Chlamydial IFN-γ immune evasion is linked to host infection tropism

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Chlamydiae are obligate intracellular pathogens that can exhibit a broad host range in infection tropism despite maintaining near genomic identity. Here, we have investigated the molecular basis for this unique host–pathogen relationship. We show that human and murine chlamydial infection tropism is linked to unique host and pathogen genes that have coevolved in response to host immunity. This intimate host–pathogen niche revolves around a restricted repertoire of host species-specific IFN-γ-mediated effector responses and chlamydial virulence factors capable of inhibiting these effector mechanisms. In human epithelial cells, IFN-γ induces indoleamine 2,3-dioxygenase expression that inhibits chlamydial growth by depleting host tryptophan pools. Human chlamydial strains, but not the mouse strain, avoid this response by the production of tryptophan synthase that rescues them from tryptophan starvation. Conversely, in murine epithelial cells IFN-γ induces expression of p47 GTPases, but not indoleamine 2,3-dioxygenase. One of these p47 GTPases (ligp1) was shown by small interfering RNA silencing experiments to specifically inhibit human strains, but not the mouse strain. Like human strains and their host cells, the murine strain has coevolved with its murine host by producing a large toxin possessing YopT homology, possibly to circumvent host GTPases. Collectively, our findings show chlamydial host infection tropism is determined by IFN-γ-mediated immunity.

immunity | virulence factors | pathogenesis | defense | coevolution

Hundreds of millions of people globally are afflicted by *Chlamydia trachomatis* infections. *C. trachomatis* urogenital infections are the leading cause of bacterial sexually transmitted diseases (STDs) in both industrialized and developing nations (1). *C. trachomatis* STDs are considered a risk factor in facilitating the transmission of HIV (2) and the progression of papilloma virus-caused neoplasia (3). *C. trachomatis* ocular infections cause trachoma, a disease that afflicts >300 million people and remains the world's leading cause of preventable blindness (4, 5). Because of its public health importance, *C. trachomatis* infections are the focus of intense public health control programs that have largely focused on diagnosis and antimicrobial treatment. Despite aggressive antimicrobial control measures over the past decade, the prevalence of *C. trachomatis* STDs has surprisingly increased (6). These findings argue that the most effective way to control, or eradicate, *C. trachomatis* STDs is through development of a vaccine. Unfortunately, significant progress toward this end has been hampered by the lack of a small animal model for the study of *C. trachomatis* pathogenesis and immunity. A relevant model would expedite vaccine development.

Members of the genus *Chlamydia* are characterized by an obligatory intracellular lifestyle and biphasic developmental cycle (7). The developmental cycle modulates between a highly infectious metabolically inert extracellular elementary body and an intracellular noninfectious metabolically active reticulate body. Shared biological features that allow survival in host cells include: (*i*) early modification of the nascent chlamydial vacuole engendering it nonfusogenic with lysosomes; and (*ii*) targeting of the chlamydial vacuole to the perinuclear region where the organisms intercept essential lipids from the trans-Golgi exocytic pathway (8), the latter being unique to chlamydiae. Host factors capable of inhibiting these virulence mechanisms would be important in preventing chlamydial infection.

Despite their common cell biology, chlamydiae exhibit a broad spectrum in natural host infection tropism (9). This paradigm is most evident comparing *C. trachomatis* and *Chlamydia muridarum*, human- and mouse-specific pathogens, respectively. In their natural hosts, these strains are epithelial tropic and produce infections of the urogenital tract that result in similar pathology (10). Restriction in host tropism of human strains is overcome in IFN-γ-deficient mice (11), suggesting that tropism is linked to the immune response. The paradox of this host–pathogen relationship is that human and mouse strains share 99% gene content (12), indicating that a limited number of pathogen-specific virulence genes control evasion of IFN-γ-mediated host-specific defense mechanisms. Understanding these virulence factors and host defense mechanisms are logical goals toward defining better infection models for human strains.

