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Conidia but Not Yeast Cells of the Fungal Pathogen *Histoplasma capsulatum* Trigger a Type I Interferon Innate Immune Response in Murine Macrophages▽†

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***Histoplasma capsulatum* is the most common cause of fungal respiratory infections and can lead to progressive disseminated infections, particularly in immunocompromised patients. Infection occurs upon inhalation of the aerosolized spores, known as conidia. Once inside the host, conidia are phagocytosed by alveolar macrophages. The conidia subsequently germinate and produce a budding yeast-like form that colonizes host macrophages and can disseminate throughout host organs and tissues. Even though conidia are the predominant infectious particle for *H. capsulatum* and are the first cell type encountered by the host during infection, very little is known at a molecular level about conidia or about their interaction with cells of the host immune system. We examined the interaction between conidia and host cells in a murine bone-marrow-derived macrophage model of infection. We used whole-genome expression profiling and quantitative reverse transcription-PCR (qRT-PCR) to monitor the macrophage signaling pathways that are modulated during infection with conidia. Our analysis revealed that type I interferon (IFN)-responsive genes and the beta type I IFN (IFN-β) were induced in macrophages during infection with *H. capsulatum* conidia but not *H. capsulatum* yeast cells. Further analysis revealed that the type I IFN signature induced in macrophages in response to conidia is independent of Toll-like receptor (TLR) signaling and the cytosolic RNA sensor MAVS but is dependent on the transcription factor interferon regulatory factor 3 (IRF3). Interestingly, *H. capsulatum* growth was restricted in mice lacking the type I IFN receptor, indicating that an intact host type I IFN response is required for full virulence of *H. capsulatum* in mice.**

Studying the interaction between macrophages and intracellular pathogens has provided fundamental information about the innate immune response to microbial challenge. Macrophages use a number of different receptors to recognize and phagocytose microbes, resulting in the activation of a variety of antimicrobial effector mechanisms (1, 2, 6, 7, 36, 51, 72, 79, 83). Intracellular pathogens have evolved to modulate some innate immune mechanisms and replicate within the phagosome or cytosol of the host cell. While our understanding of the macrophage response to bacterial intracellular pathogens has advanced in recent years, our knowledge of the host response to fungal intracellular pathogens is still limited. Transcriptional profiling of host cells has been used as a comprehensive method to reveal host pathways that are activated in response to infection (34, 38, 46, 50, 56). This work describes the macrophage transcriptional response to the infectious form of the fungal pathogen *Histoplasma capsulatum*.

H. capsulatum, the etiologic agent of histoplasmosis, is a primary fungal pathogen that infects healthy as well as immunocompromised individuals (14). Approximately 500,000 infections are thought to occur every year in the United States alone (23, 48, 86, 88). Immunocompromised individuals tend to develop progressive, disseminated disease that can be fatal. *H. capsulatum* is endemic in the Ohio River Valley through the midwestern United States into Texas and is a leading pathogen affecting both AIDS patients in the Midwest (76) as well as individuals taking tumor necrosis factor alpha (TNF-α) antagonists (20, 21, 32, 73).

H. capsulatum is a dimorphic fungus that is adapted to grow either in the soil or in a mammalian host. In the soil, it grows in a hyphal (or filamentous) form. The hyphae generate two types of vegetative spores, macroconidia (8 to 25 μm) and microconidia (2 to 6 μm), which are distinguished mainly on the basis of size (64). After inhalation, conidia are taken up by macrophages and other phagocytic cells (13, 23, 88). Once inside the host, conidia germinate and give rise to yeast cells, which evade phagocytic killing and multiply within alveolar macrophages (AvMs). Yeast cells use phagocytic cells as vehicles to spread to multiple organs of the reticuloendothelial system (such as the spleen, liver, lymph nodes, and bone marrow) and to other organs in patients with disseminated disease (19, 23, 36, 58). Whereas the yeast form is the parasitic form of the organism, conidia are thought to be the infectious particle of *H. capsulatum*. Thus, studying the interaction of conidia with immune cells sheds light on the initial stages of infection.

Recognition of *H. capsulatum* conidia or yeast cells by host cells and the resultant downstream signaling events are just beginning to be investigated. A number of germ line-encoded receptors (e.g., membrane-bound Toll-like receptors, or TLRs, and cytosolic NOD-like receptors, or NLRs) have been identified as critical for recognition of microbes by immune cells

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(35, 39, 62). In the case of fungi, the main surface-expressed pattern recognition receptors (PRRs) involved in detection of these organisms are TLR2 and TLR4; the mannose receptor (MR); Dectin-1, which recognizes the major fungal cell wall carbohydrate β-glucan; Dectin-2; and DC-SIGN (9–11, 57, 65, 80, 87). As of yet, the roles of these and other PRRs in the host response to *H. capsulatum* are largely unexplored, although it is known that β-glucan present in the yeast cell wall is shielded from recognition by Dectin-1 by the presence of an outer layer of α-(1–3)-glucan in particular *H. capsulatum* strains (65).

In contrast to *H. capsulatum*, much is known about the host response to a variety of other types of pathogenic agents. A critical host response to viral infection is the induction of type I interferons (IFNs), a family of cytokines (including beta IFN [IFN-β] and multiple IFN-α molecules) that signal through the type I IFN receptor (IFNAR). Type I IFN production is initiated via phosphorylation and activation of the IFN regulatory factor 3 (IRF3) and IRF7 transcription factors, which then activate expression of type I IFNs. A secondary response is stimulated when secreted IFN-β signals in an autocrine- and paracrine-type manner through the type I IFN receptor, IFNAR (composed of the IFNAR1 and IFNAR2 subunits), which in turn leads to induction of a large set of type I IFN response genes through activation of the JAK/STAT pathway (for reviews, see references 17 and 18). Type I IFNs directly induce critical antiviral effectors and influence the function of NK cells and $CD8^+$ T cells in antiviral defense. In recent years, it has been observed that bacterial and parasitic infections also induce a type I IFN signature in host cells, but signaling of these cytokines through IFNAR can benefit either the host or the pathogen (5, 12, 33, 61, 75, 85). The ability of fungi to trigger a type I IFN response is largely unknown and only beginning to be explored (8).

In this study, we used transcriptional profiling to investigate the macrophage response to infection with *H. capsulatum* conidia. Surprisingly, murine bone marrow-derived macrophages (BMDMs) induced a classic type I interferon (IFN) transcriptional signature in response to infection with *H. capsulatum* conidia, but not in response to infection with isogenic yeast cells. We showed that the transcription factor IRF3, which is required for previously characterized type I interferon responses to other stimuli, is required for the induction of IFN-β transcript in BMDMs in response to conidia, whereas the TLR adaptors MyD88 and TRIF and the cytosolic RNA-sensing adaptor MAVS are not. Interestingly, induction of the interferon-responsive gene Ifi205 was observed during infection of alveolar macrophages with conidia but not yeast cells, again suggesting that these two *H. capsulatum* cell types can elicit a different host response. Finally, mice lacking *IFNAR1* restricted the growth of *H. capsulatum* in the lungs and spleen compared to that in wild-type (WT) controls, indicating that type I IFN signaling in response to *H. capsulatum* benefits the pathogen rather than the host.

MATERIALS AND METHODS

Cell culture and bone marrow-derived macrophage infections. For bone marrow collection, 8-week-old wild-type C57BL/6 mice were obtained from Charles River Laboratories. Macrophages were differentiated from the bone marrow from femurs of 8-week-old mice for 6 days in bone marrow-derived macrophage medium (BMM) containing Dulbecco's modified Eagle's medium (DMEM)-H21, 20% fetal calf serum, 10% colony-stimulating factor (CSF) from 3T3 cells, 2 mM glutamine, 1 mM sodium pyruvate, and penicillin-streptomycin (Pen/Strep) at 37°C in 5% CO_2. Femurs from 8-week-old *myd88*$^{-/-}$ *trif*$^{-/-}$ double-knockout mice in the C57BL/6 background were obtained from the laboratory of G. Barton, University of California—Berkeley, and differentiated as described above. For all bone marrow-derived macrophage experiments, cells were grown in the same medium. Bone marrow-derived macrophages from *mavs* knockout (−/−) and *mavs* heterozygous (+/−) littermate controls were obtained from the laboratory of R. Vance, University of California—Berkeley. Bone marrow-derived macrophages from *irf3*$^{-/-}$ mice were obtained from the laboratory of J. Cox, University of California—San Francisco (UCSF).

