1 cells, there was still a significant level of CIITA mRNA expression induced by IFN-γ. IRF-1 expression is required for IFN-γ induction of CIITA. We found that IRF-1 mRNA expression is not affected by mycobacteria infection and TLR2 stimulation. These results indicate that, in THP-1 cells, inhibition of IFN-γ induction of HLA-DR by mycobacteria occurs both at the level of CIITA expression and downstream of CIITA expression. HLA-DRα and HLA-DRβ promoters are complex, containing elements that bind constitutive transcription factors that interact with CIITA to induce gene expression. We found no differences by EMSA in the expression of transcription factors binding to the promoter elements of HLA-DR, suggesting that mycobacterial infection is not altering the expression of these constitutive transcription factors.

Because transcriptional regulation in eukaryotes is influenced strongly by posttranslational modification of histones, chromatin remodeling through histone acetylation and deacetylation was examined as a mechanism for inhibition of HLA class II gene expression. In this article, we first demonstrated that inhibition of HDAC activity with butyrate and MS-275 could restore IFN-γ-inducible HLA-DR gene expression that was inhibited by mycobacterial infection or TLR2 stimulation. Inhibition of HDAC activity without mycobacterial infection did not induce HLA-DR gene expression with or without IFN-γ. These results indicate only the repressed the expression of HLA-DR gene by mycobacterial infection could be restored by inhibiting HDAC activity and suggest that mycobacterial infection may up-regulate HDAC activity at the regulatory region of the HLA-DR gene. By ChIP assay, we demonstrated that histone H4 acetylation at the HLA-DRα promoter was blocked by mycobacterial infection or stimulation with a TLR2 agonist. Thus, these results indicate a hypoacetylated state of the HLA-DRα gene promoter exists when mycobacteria-infected THP-1 cells are stimulated with IFN-γ.

One possible explanation for these observations is that mycobacterial infection causes a general effect by up-regulating HDAC gene expression. However, mRNA expression and protein expression of HDAC1, HDAC2, and HDAC3 was not changed by infection with mycobacteria or by TLR2 stimulation. We did observe a substantial increase in the expression of the HDAC corepressor, mSin3A. HDACs are the enzymatic component of multiprotein complexes. HDAC1 and HDAC2 in mammalian cells are complexed with corepressors mSin3A or NurD (nucleosome remodeling HDAC) (52). Overexpression of mSin3A but not NurD by transient transfection has been shown to completely repress CIITA-mediated MHC class II gene expression (30). A model for gene-specific repression has been proposed in which the mSin3A-HDAC complex is tethered to promoters by the interaction of mSin3A with DNA-binding transcriptional factors (53). Thus, it is likely that in the mycobacterial repression of IFN-γ-induced HLA-DR expression, the mSin3A-HDAC complex is recruited to HLA-DRA and HLA-DRB promoters by the interaction of mSin3A with an unidentified transcription factor. Because mSin3A association with the HLA-DRα promoter occurs only in infected cells that are stimulated with IFN-γ, the most likely candidate is CIITA. CIITA promotes HLA-DR transcription by interacting with components of the basal transcription machinery and chromatin remodeling enzymes, including the histone acetyltransferase CBP/p300. Our ChIP data shows that CBP is not associated with the HLA-DRα promoter in mycobacteria-infected cells stimulated with IFN-γ. Thus, mSin3A and CBP may compete for interaction with CIITA and determine up-regulation or down-regulation of HLA-DR expression. A similar activator-repressor switching has been shown to occur in the regulation of the c-*fos* promoter by the Elk-1 transcription factor (54). Initially following growth factor stimulation, Elk-1 is recruited to the serum-responsive element of the c-*fos* promoter and associates with coactivators and histone acetyltransferases, resulting in up-regulation of c-*fos* expression. Following a temporal delay, the mSin3A/HDAC1 complex interacts with Elk-1, resulting in repression of c-*fos* transcription. Other possible interacting partners for mSin3A include transcriptional repressors that interact with X2 and Y boxes. Transcriptional repressors that interact with the X2 box of the human HLA-DPA promoter and the Y box of the mouse I-A β promoter have been described previously (55, 56).