Here, we show that infection tropism of epithelial cells is strongly linked to a small subset of host-specific IFN-γ-mediated anti-chlamydial effector molecules and pathogen-specific virulence genes that interfere with these host defense mechanisms. Collectively, the data imply that murine and human chlamydial strains have coevolved with their respective mammalian hosts primarily to avoid IFN-γ-mediated defense mechanisms. The results suggest a logical strategy for genetically engineering humanized mouse strains of greater susceptibility to infection by human strains, which would provide superior hosts for the study of *C. trachomatis* pathogenesis and immunity.

Materials and Methods

Cells and Chlamydial Culture. Murine oviduct epithelial cells (MECs) BM12.4 were cultured as described (13). *C. muridarum* strain Nigg (mouse pneumonitis, MoPn), the human genital tract isolate(s) *C. trachomatis* serovar D strain UW-3/CX, and lymphogranuloma venereum (L2)/434/BU serovar were grown as described (14, 15).

Infection of Mice. Six- to 8-week-old female C57BL/6, C57BL/6 *ifn*-$\gamma^{-/-}$, and C57BL/6 *nos2*$^{-/-}$ mice were purchased from The Jackson Laboratory. C57BL/6 *indo*$^{-/-}$ mice were provided by A. Mellor (Medical College of Georgia, Augusta) (16). Mice were injected intravaginally and monitored for cervicovaginal shedding as described (11).

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Abbreviations: IFU, inclusion-forming units; MoPn, mouse pneumonitis; L2, lymphogranuloma venereum; iNOS, inducible NO synthase; IDO, 2,3-indoleamine dioxygenase; MEC, murine oviduct epithelial cell; p.i., postinfection; siRNA, small interfering RNA; KO, knockout.

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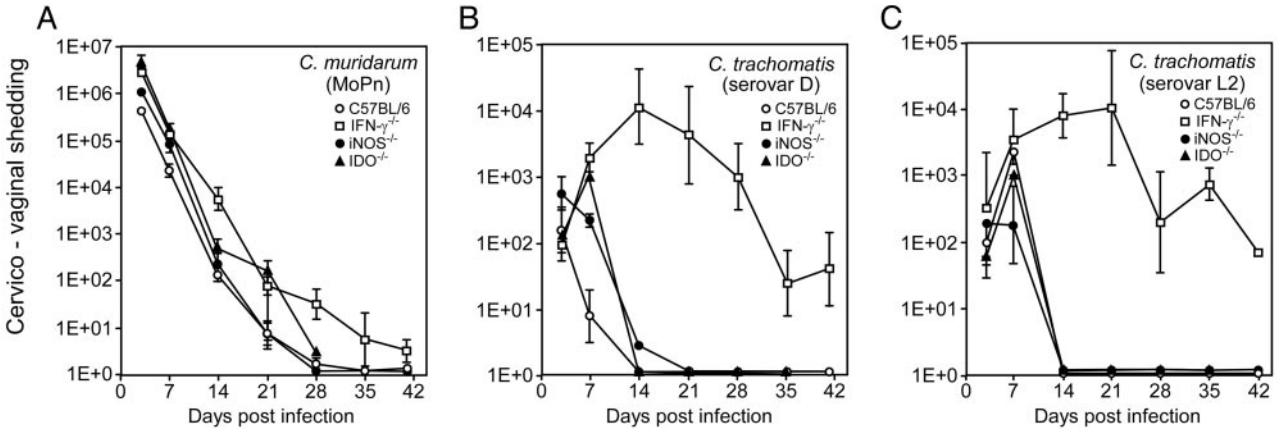

Fig. 1. MoPn and *C. trachomatis* differ in susceptibility to IFN-γ *in vivo*. C57BL/6 (○), C57BL/6 *nos2*$^{-/-}$ (●), C57BL/6 *ifn-γ*$^{-/-}$ (□), and C57BL/6 *indo*$^{-/-}$ (▲) mice were infected with MoPn (*A*), serovar D (*B*), or serovar L2 (*C*). The vaginal vault was swabbed at intervals after infection, and shed IFU were enumerated. SD is depicted by bars.