Bone marrow-derived macrophages were seeded at 7×10^5 cells/well in 6-well dishes or at 2×10^5 cells/well in 24-well dishes in BMM. After 16 to 20 h of growth, macrophages were infected with conidia or yeast cells resuspended in DMEM or phosphate-buffered saline (PBS). Conidia or yeast cells were centrifuged onto macrophages and incubated at 37°C in 5% CO_2 for the times indicated. For quantitative reverse transcription-PCR (qRT-PCR), macrophages were infected at a multiplicity of infection (MOI) of 10 for the times indicated and then were washed twice in prewarmed BMM prior to collection in RNeasy minikit cell lysis reagent (Qiagen). For microarray time course experiments, macrophages were washed 1 h postinfection with prewarmed medium and then collected at the indicated time points in cell lysis reagent.

Mice. *ifnar1*$^{-/-}$ mice that were back-crossed for at least 8 generations to the C57BL/6 background were obtained from the laboratory of J. Cox (74). Age- and sex-matched WT (C57BL/6) mice for infections were purchased from Charles River Laboratories. All mice were handled according to protocols approved by the UCSF Institutional Animal Care and Use Committee.

Histoplasma growth and conidia purification. *Histoplasma* strains were thawed from frozen stocks as yeast cells onto *Histoplasma*-macrophage medium (HMM) at 37°C with 5% CO_2 and passaged up to 3 times on plates. For infection of macrophages, yeast cells were grown to early log phase in HMM and washed and resuspended in warm PBS. Clumps of cells were pelleted by centrifugation of 50 ml of culture at 50 g for 5 min in a conical tube. The top 10 ml, which was enriched for single cells, doublets, and triplets, was collected, counted on an improved-Neubauer-phase hemacytometer, and diluted in PBS for infection.

Conidia from the G217B, G184AR, and G184AS strains were obtained by plating approximately 3×10^7 yeast cells on 15-cm petri plates containing synthetic medium 1 (3) or on Bird agar (http://www.fgsc.net/fgn51/fgn51metz.html) supplemented with cysteine-HCl and penicillin-streptomycin (Pen/Strep) as indicated. The G186AR strain, which grows poorly on synthetic media, was grown on Sabouraud dextrose agar to produce conidia. Plates were sealed in parafilm and cultured at room temperature for 4 to 12 weeks in a biosafety level 3 facility: G184AR and G184AS strains required 10 weeks to produce reasonable numbers of conidia, whereas G217B and G186AR routinely produced adequate yields of conidia within 4 to 5 weeks of incubation. Conidia were harvested by flooding the plates with PBS and dislodging the conidia with a bent glass rod. Mycelial fragments were removed from the conidial suspension by filtration through sterile glass wool. Conidia were pelleted by centrifugation at $2{,}000 \times g$, at 4°C for 10 min, washed, resuspended in PBS or PBS with Pen/Strep, and stored at 4°C until use. Conidia were heat killed by incubation in PBS at 95°C for 20 min. Conidial viability was confirmed by plating serial dilutions on brain heart infusion (BHI) agar with 10% sheep blood, 0.05% cysteine-HCl, and 10 μg/ml gentamicin and incubating for at least 10 days at 30°C.

RNA preparation. Macrophage RNA was purified using a RNeasy minikit and Qiashredder columns (Qiagen) according to the manufacturer's instructions with the following modification. Macrophage lysates were centrifuged for 5 min at 14,000 rpm to pellet any yeast cells or conidia prior to loading onto Qiashredder colums. For qRT-PCR analysis, RNA was treated with RQ1 RNase-free DNase I (Promega) for 20 min at room temperature. RQ1 stop solution was added, and reaction mixtures were incubated at 65°C for 15 min to inactivate the DNase I enzyme.

Microarray analysis. Total RNA was amplified to generate anti-sense RNA (aRNA) using the amino allyl MessageAmp II aRNA kit (Ambion). Each sample was labeled with Cy5 and competitively hybridized to a reference sample consisting of a pool of experimental samples labeled with Cy3. The aRNAs were fragmented with RNA fragmentation reagent (Ambion) according to the manufacturer's instructions prior to hybridizing the samples to microarrays. Microarrays were printed at the UCSF Center for Advanced Technology, using the MEEBO (Mouse Exonic Evidence-Based Oligonucleotide) 70-mer oligonucleotide set (Illumina; for more details, see http://alizadehlab.stanford.edu/). Microarrays were scanned using Gene Pix Pro 6.0 software on an Axon 4000B scanner (Molecular Devices). Grids were generated for each array with Gene Pix 6.0 (Molecular Devices), and the data were uploaded to the NOMAD database

(http://ucsf-nomad.sourceforge.net/) for quality control and normalization. Significantly induced genes were determined using the MeV implementation of SAM (Significance Analysis of Microarrays) with a false discovery rate of less than 5%. Information linked to each unique Oligo ID can be accessed at http://meebo.ucsf.edu:8080/meebo/meeboInfo.jsp?oligoid = (insert Oligo ID here). The data were organized for presentation with XCluster (http://genome-www5.stanford.edu/download/) and Java Treeview software (22, 70).

qRT-PCR analysis. For qRT-PCR, 1 μg of DNase I-treated total macrophage RNA was reverse transcribed with Affinity Script multitemperature reverse transcriptase (Stratagene) and 500 ng oligo($dT_{19}V$) (Integrated DNA Technologies, San Diego, CA) for 2 h. cDNA was diluted 3- to 4-fold with pyrogen-free water. Two microliters of diluted cDNA was used in each 25-μl reaction. Reactions were run on an Mx3000P machine (Stratagene), and MxPro software (Stratagene) was used to determine threshold and threshold cycle (C_T) values. qRT-PCR data were normalized to hypoxanthine phosphoribosyltransferase 1 (HPRT1) expression using the Pfaffl method (63). The IFN-β expression shown is relative to that of the mock-infected control unless otherwise indicated. Data are representative of at least 3 (in many cases 4 or more) independent experiments. Error bars represent the standard error of the mean for replicate qRT-PCRs. The primers used in this study were IFNb-F (CTGGAGCAGCTGAATGGAAAG), IFNb-R (CTTGAAGTCCGCCCTGTAGGT), mHPRT1-F (AGGTTGCAAGCTTGCTGGT), and mHPRT1-R (TGAAGTACTCATTATAGTCAAGGGCA).

Quantitation of phagocytosis and cell staining. For immunostaining, macrophages were seeded in 24-well dishes on 12-mm coverslips and infected as described above. After 2 h, macrophages were washed twice with BMM to remove unbound conidia or yeast cells. Coverslips were fixed in phosphate-buffered saline (PBS) with 3.7% formaldehyde for 5 min and then washed in PBS and stored at 4°C until stained. Extracellular conidia or yeast cells were detected with 1:300 anti-*Histoplasma* mold or 1:500 anti-*Histoplasma* yeast cell antibodies (a kind gift of Joseph Wheat, Miravista Labs) in PBS with 1% bovine serum albumin (BSA) for 30 min at room temperature. Goat anti-rabbit Alexa 594 (Molecular Probes) secondary antibody was used at 1:500, concanavalin A-fluorescein isothiocyanate (FITC) (Invitrogen) was used at 1:400 to stain macrophages, and 10 μg/ml calcofluor (fluorescence brightener 28; Sigma-Aldrich) was used to stain all fungal cells (4, 47). Fluorescence images were obtained on a Zeiss Axiovert 200 inverted microscope using Axiovision 4.4 software. Red, green, and blue fluorescent channels were merged with Adobe Photoshop. Cytochalasin D was resuspended in dimethyl sulfoxide (DMSO) and used at a 5 μM final concentration. At least 100 macrophages were evaluated per condition. To visualize germination during macrophage infection, coverslips seeded with conidium-infected macrophages were fixed at 24 h postinfection (hpi) in PBS with 3.7% formaldehyde for 5 min before staining with periodic acid-Schiff (PAS) base (Sigma).