In these studies, we also observed that stimulation of TLR2 with Pam_3CSK_4 exerted the same effect on HLA-DR expression as infection with *M. avium* or treatment with irradiated *M. tuberculosis*. This suggests that TLRs are involved in initiating the inhibition of HLA-DR expression in mycobacteria-infected THP-1 cells. This is consistent with the observations that mycobacteria does not need to be alive to inhibit IFN-γ-induced gene expression (7, 10). We and others (16–18) have examined the effects of TLR2 stimulation on IFN-γ-induced gene expression in mouse macrophages. These studies showed that TLR2 stimulation of mouse macrophages inhibited IFN-γ-induced expression of several genes, including FcγRI, IRF-1, CIITA, and MHC class II genes. These studies also showed that TLR2 stimulation does not inhibit IFN-γ-induced STAT1α phosphorylation. However, our studies (18) showed that TLR2 stimulation of mouse macrophages increased phosphorylation and protein expression of STAT1β, which is transcriptionally inactive and inhibits IFN-γ-induced gene expression by acting as a dominant negative mutant. The increased expression of STAT1β was shown to result from increased stability of STAT1β mRNA in TLR2-stimulated macrophages. The studies in the present report with PMA-differentiated THP-1 cells differ from these previous mouse studies in that only MHC class II gene expression is inhibited by TLR2 stimulation. However, our PMA-differentiated THP-1 cells do not express the STAT1β protein. Only STAT1α was observed on Western blots (Fig. 2). We do not know why our THP-1 cells did not express STAT1β. Human primary macrophages express both STATα and STAT1β (9). Thus, the lack of STAT1β could be unique to THP-1 cells or due to an unknown effect of PMA treatment on RNA splicing.

The absence of global inhibition of IFN-γ-induced genes in differentiated human THP-1 cells has enabled us to identify a novel mechanism by which mycobacteria inhibit IFN-γ-induced MHC class II expression. Our studies suggest that mycobacteria through TLR2 signaling interferes with chromatin remodeling by up-regulating expression of the corepressor mSin3A, which following IFN-γ stimulation associates with HLA-DRα promoter and recruits HDACs, resulting in inhibition of histone acetylation. Additional investigation needs to be done to elucidate the mechanism by which TLR2 signaling regulates mSin3A expression and how mSin3A associates with the MHC class II promoter. In conclusion, the IFN-γ activation of macrophages is inhibited by mycobacteria through a number of different mechanisms. These include the down-regulation of the expression of the IFN-γ receptor following mycobacteria infection of mouse macrophages (7, 47), differential expression of the transcriptionally inactive STAT1β (18), and up-regulation of HDAC corepressor mSin3A expression.

Disclosures

The authors have no financial conflict of interest.

References

1. Ellner, J. J., M. J. Goldberger, and D. M. Parenti. 1991. *Mycobacterium avium* infection and AIDS: a therapeutic dilemma in rapid evolution. *J. Infect. Dis. 163:1326*.
2. Bermudez, L. E. 1994. Immunobiology of *Mycobacterium avium* infection. *Eur. J. Clin. Microbiol. Infect. Dis. 13:1000*.
3. Boehm, U., T. Klamp, M. Groot, and J. C. Howard. 1997. Cellular responses to IFN-γ. *Annu. Rev. Immunol. 15:749*.
4. Nathan, C. F., H. W. Murray, M E. Wiebe, and B. Y. Rubin. 1983. Identification of interferon-γ as the lymphokine that activates human macrophage oxidative metabolism and antimicrobial activity. *J. Exp. Med. 158:670*.
5. Cooper, A. M., D. K. Dalton, T. A. Stewart, J. P. Griffin, D. G. Russell, and I. M. Orne. 1993. Disseminated tuberculosis in interferon γ disrupted mice. *J. Exp. Med. 178:2243*.
6. Flynn, J. L., J. Chan, K. J. Triebold, D. K. Dalton, T. A. Stewart, and B. R. Bloom. 1993. An essential role for interferon in resistance to *Mycobacterium tuberculosis* infection. *J. Exp. Med. 178:2249*.