Nitrite Assays, Inducible NO Synthase (iNOS) and 2,3-Indoleamine Dioxygenase (IDO) Expression, and RT-PCR. RNA for RT-PCR detection of iNOS, IDO, and β-actin was extracted with Trizol (Invitrogen), DNaseI-treated, and amplified with gene-specific primers (Table 1, which is published as supporting information on the PNAS web site) by standard procedures. Detailed methods for iNOS, nitrate, and IDO assays are described in *Supporting Text*, which is published as supporting information on the PNAS web site.

Microarray Analysis. MECs were infected with MoPn or L2 at a multiplicity of infection of 1 and cultured in medium without or containing 20 units/ml of IFN-γ. Total RNA was extracted 6 h postinfection (p.i.) with Trizol and purified with the RNeasy mini kit (Qiagen, Valencia, CA). Six micrograms of the total RNA was amplified according to the Affymetrix (Santa Clara, CA) eukaryotic target preparation protocol, and 10 μg of amplified-labeled RNA was hybridized to a Mouse 430A Chip (Affymetrix). Details of microarray analysis and Taqman quantitative RT-PCR analysis are described in *Supporting Text*. MIAME-formatted microarray data are available in *Supporting Data Set*, which is published as supporting information on the PNAS web site.

Sphingomyelin Trafficking. MECs were seeded onto 12-mm glass coverslips in 24-well plates at a density of 8×10^4 cells per well. Cells were treated with 40 units/ml of IFN-γ for 4 h then infected with L2 at a multiplicity of infection of 1. Sphingomyelin labeling was performed as described (8) by using the trans-Golgi selective stain 6-(*N*-[{7-nitrobenzo-2-oxa-1,3-diazol-4-yl}amino]caproyl) sphingosine (Invitrogen). Nuclei and chlamydial inclusions were labeled with the DNA dye DRAQ5 (1 μM; Alexis Biochemicals, Lausen, Switzerland) for 5 min before imaging.

Small Interfering RNA (siRNA) Analysis. The siRNA were designed from GenBank sequences and purchased from Qiagen. Details of siRNA analysis are provided in *Supporting Text*.

Results and Discussion

Chlamydial Growth in the Urogenital Tract of Female Mice. We investigated susceptibility of C57BL/6 and isogenic IFN-γ (*ifn-γ*$^{-/-}$), iNOS (*nos2*$^{-/-}$), and IDO (*indo*$^{-/-}$) knockout (KO) female mice to infection by the human *C. trachomatis* serovars D and L2 and the mouse MoPn strain. After intravaginal challenge, the cervico-vaginal shedding profiles of human and mouse strains in WT and gene KO mice differed dramatically (Fig. 1). MoPn was highly infectious for control mice, yielding infectious burdens of 10^6 inclusion-forming units (IFU) during the first 7 days p.i. (Fig. 1*A*). Infection then decreased exponentially until 28 days p.i., when mice became culture negative. Interestingly, MoPn infection did not substantially differ in IFN-γ, iNOS, or IDO KO mice (Fig. 1*A*). In contrast, human strains (D and L2) were poorly infectious for the genital tract of immuncompetent mice. Shedding was short-lived (7–10 d) and yielded small numbers (10^2 IFU) of infectious organisms (Fig. 1 *B* and *C*).