Isolation and infection of AvMs. Bronchoalveolar lavage (BAL) was performed to obtain AvMs from the lungs of 8- to 12-week-old female C57BL/6 mice. Briefly, mice were sacrificed by cervical dislocation and lungs were flushed with a total of 20 ml BAL solution (5 mM EDTA in PBS without Ca^{2+} and Mg^{2+}). Cells were pelleted and resuspended in AKT solution (150 mM NH_4Cl, 10 mM $KHCO_3$, 0.1 mM EDTA in double-distilled water [ddH_2O], pH 7.2 to 7.4) to lyse contaminating red blood cells. The remaining cells were pelleted and resuspended in high-glucose DMEM (UCSF Cell Culture Facility) supplemented with 10% fetal bovine serum (HyClone; Thermo Fisher [www.hyclone.com]), penicillin, and streptomycin (UCSF Cell Culture Facility). AvMs were seeded at a density of 6.0×10^5 cells/well in 24-well plates and allowed to settle overnight prior to infection with G217B yeast cells or conidia at an MOI of 10. As a positive control for induction of Ifi205, AvMs were treated for 2 h with 100 ng/ml lipopolysaccharide (LPS) (Sigma). Four hours postinfection, AvM total RNA samples were isolated using the RNeasy minikit (Qiagen) and 250 ng total RNA was reverse transcribed using the Affinityscript quantitative PCR (qPCR) cDNA synthesis kit (Agilent). qRT-PCR analysis of Ifi205 expression in AvMs was performed using the same methods as for analysis of IFN-β expression in BMDMs, with the exception that reactions were carried out using SYBR green qPCR master mix (Applied Biosystems). The primer sequences are CATCTTCGGCTTCATCTAAC for Ifi205 fwd and ACATGGAAATACTGGCTCAC for Ifi205 rev.

Mouse infections. Mice were anesthetized with isoflurane and infected intranasally with 2×10^6 conidial CFU (from the G217B strain) or 2×10^4 yeast CFU (from the G217B strain) in a volume of 25 to 40 μl PBS. Since germination of conidia must occur before they give rise to actively dividing yeast cells, and because 100% of the conidia do not germinate, different numbers of infectious particles for conidia and yeast cells were selected to allow a similar progression of fungal burden and disease in both cases. At the indicated time points, mice were euthanized using CO_2 inhalation followed by cervical dislocation. Lungs and spleens were homogenized in Hank's medium supplemented with 10 μg/ml gentamicin with disposable 15-ml conical homogenizers. Dilution series were plated on brain-heart infusion (BHI) agar with 10% sheep blood, 0.05% cysteine, and 10 μg/ml gentamicin at 30°C for 10 to 14 days before enumeration of CFU. *P* values were calculated using the Mann-Whitney rank sum test.

For histopathological analysis of infected tissues, age- and sex-matched WT and *ifnar*$^{-/-}$ mice of the C57BL/6 background were infected intranasally with a suspension of 2×10^6 G217B conidial CFU in sterile PBS. Mice were weighed and monitored for symptoms at regular intervals. At the indicated time points, two mice per strain were euthanized as described above. Postmortem, the trachea was cannulated and the lungs were inflated *in situ* with 0.7 ml of 10% formalin–PBS. The lungs were removed and fixed in 10% formalin–PBS before serial dehydration and paraffin embedding. Five-micrometer parasagittal sections were taken at 100-μm intervals from the right lungs. At each level, sections were stained for hematoxylin and eosin (H&E) or periodic acid-Schiff (PAS) (Sigma-Aldrich). Sections were analyzed using light microscopy and photographed using a Leica DM1000 with a Leica DFC290 color camera. Images of sections were montaged using Photoshop CS3 (Adobe Systems, Inc.) for additional comparative analysis of inflammatory regions.

Microarray data accession number. The GEO accession number for the microarray data is GSE20022.

RESULTS

Phagocytosis of conidia and infection of murine bone-marrow derived macrophages. Although numerous studies have documented the interaction between macrophages and *Histoplasma* yeast cells (59, 60), there has been only limited analysis of infection of macrophages with conidia. We generated conidia from the virulent laboratory strain G217B, which has been studied extensively in the yeast form. G217B yeast cells were induced to form filaments and sporulate by incubation on synthetic sporulation medium or on Sabouraud dextrose agar (see Materials and Methods) at room temperature. Under these conditions, near-pure populations of microconidia (>95%) were produced, with the remaining cells in the preparation being macroconidia. To determine whether G217B microconidia (hereafter referred to as conidia) were efficiently ingested by BMDMs, we infected macrophages with conidia or yeast cells (which are known to be efficiently phagocytosed by macrophages) at a multiplicity of infection (MOI) of 3 or 5. After a 2-h incubation period, we used polyclonal antibodies and calcofluor white to detect *Histoplasma* yeast cells and conidia (see Materials and Methods). Only external *Histoplasma* cells were accessible to the antibodies, whereas both external and internal fungal cells were accessible to calcofluor white (47), which binds to chitin in the fungal cell wall (Fig. 1A). Quantitation of the staining revealed that conidia and yeast cells were phagocytosed by wild-type macrophages with similar efficiencies (85.8% of yeast cells and 86.4% of conidia associated with macrophages were internalized). Germination of conidia to give rise to yeast cells was observed approximately 16 to 24 h postinfection (hpi) by staining the infected macrophages with periodic acid-Schiff base (PAS) (Fig. 1B). Ultimately, infection of macrophages with conidia resulted in lysis of the macrophage monolayer, as is observed for infection of BMDMs with *H. capsulatum* yeast cells (data not shown).

Macrophages infected with conidia express type I interferon response genes. To identify host signaling pathways induced specifically in response to infection by conidia, we used Mouse Exonic Evidence-Based Oligonucleotide (MEEBO) microarrays (Illumina) to determine the transcriptional profile of murine BMDMs infected with G217B conidia or yeast cells. Mac-

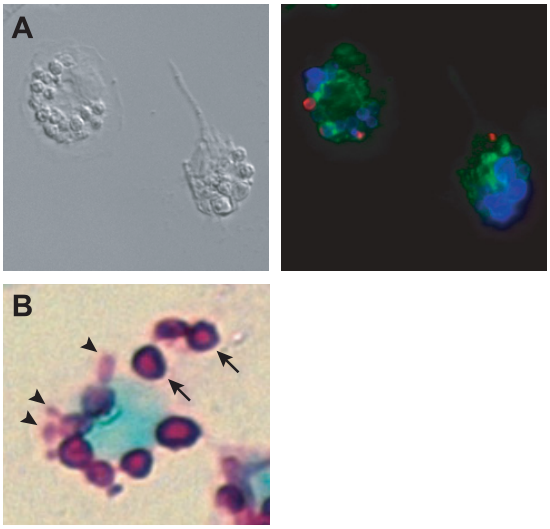

FIG. 1. Conidia are internalized by macrophages and germinate intracellularly to give rise to yeast cells. (A) Differential interference contrast (DIC) image (left) and immunofluorescence staining (right) of macrophages infected with conidia of the G217B strain. Both internal and external conidia are stained in blue with calcofluor, whereas only external conidia are stained in red with anti-*Histoplasma* antibodies. Macrophages are stained green using concanavalin A-FITC. (B) Periodic acid-Schiff (PAS) staining of conidia that have germinated and are producing yeast cells within macrophages 24 hpi. With PAS, conidia typically stain a darker magenta color than yeast cells. Three representative yeast cells are indicated with arrowheads, and two representative conidia are indicated with arrows.