7. Hussain, S., B. S. Zwilling, and W. P. Lafuse. 1999. *Mycobacterium avium* infection of mouse macrophages inhibits IFN-γ Janus kinase-STAT signaling and gene induction by down-regulation of the IFN-γ receptor. *J. Immunol. 163:2041*.
8. Hmama, Z., R. Gabathuler, W. A. Jefferies, G. de Jong, and N. E. Reiner. 1998. Attenuation of HLA-DR expression by mononuclear phagocytes infected with *Mycobacterium tuberculosis* is related to intracellular sequestration of immature class II heterodimers. *J. Immunol. 161:4882*.
9. Ting, L. M., A. C. Kim, A. Cattamanchi, and J. D. Ernst. 1999. *Mycobacterium tuberculosis* inhibits IFN-γ transcriptional responses without inhibiting activation of STAT1. *J. Immunol. 163:3898*.
10. Kincaid, E. Z., and J. D. Ernst. 2003. *Mycobacterium tuberculosis* exerts gene-selective inhibition of transcriptional responses to IFN-γ without inhibiting STAT1 function. *J. Immunol. 171:2042*.
11. Darnell, J. E., Jr., I. M. Kerr, and G. R. Stark. 1994. Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. *Science 264:1415*.
12. Ihle, J. N. 1996. STATs: signal transducers and activators of transcription. *Cell 84:331*.
13. Schindler, C., and J. E. Darnell, Jr. 1995. Transcriptional responses to polypeptide ligands: the JAK-STAT pathway. *Annu. Rev. Biochem. 64:621*.
14. Heldwein, K. A., and M. J. Fenton. The role of Toll-like receptors in immunity against mycobacterial infection. *Microbes Infect. 4:937*.
15. Underhill, D. M., A. Ozinsky, K. D. Smith, and A. Aderem. 1999. Toll-like receptor-2 mediates mycobacteria-induced proinflammatory signaling in macrophages. *Proc. Natl. Acad. Sci. USA 96:14459*.
16. Pai, R. K., M. Convery, T. A. Hamilton, W. H. Boom, and C. V. Harding. 2003. Inhibition of IFN-γ-induced class II transactivator expression by a 19-kDa lipoprotein from *Mycobacterium tuberculosis:* a potential mechanism for immune evasion. *J. Immunol. 171:175*.
17. Gehring, A. J., R. E. Rojas, D. H. Canaday, D. L. Lakey, C. V. Harding, and W. H. Boom. 2003. The *Mycobacterium tuberculosis* 19-kilodalton lipoprotein inhibits γ interferon-regulated HLA-DR and FcγR1 on human macrophages through Toll-like receptor 2. *Infect. Immun. 71:4487*.
18. Alvarez, G. R., B. S. Zwilling, and W. P. Lafuse. 2003. *Mycobacterium avium* inhibition of IFN-γ signaling in mouse macrophages: toll-like receptor 2 stimulation increases expression of dominant-negative STAT1β by mRNA stabilization. *J. Immunol. 171:6766*.
19. Chang, C. H., J. D. Fontes, M. Peterlin, and R. A. Flavell. 1994. Class II transactivator (CIITA) is sufficient for the inducible expression of major histocompatibility complex class II genes. *J. Exp. Med. 180:1367*.
20. Otten, L. A., V. Steimle, S. Bontron, and B. Mach. 1998. Quantitative control of MHC class II expression by the transactivator CIITA. *Eur. J. Immunol. 28:473*.
21. Steimle, V., C. A. Siegrist, A. Mottet, B. Lisowska-Grospierre, and B. Mach. 1994. Regulation of MHC class II expression by interferon-γ mediated by the transactivator gene CIITA. *Science 265:106*.
22. Zhu, X. S., M. W. Linhoff, G. Li, K. C. Chin, S. N. Maity, and J. P. Ting. 2000. Transcriptional scaffold: CIITA interacts with NF-Y, RFX, and CREB to cause stereospecific regulation of the class II major histocompatibility complex promoter. *Mol. Cell. Biol. 20:6051*.
23. Beresford, G. W., and J. M. Boss. 2001. CIITA coordinates multiple histone acetylation modifications at the HLA-DRA promoter. *Nat. Immunol. 2:652*.