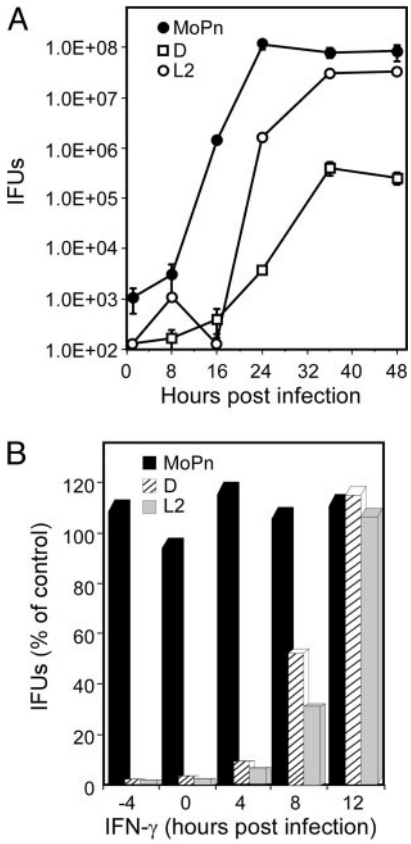

Fig. 2. IFN-γ inhibits *C. trachomatis* growth in MECs. (*A*) Recoverable IFU of chlamydial strains from MECs. MECs were infected at a multiplicity of infection of 0.2, and recoverable IFU were harvested at various intervals p.i. MoPn, ●; serovar D, □; L2, ○. (*B*) Temporal effect of IFN-γ on recoverable IFU. Time of IFN-γ addition (20 units/ml) is indicated below the graph. MoPn, black bars; serovar D, hatched bars; L2, gray bars.