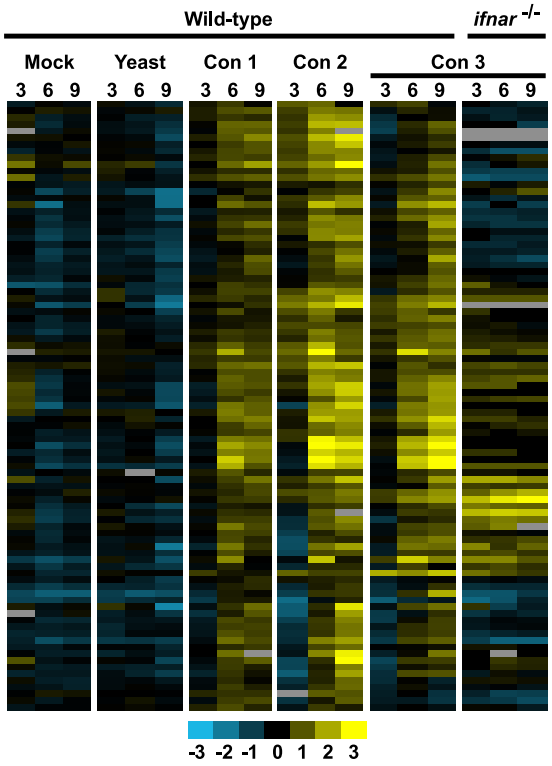

FIG. 2. Heat map of type I IFN response genes induced by macrophages infected with *Histoplasma* conidia but not yeast. C57BL/6 (WT) macrophages were subjected to either mock infection, infection with *H. capsulatum* yeast cells, or infection with two independent preparations of *H. capsulatum* conidia (Con 1 and Con 2). WT and *ifnar1*$^{-/-}$ macrophages were mock infected (data not shown) or infected with a third preparation of G217B conidia (Con 3). At 3, 6, and 9 hpi, macrophages were subjected to gene expression profiling. Genes with statistically significant induction in two independent wild-type macrophage infection experiments relative to the mock infection are shown. Yellow indicates gene upregulation, blue indicates downregulation, and black indicates no change. The color bar indicates the $\log_2$ scale.

rophages were infected at an MOI of 5, and RNA was harvested at 0, 3, 6, and 9 hpi. As expected, we found that infection with both conidia and yeast cells resulted in induction of general inflammatory response genes, including chemokines and cytokines (C. A. Berkes et al., unpublished data). However, a group of 74 genes were significantly induced only in macrophages infected with conidia (Fig. 2; see Table S1 in supplemental material for the gene list). Many of these genes are known to be induced by type I IFNs, suggesting that macrophages were producing type I IFNs specifically in response to infection with *H. capsulatum* conidia. Induction of type I IFN response genes during infection of macrophages with conidia is interesting because previous reports of type I IFN responses to fungal infection are limited, although signaling through IFNAR1 has been shown to play a critical role in host survival during infection with the fungal pathogen *Cryptococcus neoformans* (8).

To test whether the type I IFN signaling pathway is required for the transcriptional response of macrophages to conidia, we infected macrophages deficient in the type I IFN receptor (*ifnar1*$^{-/-}$ macrophages) with conidia and examined the resultant transcriptional response. Cells lacking the type I IFN receptor are capable of primary induction of type I IFNs but are deficient in the secondary response that amplifies the primary signal and results in the expression of downstream genes (37, 81). *ifnar1*$^{-/-}$ macrophages were unable to mount a wild-type transcriptional response to *H. capsulatum* conidia (Fig. 2), strongly suggesting that the production of type I IFNs and subsequent signaling through IFNAR are required for the transcriptional response to conidia.

***H. capsulatum* conidia trigger the induction of IFN-β transcript in macrophages.** To confirm our transcriptional profiling data, we used qRT-PCR as a sensitive assay to detect IFN-β expression in infected macrophages. WT macrophages were infected with G217B conidia at an MOI of 10, and RNA was harvested at multiple time points between 1 and 6 hpi. Maximal (12-fold) induction of IFN-β occurred between 3 and 4 hpi and declined by 6 hpi (Fig. 3A). Over the course of multiple experiments, we routinely observed that infection with G217B conidia at an MOI of 10 resulted in a range of IFN-β induction that was largely dependent on the age of the conidia—i.e., conidia purified from plates incubated for a longer period (e.g., 10 weeks) stimulated higher levels of IFN-β message than conidia purified from plates incubated for shorter periods (e.g., 4 weeks). We were not able to detect IFN-β protein production by enzyme-linked immunosorbent assay (ELISA) (data not shown), although the dependence of the host transcriptional signature on IFNAR (Fig. 2) strongly suggests that type I IFN proteins are produced and signal through IFNAR during in-

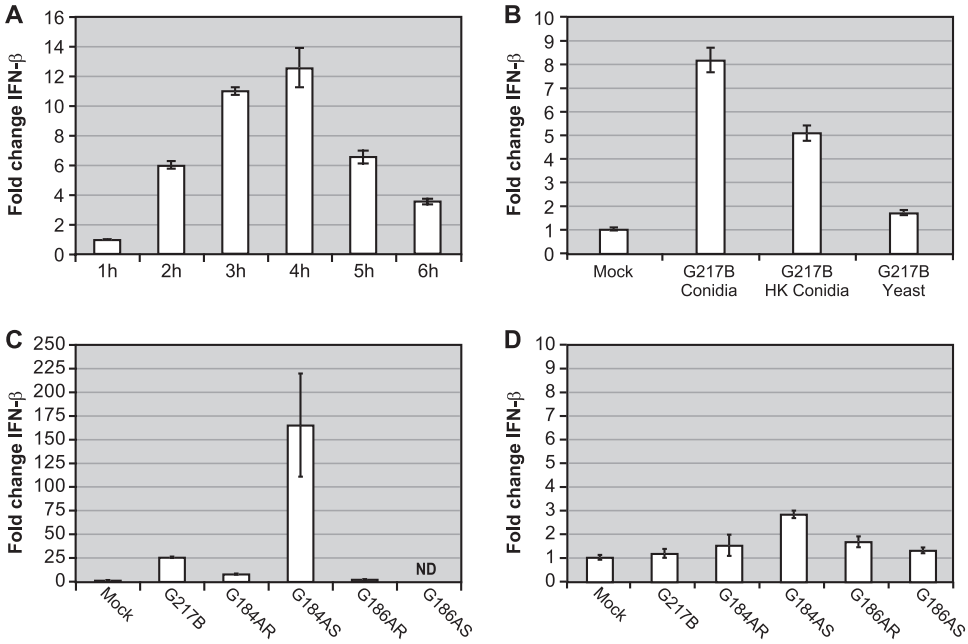

FIG. 3. Expression level of IFN-β in conidium- and yeast-infected macrophages. (A) qRT-PCR analysis to determine fold induction of IFN-β was performed on macrophage samples at various time points after infection with G217B conidia at an MOI of 10. (B) qRT-PCR analysis to determine fold induction of IFN-β at 3 hpi in macrophages infected with live or heat-killed (HK) conidia or with live yeast cells at an MOI of 10. (C and D) Macrophage lysates were subjected to qRT-PCR to detect relative induction of IFN-β after mock infection or infection with G217B, G184AR, G184AS, G186AR, or G186AS conidia (C) or yeast cells (D) at an MOI of 10. ND, not determined.

fection of bone-marrow derived macrophages with *Histoplasma* conidia.