24. Fontes, J. D., S. Kanazawa, D. Jean, and B. M. Peterlin. 1999. Interactions between the class II transactivator and CREB binding protein increase transcription of major histocompatibility complex class II genes. *Mol. Cell. Biol. 19:941*.
25. Kretsovali, A., T. Agalioti, C. Spilianakis, E. Tzortzakaki, M. Merika, and J. Papamatheakis. 1998. Involvement of CREB binding protein in expression of major histocompatibility complex class II genes via interaction with the class II transactivator. *Mol. Cell. Biol. 18:6777*.
26. Ogryzko, V. V., R. L. Schiltz, V. Russanova, B. H. Howard, and Y. Nakatani. 1996. The transcriptional coactivators p300 and CBP are histone acetyltransferases. *Cell 87:953*.
27. Bannister, A. J., and T. Kouzarides. 1996. The CBP co-activator is a histone acetyltransferase. *Nature 384:641*.
28. Moreno, C. S., G. W. Beresford, P. Louis-Plence, A. C. Morris, and J. M. Boss. 1999. CREB regulates MHC class II expression in a CIITA-dependent manner. *Immunity 10:143*.
29. Osborne, A., H. Zhang, W. M. Yang, E. Seto, and G. Blanck. 2001. Histone deacetylase activity represses γ interferon-inducible HLA-DR gene expression following the establishment of a DNase I-hypersensitive chromatin conformation. *Mol. Cell. Biol. 21:6495*.
30. Zika, E., S. F. Greer, X.-S. Zhu, and J. P. Y. Ting. 2003. Histone deacetylase 1/mSin3A disrupts γ interferon-induced CIITA function and major histocompatibility complex class II enhanceosome formation. *Mol. Cell. Biol. 23:3091*.
31. Chomczynski, P., and N. Sacchi. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem. 162:156*.
32. Lafuse, W. P., D. Brown, L. Castle, and B. S. Zwilling. 1995. Cloning and characterization of a novel cDNA that is IFN-γ-induced in mouse peritoneal macrophages and encodes a putative GTP-binding protein. *J. Leukocyte Biol. 57:477*.
33. Horvath, C. M., Z. Wen, and J. E. Darnell, Jr. 1995. A STAT protein domain that determines DNA sequence recognition suggests a novel DNA-binding domain *Genes Dev. 9:984*.
34. Livak, K. J., and T. D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-[\mathrm{delta}]\Delta C(T)}$ method. *Methods 25:402*.
35. Means, T. K., S. Wang, E. Lien, A. Yoshimura, D. T. Golenbock, and M. J. Fenton. 1999. Human toll-like receptors mediate cellular activation by *Mycobacterium tuberculosis*. *J. Immunol. 163:3920*.
36. Lien, E., T. J. Sellati, A. Yoshimura, T. H. Flo, G. Rawadi, R. W. Finberg, J. D. Carroll, T. Espevik, R. R. Ingalls, J. D. Radolf, and D. T. Golenbock. 1999. Toll-like receptor 2 functions as a pattern recognition receptor for diverse bacterial products. *J. Biol. Chem. 274:33419*.
37. Aliprantis, A. O., R. B. Yang, M. R. Mark, S. Suggett, B. Devaux, J. D. Radolf, G. R. Klimpel, P. Godowski, and A. Zychlinsky. 1999. Cell activation and apoptosis by bacterial lipoproteins through Toll-like receptor-2. *Science 285:736*.
38. Strahl, B. D., and C. D. Allis. 2000. The language of covalent histone modifications. *Nature 403:41*.
39. Yamagoe, S., T. Kanno, Y. Kanno, S. Sasaki, R. M. Siegel, M. J. Lenardo, G. Humphrey, Y. Wang, Y. Nakatani, B. H. Howard, and K. Ozato. 2003. Interaction of histone acetylases and deacetylases in vivo. *Mol. Cell. Biol. 23:1025*.