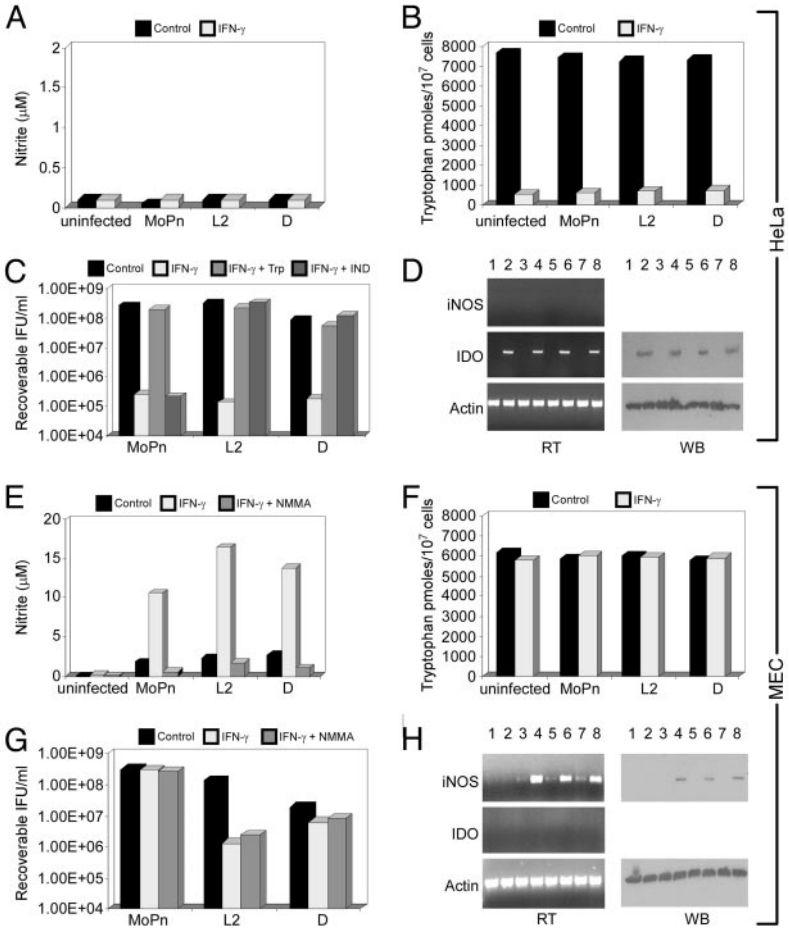

Fig. 3. IFN-γ-mediated inhibition of chlamydial growth in MECs is independent of iNOS and IDO. (*A–D*) Results in HeLa cells. (*E–H*) Results in MECs. (*A* and *E*) HeLa cells or MECs were infected with chlamydiae without IFN-γ (black bars), with IFN-γ (light gray bars), or with IFN-γ and N^G-monomethyl-L arginine (L-NMMA) (dark gray bars) (*E* only), and nitrite levels were determined. (*B* and *F*) HeLa cells or MECs were infected with chlamydiae and IFN-γ (gray bars) or without IFN-γ (black bars), and tryptophan pools were measured 24 h p.i. (*C*) HeLa cells were infected with chlamydiae without IFN-γ (black bars), with IFN-γ (light gray bars), with IFN-γ and tryptophan (midgray bars), or with IFN-γ and indole (dark gray bars). (*D*) HeLa cells were mock-infected (lane 1), mock-infected with IFN-γ (lane 2), L2-infected (lane 3), L2-infected with IFN-γ (lane 4), MoPn-infected (lane 5), MoPn-infected with IFN-γ (lane 6), serovar D-infected (lane 7), and serovar D-infected with IFN-γ (lane 8). RNA or proteins were harvested 24 h p.i. and detected by RT-PCR (*Left*) or Western blot (*Right*). (*G*) MECs were infected with chlamydiae and grown in normal infection medium (black bars), with IFN-γ (light gray bars), or with IFN-γ and L-NMMA (dark gray bars). (*H*) Identical to *D* except the experiment was performed in MECs with mouse-specific PCR primers and antibodies.

However, IFN-γ KO mice challenged with *C. trachomatis* had markedly more severe infections (Fig. 1 *B* and *C*) with significantly increased cervico-vaginal shedding (10^3 to 10^4 IFU) that extended over a period of 3–4 weeks. Infection of iNOS and IDO KO mice with the human strains did not significantly differ from controls. Thus, immune-mediated clearance of the MoPn strain from the female urogenital mucosae is independent of IFN-γ, iNOS, or IDO. In contrast, immune clearance of human strains highly depends on IFN-γ, but is independent of iNOS and IDO.

Chlamydial Growth in Epithelial Cells. To understand differential sensitivity of mouse and human strains to IFN-γ-mediated immunity *in vivo*, we undertook *in vitro* studies with cultured human HeLa and primary MECs (13). HeLa cells are permissive hosts for murine and human strains; however, susceptibility of MECs to infection by these strains has not been investigated. All three strains grew readily in MECs. There were differences in growth rates and burst sizes (Fig. 2*A*), but these were reflective of the growth characteristics of these strains in HeLa cells (data not shown). We concluded that the highly attenuated *in vivo* growth of human strains (Fig. 1) was not caused by their inability to productively infect MECs.