To determine whether induction of IFN-β transcript by *Histoplasma* is an active process that requires viable spores, we compared the IFN-β responses of WT macrophages infected with live or heat-killed G217B conidia (Fig. 3B). Whereas infection with G217B yeast cells failed to induce IFN-β, infection with heat-killed conidia induced intermediate levels of IFN-β transcript compared to infection with live conidia. Thus, induction of IFN-β does not fully depend on conidial viability and at least partially reflects a heat-resistant property of conidia.

Conidia from evolutionarily diverged *Histoplasma* strains trigger induction of IFN-β transcript in macrophages. Molecular studies of *H. capsulatum* biology and pathogenesis have largely taken place in three distinct strains: the North American clinical isolate G217B and the Latin American clinical isolates G186AR and G184AR ("R" indicates that the yeast form of the organism has a rough colony morphology). These strains were originally classified on the basis of the polysaccharide composition of their cell walls (16, 67–69), which is a microbial property that could influence the host immune response. G217B yeast cells lack α-(1,3)-glucan in their cell wall, whereas the cell walls of G186AR and G184AR yeast cells are rich in α-(1,3)-glucan. Variants of G186AR and G184AR that lack α-(1,3)-glucan (the so-called "smooth" G186AS and G184AS strains) are avirulent (44, 45), whereas G217B is virulent despite its lack of α-(1,3)-glucan. Recent molecular phylogeny studies confirmed that G217B is in a phylogenetic clade that is significantly diverged from the G186AR and G184AR lineages (40). To determine whether the IFN-β induction by conidia was a property restricted to the G217B strain or whether spores and yeast cells from other strains could induce IFN-β, we attempted to generate conidia from the G186AR, G186AS, G184AR, and G184AS strains. Like many strains that have undergone extensive laboratory passaging, our stock of the G186AS strain failed to produce conidia (data not shown). However, we were able to produce conidia from G184AR, G184AS, and G186AR yeast cells, as described in Materials and Methods. All of these strains, including G217B, were plated simultaneously and grown for approximately 10 weeks at room temperature. Macrophages were infected with G217B, G184AR, G186AR, or G184AS conidia, and qRT-PCR was used to detect IFN-β induction 4 h after infection (Fig. 3C). Infection with G217B conidia resulted in approximately 25-fold induction of IFN-β, but infection with G186AR conidia failed to induce significant levels of IFN-β. Interestingly, whereas G184AR conidia induced modest levels of IFN-β (7.5-fold), infection with G184AS conidia resulted in a 150-fold induction of IFN-β transcript. These data suggest that the unknown microbial property that triggers production of IFN-β by host cells is enhanced in the smooth G184AS strain and is masked in the rough G184AR and G186AR strains, although the molecular basis of this difference is unknown. To determine if α-(1,3)-glucan modulates type I IFN production, we attempted to generate conidia from the G186A *ags1*Δ strain (66), which is smooth because these cells produce no α-(1,3)-glucan due to a deletion in the α-(1,3)-glucan synthase. However, like many laboratory strains, the *ags1*Δ strain failed to sporulate (data not shown). No yeast cells from any strains tested, including G217B, G184AR, G184AS, G186AR, and G186AS, were capable of inducing appreciable levels of IFN-β

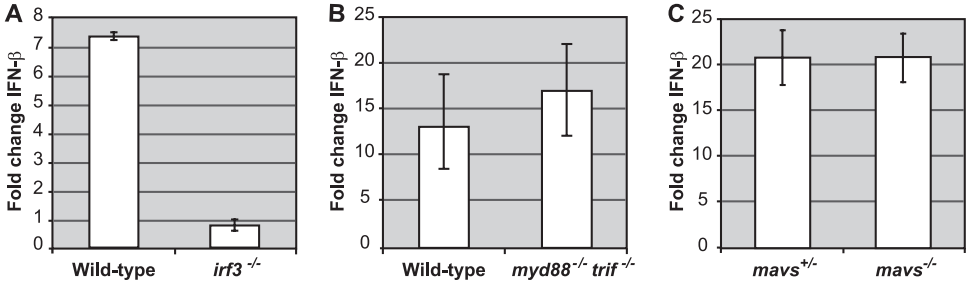

FIG. 4. The type I IFN response to conidia is dependent on IRF3 and independent of MyD88, TRIF, and MAVS. qRT-PCR was used to determine fold IFN-β induction in *irf3*$^{-/-}$ macrophages (A), *myd88*$^{-/-}$ *trif*$^{-/-}$ macrophages (B), and *mavs*$^{+/-}$ and *mavs*$^{-/-}$ macrophages (C) infected with G217B conidia at an MOI of 10.

transcript in macrophages (Fig. 3D), again suggesting that production of IFN-β is a specific characteristic of infection with *H. capsulatum* conidia but not their isogenic yeast cells.

The type I IFN response of BMDMs is independent of MyD88 and TRIF signaling and the adaptor protein MAVS but dependent on IRF3. Canonical production of type I IFNs by macrophages during infection occurs in response to signaling through host Toll-like receptors (TLRs) or a cytosolic nucleic acid detection pathway (42, 77). The induction of IFN-β through either of these pathways is dependent on the transcription factor IRF3. We observed that IFN-β induction during infection with conidia was completely dependent on IRF3 (Fig. 4A), indicating that production of IFN-β transcript during infection with conidia is likely to occur via known pathways.

To determine whether host TLR signaling was required for the type I IFN response to conidia, we utilized macrophages from mice lacking TLR adaptor molecules MyD88 and TRIF. *myd88*$^{-/-}$ *trif*$^{-/-}$ macrophages, which are deficient in TLR signaling, were fully capable of inducing IFN-β in response to infection with G217B conidia (Fig. 4B), suggesting that TLR signaling is not required for IFN-β production by macrophages in response to *Histoplasma* conidia.

Cytosolic detection of microbial nucleic acids by host cells also results in production of IFN-β. Sensing of RNA by the cytosolic RNA receptors RIG-I and MDA5 requires the innate immune signaling adaptor MAVS, which is required for type I IFN production in response to viral infection (25, 43, 52, 71, 91). Levels of induction of IFN-β transcript by infection with conidia in *mavs*$^{-/-}$ and *mavs*$^{+/-}$ littermate control macrophages were comparable (Fig. 4C), indicating that cytosolic detection of conidial RNA is unlikely to be responsible for production of IFN-β by host cells. It is currently unknown whether cytosolic sensing of conidial DNA contributes to the type I IFN response.

Phagocytosis is required for IFN-β induction in conidium-infected macrophages. Since TLR signaling is dispensable for IFN-β production in response to conidial infection, our data suggested that cytosolic sensing of a conidial molecule(s) might be required for production of IFN-β by host macrophages. If so, it is likely that phagocytosis of conidia would be necessary to trigger a type I IFN response in macrophages. Macrophages were pretreated with either DMSO (control) or 5 μM actin polymerization inhibitor cytochalasin D (15, 24), infected with G217B conidia, and then subjected to staining as described in Materials and Methods to determine internalization of fungal cells. Cytochalasin-treated macrophages were still associated with conidia but were unable to phagocytose them (Table 1). In contrast to DMSO-treated control cells, cytochalasin-treated macrophages showed a 25-fold reduction in production of IFN-β by qRT-PCR when infected with G217B, suggesting that phagocytosis of conidia is required for the type I response (Table 1). Cytochalasin-treated macrophages exposed to LPS were capable of inducing IFN-β, indicating that the cytochalasin treatment did not generally inhibit IFN-β expression in these cells (data not shown).