40. Wu, C. 1997. Chromatin remodeling and the control of gene expression. *J. Biol. Chem. 272:28171*.
41. Hu, E., E. Dul, C.-M. Sung, Z. Chen, R. Kirkpatrick, G.-F. Zhang, K. Johanson, R. Liu, A. Lago, G. Hofmann, et al. 2003. Identification of a novel isoform-selective inhibitors within class I histone deacetylases. *J. Exp. Pharmacol. Exp. Therap. 307:720*.
42. Mach, B., V. Steimle, E. Martinez-Soria, and W. Reith. 1996. Regulation of MHC class II genes: lessons from a disease. *Annu. Rev. Immunol. 14:301*.
43. Benosit, C., and D. Mathis. 1990. Regulation of major histocompatibility complex class II genes: X, Y and other letters of the alphabet. *Annu. Rev. Immunol. 8:681*.
44. Hassig, C. A., T. C. Fleischer, A. N. Billin, S. L. Schreiber, and D. E. Ayer. 1997. Histone deacetylase activity is required for full transcriptional repression by mSin3A. *Cell 89:341*.
45. Laherty, C. D., W. M. Yang, J. M. Sun, J. R. Davie, E. Seto, and R. N. Eisenman. 1997. Histone deacetylases associated with the mSin3 corepressor mediate mad transcriptional repression. *Cell 89:349*.
46. Nagy, L., H. Y. Kao, D. Chakravarti, R. J. Lin, C. A. Hassig, D. E. Ayer, S. L. Schreiber, and R. M. Evans. 1997. Nuclear receptor repression mediated by a complex containing SMRT, mSin3A, and histone deacetylase. *Cell 89:373*.
47. Curry, H., G. R. Alavarez, B. S. Zwilling, and W. P. Lafuse. 2004. Toll-like receptor 2 stimulation decreases IFN-γ receptor expression in mouse RAW264.7 macrophages. *J. Interferon Cytokine Res. 24:699*.
48. Luder, C. G., W. Walter, B. Beuerle, M. J. Maeurer, and U. Gross. 2001. *Toxoplasma gondii* down-regulates MHC class II gene expression and antigen presentation by murine macrophages via interference with nuclear translocation of STAT1α. *Eur. J. Immunol. 31:1475*.
49. Nandan, D., and N. E. Reiner. 1995. Attenuation of γ interferon-induced tyrosine phosphorylation in mononuclear phagocytes infected with *Leishmania donovani:* selective inhibition of signaling through Janus kinases and Stat1. *Infect. Immun. 63:4495*.
50. Lee, E. H., and Y. Rikihisa. 1998. Protein kinase A-mediated inhibition of γ interferon-induced tyrosine phosphorylation of Janus kinases and latent cytoplasmic transcription factors in human monocytes by *Ehrlichia chaffeensis*. *Infect. Immun. 66:2514*.
51. Wojciechowski, W., J. DeSanctis, E. Skamene, and D. Radzioch. 1999. Attenuation of MHC class II expression in macrophages infected with *Mycobacterium bovis* bacillus Calmette-Guerin involves class II transactivator and depends on the *Nramp1* gene. *J. Immunol. 163:2688*.
52. Ayer, D. 1999. Histone deacetylases: transcriptional repression with SINers and NuRDs. *Trends Cell Biol. 9:193*.
53. Pazin, M. J., and J. T. Kadonaga. 1997. What's up and down with histone deacetylation and transcription. *Cell 89:325*.
54. Yang, S.-H., E. Vickers, A. Brehm, T. Kouzarides, and A. D. Sharrocks. 2001. Temporal recruitment of the mSin3A-histone deacetylase corepressor complex to the ETS domain transcription factor Elk-1. *Mol. Cell. Biol. 21:2802*.
55. Loberras, J., R. A. Maki, and A. Celada. 1995. Repression of major histocompatibility complex I-A β gene expression by dbpA and dbB (mYB-1) proteins. *Mol. Cell. Biol. 15:5092*.
56. Song, Z., S. Krisha, D. Thanos, J. L. Strominger, and S. J. Ono. 1994. A novel cysteine-rich sequence-specific DNA-binding protein interacts with the conserved X-box motif of the human major histocompatibility complex class II genes via a repeated Cys-His domain and functions as a transcriptional repressor. *J. Exp. Med. 180:1763*.