Next, we assessed the effect of IFN-γ on chlamydial growth in MECs. MECs were pretreated with IFN-γ, or at various times p.i., and recoverable IFU were assayed. IFN-γ treatment did not inhibit growth of MoPn in MECs (Fig. 2*B*). In contrast, IFN-γ pretreatment or treatment up to 4 h after infection dramatically inhibited the human strains (>95% reduction in IFU). However, IFN-γ treatment of MECs at 8 h p.i. was less inhibitory (30–40% reduction) and failed to inhibit chlamydiae entirely when the cytokine was added 12 h or longer p.i. (Fig. 2*B*). Inhibition was partially reversible as removal of IFN-γ from infected cells as late as 24 h p.i. rescued 80% of the input infectivity (unpublished data). Thus, although highly inhibitory to human strains, IFN-γ was not bactericidal.

IFN-γ Inhibition of Human Strains Is IDO-Mediated in HeLa Cells and IDO- and iNOS-Independent in MECs. To define the IFN-γ-mediated factors that inhibit human strains in MECs, we conducted parallel experiments in HeLa cells and MECs under conditions of IFN-γ treatment alone, infection, or infection in combination with IFN-γ treatment (Fig. 3). We analyzed: (*i*) iNOS and IDO mRNA and protein expression, (*ii*) medium nitrite (NO) and intracellular tryptophan concentrations, and (*iii*) chlamydial growth. Infection, IFN-γ treatment, or infection and IFN-γ treatment did not induce iNOS mRNA or protein in HeLa cells or increase NO levels in the culture medium (Fig. 3 *A* and *D*). IFN-γ treatment of HeLa cells did induce expression of IDO mRNA and protein (Fig. 3*D*) and depleted intracellular tryptophan pools (Fig. 3*B*). Tryptophan depletion inhibited growth of all three chlamydial strains in HeLa cells (Fig. 3*C*) (12, 17). Infectivity of both human and mouse strains was rescued by the addition of exogenous tryptophan, but importantly, only the infectivity of human strains was rescued by exogenous indole (Fig. 3*C*), demonstrating the expression of a functional tryptophan synthase enzyme (17, 18) by the human strains. Unlike HeLa cells, MECs treated with IFN-γ produced iNOS mRNA and protein that resulted in NO production (Fig. 3 *E–H*). Expression of iNOS required both IFN-γ and infection. In contrast, IFN-γ, infection, or IFN-γ and infection failed to induce IDO in MECs (Fig. 3 *F* and H). Despite significant iNOS expression in infected MECs, NO had a minimal effect on intracellular chlamydial growth.