Alveolar macrophages induce an interferon-responsive gene in response to infection with conidia but not yeast cells. By probing the transcriptional profile of bone marrow-derived macrophages during infection with conidia or yeast cells, we were able to uncover differential responses elicited in host cells by these two fungal cell types. To perform an initial investigation to determine whether conidia and yeast cells might elicit different responses in a lung macrophage, we isolated alveolar macrophages (AvMs) from 30 mice by BAL. Macrophages were infected with either conidia or yeast cells, and host RNA was harvested at 4 hpi to examine early transcriptional responses. No detectable IFN-β transcript was observed by qRT-PCR during infection of AvMs with either conidia or yeast cells (data not shown). However, we were able to detect a reproducible 6-fold induction of interferon-responsive gene Ifi205 (53) in AvMs infected with conidia but not yeast cells (Fig. 5); Ifi205 was also induced by BMDMs in response to conidia but not yeast cells (see Table S1 in the supplemental material). This experiment supports the idea that conidia and yeast cells could provoke different transcriptional responses in host cells during infection.

Signaling through the type I IFN receptor IFNAR1 contributes to the pathogenesis of *H. capsulatum* during host infection. The observation that infection with *H. capsulatum* conidia

TABLE 1. Cytochalasin D treatment-inhibited internalization of conidia and induction of type I IFNs

Treatment group	Mean ± SD % of conidia ingested	Avg ± SD relative IFN-β induction
G217B conidia + DMSO	68.5 ± 2.0	31.2 ± 2.7
G217B conidia + 5 μM cytochalasin D	8.6 ± 0.5	1.23 ± 1.5

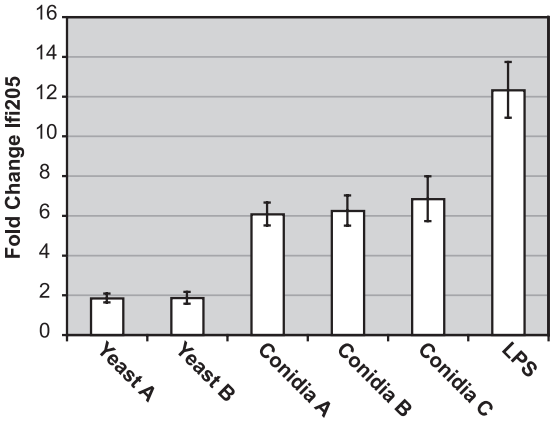

FIG. 5. Induction of Ifi205 in AvMs infected with G217B conidia. qRT-PCR analysis to determine fold induction of Ifi205 was performed on AvM samples 4 h after infection with G217B conidia or yeast cells at an MOI of 10. Fold changes are calculated relative to the level in the mock-infected control. Error bars represent the standard error of the mean for replicate qRT-PCRs. The data shown include two (yeast cell A and B) or three (conidia A, B, and C) biological replicates.

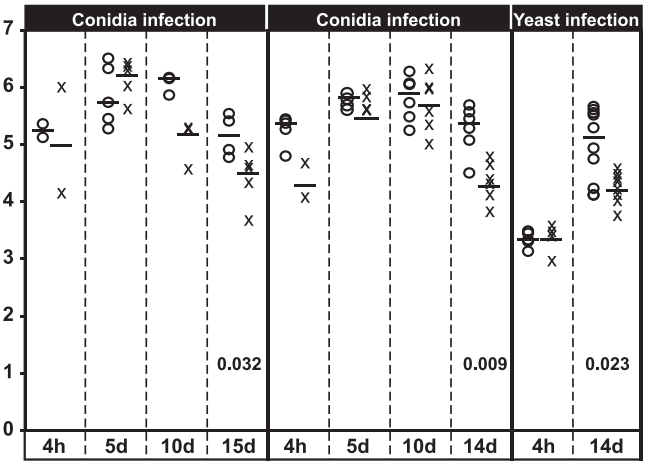

FIG. 6. Host type I IFN signaling is required for maximal fungal burden in host tissues following infection with *H. capsulatum*. WT and *ifnar1*$^{-/-}$ mice were subjected to intranasal infection with G217B conidia or yeast cells. Two representative conidial infections and one representative yeast cell infection are shown. Lungs from infected animals were harvested at the indicated hours (h) or days (d) postinfection and assessed for CFU. Horizontal bars indicate mean $\log_{10}$ CFU values. Significant *P* values ($P \leq 0.05$) for WT versus *ifnar1*$^{-/-}$ comparisons are indicated on the figure.

triggered a type I IFN signature in bone marrow-derived macrophages raises the possibility that type I IFNs could influence the outcome of *H. capsulatum* infection in the mouse, although the production of type I IFNs *in vivo* and the cell types that produce them have not been investigated. For other pathogens, examination of the outcome of infection in the *ifnar1*$^{-/-}$ mice, which are deficient in the secondary response that results in robust expression of interferon-dependent genes (37, 81), has been used as an initial query to shed light on the role of type I IFN signaling during infection. Interestingly, in response to infection with bacterial pathogens, this type of approach has been used to show that host type I IFN signaling confers either resistance or susceptibility, depending on the bacterial pathogen in question (5, 61, 74). To determine whether type I IFN signaling contributes to the outcome of *H. capsulatum* infection, we subjected WT and *ifnar1*$^{-/-}$ mice to an intranasal infection with 2×10^6 CFU of G217B conidia. Lungs and spleens from infected animals were harvested for enumeration of CFU at 5, 10, and 14 days postinfection (dpi). Whereas the level of fungal burden was not significantly different between the WT and mutant mouse strains at 5 and 10 dpi (based on a *P* value of ≤ 0.05), the fungal burden was reproducibly lower in the *ifnar1*$^{-/-}$ mice in both the lungs (Fig. 6) and spleen (data not shown) by 14 dpi. These data indicate that signaling through the type I IFN receptor is required for full virulence of *Histoplasma* conidia.

Since we observed decreased fungal burden in *ifnar1*$^{-/-}$ mice at later time points in infection when conidia have germinated to give rise to yeast cells, we were interested to know if infection of wild-type and *ifnar1*$^{-/-}$ mutant mice with *Histoplasma* yeast cells would give a comparable difference in fungal burden. We observed that during mouse infections with *H. capsulatum* yeasts, the fungal burden was significantly lower in the lungs of *ifnar1*$^{-/-}$ mice at 14 dpi (Fig. 6). These data indicate that signaling through the type I IFN receptor is required for maximal disease burden during *Histoplasma* infection.

Histological examination of lung sections from WT and mutant mice infected with *Histoplasma* conidia revealed significant differences in the inflammatory infiltrate (Fig. 7). Infected lungs of both WT and *ifnar1*$^{-/-}$ mice had a similar pattern of inflammation centered around the bronchioles (Fig. 7A, B, E, and F); however, the lungs of WT mice contained a denser inflammatory infiltrate as well as larger foci of inflammation. Additionally, there were differences in the compositions of the inflammatory infiltrate between the two infected mouse strains (Fig. 7C and D). In WT lungs at 5 dpi, the infiltrate consisted largely of granulocytes and lymphocytes with numerous eosinophils. In contrast, at the same time point, the *ifnar1*$^{-/-}$ infiltrate was largely composed of macrophages, with only a minor lymphocytic component. Giant cells, which presumably result from coalescence of infected macrophages, were observed in nearly all the inflammatory foci of WT lungs (Fig. 7C and 8), but were not found in the *ifnar1*$^{-/-}$ lungs (Fig. 7D). By 14 dpi, the extent of inflammation had decreased relative to 5 dpi, but was still higher in wild-type mice than in *ifnar1*$^{-/-}$ mice (Fig. 7E, F, G, and H). The uninfected lung sections from WT and *ifnar1*$^{-/-}$ mice did not look appreciably different (data not shown). Taken together with the CFU data (Fig. 6), these experiments indicate that signaling through the type I IFN receptor is required for the normal extent and character of the inflammatory response to *Histoplasma* as well as maximal fungal burden in host tissues during *Histoplasma* infection.

DISCUSSION

H. capsulatum is an environmental fungus that is able to colonize a number of mammalian species via inhalation of infectious spores (conidia). As a primary pathogen, *H. capsu-*

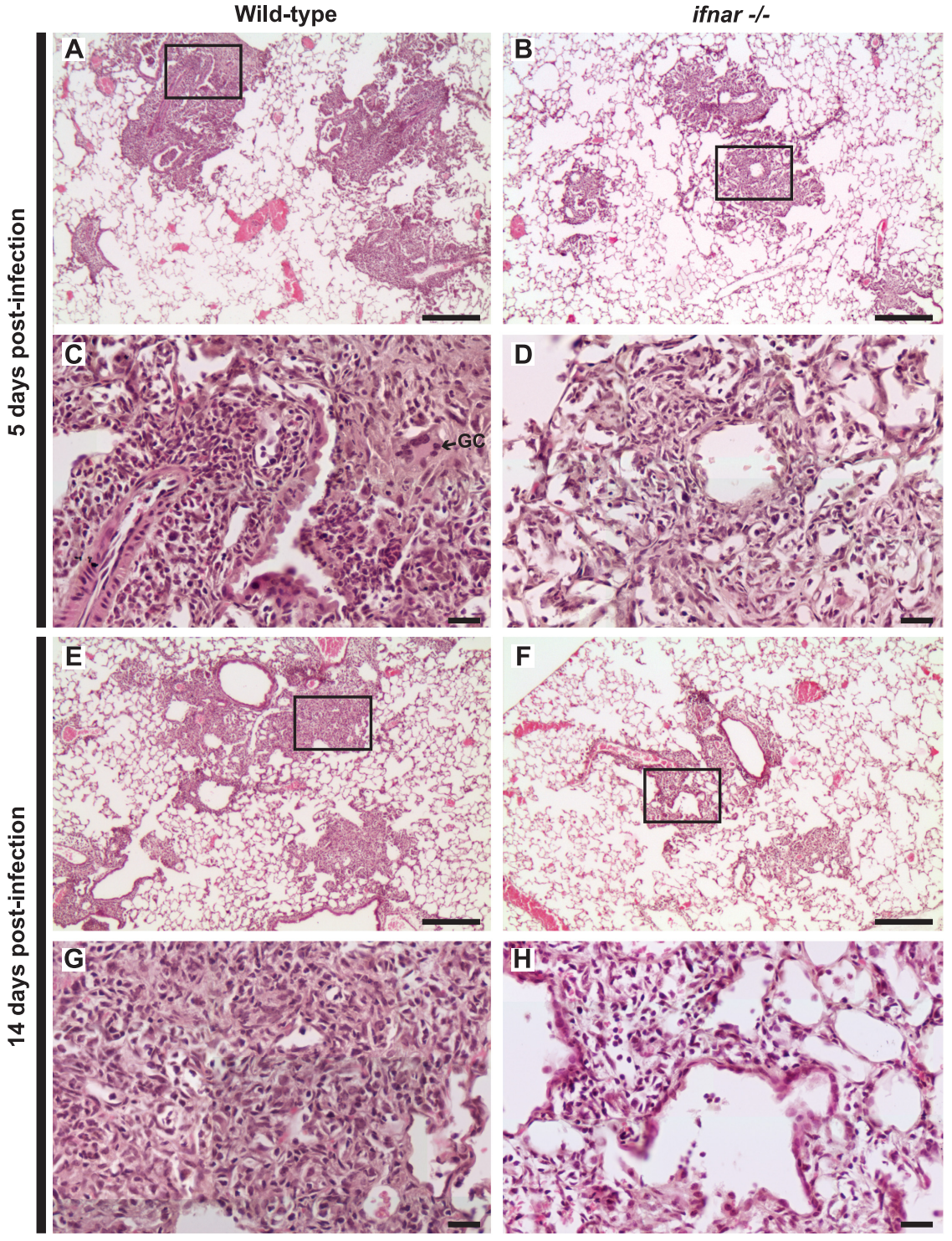

FIG. 7. Wild-type mice have a more extensive inflammatory response to *H. capsulatum* conidia than *ifnar1*$^{-/-}$ mice. Shown are hematoxylin-and-eosin-stained lung sections from mice infected with G217B conidia. Panels A and B are low-power images of representative inflammatory foci at 5 dpi in WT (A) or *ifnar*$^{-/-}$ (B) mice. WT inflammatory foci are larger and more densely packed with immune cells. Scale bar, 200 μm. Panels C and D are high-power views of boxed regions from panels A and B. WT infiltrate contains many neutrophils, macrophages and eosinophils, with giant cells (GC) also present. Scale bar, 20 μm. (E and F) Low-power images of representative inflammatory foci at 14 dpi in WT (E) or *ifnar*$^{-/-}$ (F) mice. Again, WT mouse inflammatory foci are larger and more densely packed than those of *ifnar*$^{-/-}$ mice. Scale bar, 200 μm. Panels G and H are high-power views of boxed regions from panels E and F. The WT shows more densely organized macrophage and lymphocytic inflammation. Scale bar, 20 μm.

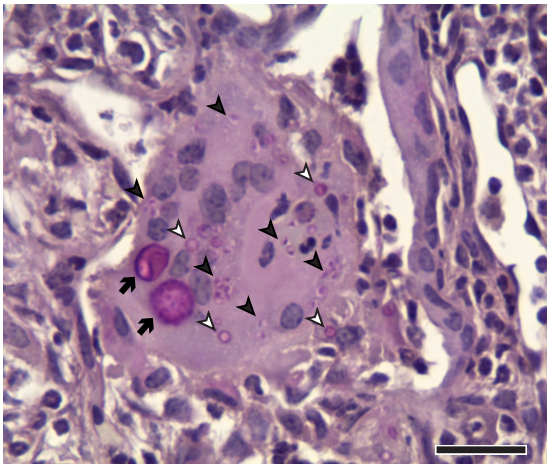

FIG. 8. Giant cell formation in conidium-infected lungs of wild-type mice. Shown is high magnification of a giant cell containing *H. capsulatum* macroconidia (black arrows), microconidia (white arrowheads), and yeast cells (black arrowheads) observed in the lungs of conidium-infected WT mouse strains at 5 dpi. Conidial forms could represent ungerminated cells or conidial remnants that persist after germination. Scale bar, 20 μm.

latum causes significant morbidity among healthy individuals (14), but little is understood about the host response to this intracellular fungus. This study represents the first examination of the macrophage transcriptional profile in response to *H. capsulatum* infectious particles. We found that infection of macrophages with conidia results in induction of IFN-β transcript, as well as induction of a classic type I IFN secondary response signature. These data are one of the first demonstrations of type I IFN induction in macrophages in response to an infection with fungal cells. Even more interesting is that induction of a type I IFN signature by macrophages in response to *H. capsulatum* occurred only in response to conidia; the yeast form of the organism, which is produced within the host as conidia germinate, was unable to stimulate this response, even at an MOI of 10 (data not shown). Similarly, a more limited examination of the alveolar macrophage response revealed that infection with conidia but not yeast induced the interferon-responsive gene Ifi205. Since conidia represent the most common infectious particle, they are likely to be the initial *H. capsulatum* cell encountered by host macrophages. These data suggest that in a natural infection, conidia could trigger early differential immune responses that influence the progression of *H. capsulatum* infection.

Type I IFN induction is elicited either in response to activation of TLRs or in response to cytosolic receptors (77). Since induction of IFN-β in response to conidia is independent of TLR signaling, it is likely that a cytosolic response pathway might be engaged by an unknown conidial component. Although *H. capsulatum* yeast cells are known to remain in the phagosome of macrophages during infection, the subcellular location of *H. capsulatum* conidia has not been investigated. Of note, some pathogens can trigger cytosolic signaling pathways despite being confined to the phagosome (55): for example, the bacterial pathogen *Mycobacterium tuberculosis* is able to access cytosolic signaling pathways to stimulate IFN-β despite its localization in the phagosome of macrophages (74).