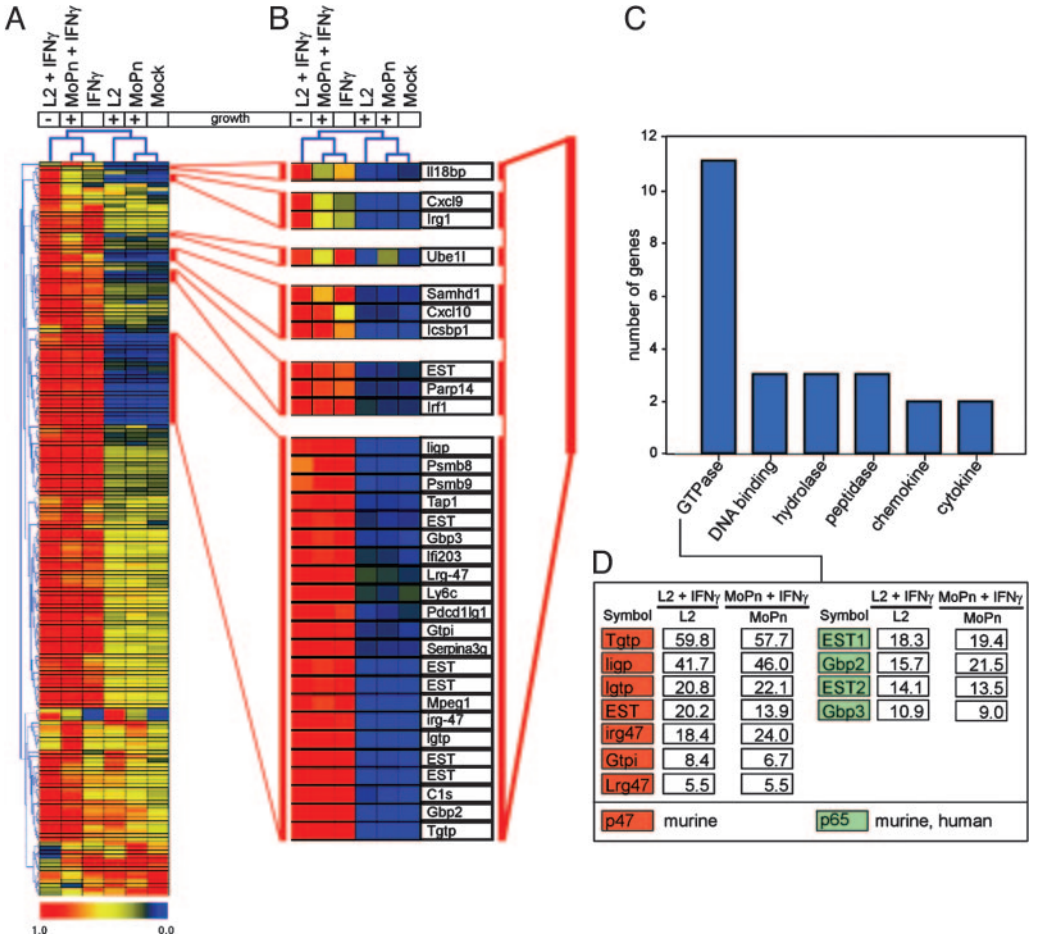

Fig. 4. Comparative microarray analysis of IFN-γ-inhibited chlamydial growth in MECs. (*A* and *B*) Hierarchical clustering of gene expression. Median signal intensities of significantly up- and down-regulated genes were normalized so that the maximum expression level was equal to 1; experiments and genes were clustered. Experimental conditions are indicated above the columns. Symbols below experimental conditions indicate whether the condition was permissive (+) or inhibitory (−) to chlamydiae. Rows indicate individual genes. Red genes are highly expressed, blue genes are lowly expressed. (*B*) Genes highly differentially expressed between L2 infection (permissive) and L2 infection plus IFN-γ (inhibitory). (*C*) Classification of genes in *B* based on Gene Ontology molecular function; the six most highly populated functional groups are depicted. (*D*) Detail of p47 and p65 GTPase expression is shown in orange and green, respectively. Ratio of expression between L2 vs. L2 plus IFN-γ and MoPn vs. MoPn plus IFN-γ is given in the open boxes.

Inhibition of iNOS with the competitive inhibitor N^G-monomethyl-L arginine decreased nitrite levels (Fig. 3*E*), but failed to significantly reverse IFN-γ-mediated inhibition (Fig. 3*G*). These results strongly argue that the IFN-γ-mediated inhibition of human strains in MECs was not caused by NO. Further, these results closely parallel those observed *in vivo* with KO mice (Fig. 1). Collectively, these results present overwhelming evidence that IFN-γ-mediated inhibition of *C. trachomatis* in MECs is independent of iNOS and IDO.

Identification of Candidate Murine-Specific Antichlamydial Inhibitors by DNA Microarray Analysis. We used comparative microarray analysis to identify genes expressed by IFN-γ-treated infected MECs that were nonpermissive for growth of human strains (Fig. 4). Gene expression profiles of MECs infected with murine or human chlamydiae (L2) and treated with IFN-γ, or MECs treated with IFN-γ but not infected, were remarkably similar (Fig. 4*A*). Moreover, mRNA expression profiles of cells infected with either strain in the absence of IFN-γ treatment were also very similar to untreated controls (Fig. 4*A*), suggesting that IFN-γ was the major source of gene induction during the 8-h time period that was strongly inhibitory to the growth of human strains. Clustering analysis revealed 32 genes, differing by a 5.5-fold or higher expression levels between L2-infected IFN-γ-treated cells (inhibitory to growth) and cells infected in the absence of IFN-γ (Fig. 4*B*). This gene set contained a dominant representation of genes encoding GTPase proteins (Fig. 4*C*). Considering the ontology of all genes on the microarray, enrichment of the GTPase cluster was highly significant ($P < 10^{-10}$). The cluster contained all six characterized murine p47 family members (Iigp1, Tgtp, Lrg-47, Irg-47, Igtp, and Gtpi) and two p65 GTPases (Gbp2 and Gbp3) (19). Although p65 GTPases are conserved in humans and mice, functional p47 GTPases have been described only in mice (19, 20). This transcriptome analysis strongly implicated p47 GTPases as the potent antichlamydial factors produced by MECs.

GTPase Expression Is Induced by IFN-γ and Chlamydial Infection. To determine whether the elevated transcription of GTPases resulted in protein expression, we analyzed p47 GTPases protein expression by Western blot. IFN-γ treatment rapidly increased p47 (Iigp1, Gtpi, Lrg-47, Tgtp, and Igtp) and p65 (Gbp2) protein levels (Fig. 8, which is published as supporting information on the PNAS web site). Infection alone failed to induce detectable GTPase expression within the first 12-h period, although low to moderate levels were present in infection-only cultures by 24 h. However, it is unlikely that these GTPases would be inhibitory at this late stage because IFN-γ-mediated inhibition of growth is restricted to the first 12 h of infection (Fig. 2). Moreover, these results show that neither the human nor murine strain inhibit the expression of p47 GTPases.