It is unclear which feature of conidia is recognized by host macrophages, although we did observe that the unknown inducing factor was partially resistant to heat treatment. The host sensors required for the response are also unknown. Type I IFN production is triggered by signaling through cytosolic receptors that recognize nucleic acids, including DNA, RNA, cyclic-di-GMP, and cyclic-di-AMP (41, 55, 78, 89, 92). We have shown that induction of type I IFNs in response to conidia is independent of the adaptor MAVS, which is required for recognition of pathogen RNA by the RNA helicases RIG-I and MDA5. Thus, in contrast to the bacterial pathogen *Legionella pneumophila* (54), it seems unlikely that pathogen RNA contributes to the induction of the type I IFN response to *H. capsulatum* conidia. The role of conidial DNA in the induction of the type I IFN response has not been tested, and DNA remains a viable candidate ligand that could be sensed by host receptors. In this model, some unknown aspect of conidial but not yeast cell biology would allow fungal DNA to access the cytosol. In the case of the bacterial pathogen *Listeria monocytogenes*, introduction of bacterial genomic DNA into the cytosol of macrophages is sufficient to induce IFN-β, but this transcriptional response is enhanced by co-delivery of muramyl dipeptide, a constituent of the bacterial cell wall peptidoglycan (46). These data suggest that recognition of multiple ligands by different cytosolic receptors can contribute to induction of type I IFNs during infection with a pathogen. Notably, in the cases of *L. monocytogenes*, *M. tuberculosis*, and several other well-studied pathogens, the host receptors required for the type I response are unknown (55). The identification of these host molecules, as well as those that participate in the response to *Histoplasma* conidia, will shed light on common and distinct host pathways that are utilized to sense and respond to a diversity of pathogens.

The magnitude of induction of IFN-β by *H. capsulatum* conidia varied with respect to age and strain background. "Older" spores were more likely to induce higher levels of IFN-β, suggesting that these spores might accumulate higher levels of the inducing factor or activity that is recognized by the host. We also examined the ability of conidia from several evolutionarily diverged *H. capsulatum* strains to induce IFN-β. Whereas the North American G217B conidia induced intermediate levels of IFN-β, the "rough" Latin American G184AR strain induced only modest levels of IFN-β, and G186AR conidia did not appear to induce any. Interestingly, conidia from the "smooth" variant of G184AR, termed G184AS, induced high levels of IFN-β. (We were unable to produce conidia from the G186AS strain to test whether enhanced IFN-β production is a common property of smooth strains.) Although the molecular differences between the rough and smooth variants have not been characterized, it is known that the cell walls of the yeast form of the rough and smooth strains are fundamentally different: the rough yeast strains express the cell wall carbohydrate α-(1,3)-glucan, whereas the smooth strains do not. α-(1,3)-Glucan is thought to be specific to yeast cells, so unless α-(1,3)-glucan has a previously unsuspected role in conidial biology, it is likely that some other undetermined property of the G184AS smooth variant is contributing to the increased induction of IFN-β. In either case, the rough conidia either fail to accumulate the inducing factor or shield that factor from recognition by host cells.

During a natural infection, conidia are inhaled by the host, undergo germination, and produce yeast cells that colonize the host for the remainder of the infection. We observed that only *H. capsulatum* conidia, and not yeast cells, were able to induce IFN-β transcript in bone marrow-derived macrophages. Alveolar macrophages assayed at a single time point postinfection induced expression of Ifi205, an interferon-responsive gene (53), in response to conidia but not yeast cells, which also suggests that these host cells might respond differentially to various fungal cell types. Even though we observed induction of an interferon-responsive gene at 4 hpi, we did not observe induction of IFN-β in alveolar macrophages at 4 hpi in response to either conidia or yeast cells, which suggests that induction of Ifi205 could be dependent on production of IFN-α species or that the chosen time point was not optimal for detection of IFN-β transcript. Of note, Ifi205 expression can be activated in response to either type I or type II interferons (53), so it is also formally possible, although unexpected, that type II interferons could trigger Ifi205 expression in AvMs infected with conidia. Regardless, these data are consistent with the model that conidia and yeast cells trigger nonequivalent responses in macrophages. Most studies of *Histoplasma*-host interaction have utilized yeast cells, which are an excellent model for macrophage-fungus interactions that occur after germination of conidia. Our data highlight the value of examining the interaction of host cells with conidia, which, although technically challenging, sheds light on the initial stages of a natural infection. Fungal pathogens are notorious for adopting different morphologies in response to distinct environmental stimuli, and there is precedent for a host response that is tailored to individual morphological states. For example, it has been suggested that distinct morphological forms of the fungal pathogen *Candida albicans* are differentially recognized by TLR4 and by Dectin-1 (26, 82). Certainly the conidial and yeast forms of *H. capsulatum* have notable differences that could easily influence the host response: for example, electron microscopy clearly reveals that two morphological forms display fundamental differences in the structures of their cell walls (27–31). Furthermore, we have observed that conidia and yeast cells are molecularly distinct; approximately 300 transcripts accumulate preferentially in conidia as compared to yeast cells (D. O. Inglis, M. Voorhies, and A. Sil, unpublished data).

H. capsulatum yeast cells may lack the ability to induce IFN-β in macrophages, or they may actively suppress induction of this pathway in host cells. Even though yeast cells are thought to suppress other types of innate immune responses during infection (49), preliminary coinfection experiments of WT macrophages with conidia and yeast cells did not reveal a clear ability of yeast cells to inhibit the induction of IFN-β (data not shown). Macrophages infected with heat-killed yeast cells also failed to induce IFN-β (data not shown), indicating that yeast cells are unlikely to be actively suppressing the type I IFN response of macrophages.

By comparing fungal burdens in WT and *ifnar1*-deficient mice, we determined that type I IFN signaling does not protect the host from *H. capsulatum*-associated disease. In fact, type I IFN signaling promotes maximal fungal burden in lungs and spleens at later time points during infection, regardless of whether mice were infected with conidia or yeast cells. At present, the identity of the host cells (e.g., macrophages versus plasmacytoid dendritic cells [pDCs]) that produce type I IFNs during *in vivo* infection and the kinetics of type I IFN production are unknown. Presumably, some host cells, such as pDCs, or even alveolar macrophages at different times in infection, might produce type I IFNs in response to both yeast cells and conidia, suggesting that bone marrow-derived macrophages, although a useful model for assessing host-pathogen signaling, do not reflect the full complexity of *in vivo* interactions. Given the myriad roles of type I IFNs in the host, the possible effects of induction of IFN-β cells infected with *H. capsulatum* could include modulation of (i) downstream cytokine production, (ii) apoptosis of infected macrophages, or (iii) specific aspects of the adaptive immune response to *H. capsulatum*. Interestingly, it was previously observed that chronic infection of macrophages or mice with lymphocytic choriomeningitis virus (LCMV) clone 13, which induces type I IFNs, caused sensitization of the host to *H. capsulatum* infection (84, 90). Although the possible interpretations of these data are complex, they are consistent with the model that increased levels of type I IFNs correlate with increased sensitivity to *H. capsulatum* infection.

Of note, type I IFN signaling has been shown to play both protective and sensitizing roles in response to bacterial infection (17, 55). Our data are reminiscent of the observation that organs lacking *IFNAR1* are more restrictive for bacterial growth during infection with *L. monocytogenes* and *M. tuberculosis* (5, 61, 74). In the case of the fungal pathogen *Cryptococcus neoformans* (8), *ifnar1*$^{-/-}$ mice displayed a higher fungal burden in the lungs and brain, as well a dramatic decrease in survival. Disruption of *IFNAR1* also results in increased sensitivity to infection with the fungal pathogen *Candida albicans* (K. Kuchler, personal communication). Thus, analogous to what is observed for bacterial pathogens, it may be that type I IFN signaling may play protective or sensitizing roles during fungal infections, depending on the distinct strategies used by individual pathogens to promote disease.

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