IFN-γ Treatment Fails to Promote Chlamydial Phagolysosomal Fusion but Blocks Trafficking of Sphingolipids to the Early Inclusion. p47 GTPases have potent antimicrobial activities that are believed to function by alteration of normal host cell trafficking and promotion of phagosome maturation (19, 20). Therefore, a logical explanation for the IFN-γ-mediated inhibition of L2 infection was GTPase-mediated modification of the chlamydial inclusion leading to phagosome fusion. To test this, the distribution of Lamp1 was studied in IFN-γ-treated chlamydial-infected MECs. In the presence of IFN-γ, L2 inclusions were small (Fig. 9, which was published

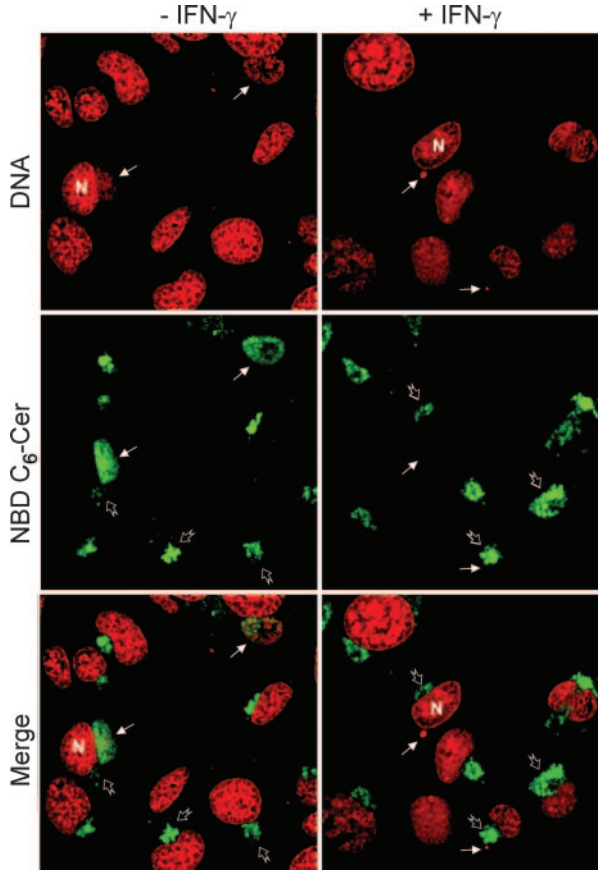

Fig. 5. *C. trachomatis* inclusions fail to incorporate sphingolipids in IFN-γ-treated MECs. MECs were infected with *C. trachomatis* (L2) for 18 h in the absence (−) or presence (+) of IFN-γ. DNA of cells and chlamydiae was labeled with Draq5 (red), and sphingolipids (green) were visualized by using the trans-Golgi selective stain 6-(*N*-[{7-nitrobenzo-2-oxa-1,3-diazol-4-yl}amino]caproyl) sphingosine. Host cell nuclei are stained red and indicated by N. Golgi stacks are labeled green and indicated by open arrows, and chlamydial inclusions are labeled red and indicated by solid arrows. The merged image shows distinct colocalization of the inclusion and sphingolipid in the absence of IFN-γ and no colocalization in the presence of IFN-γ. (Magnification: ×600.)

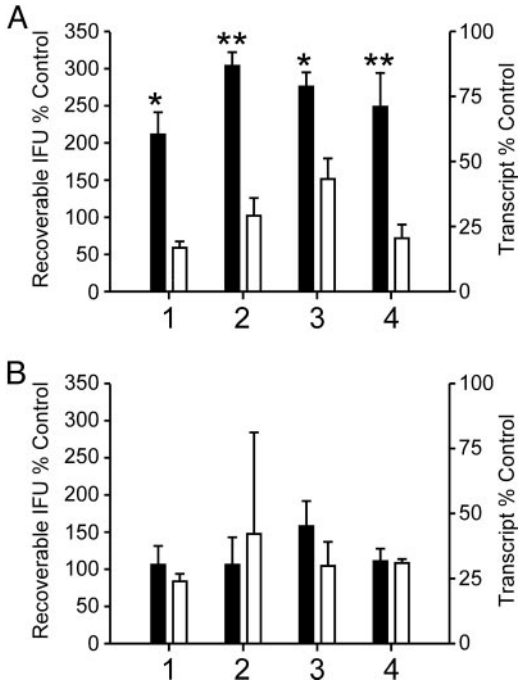

Fig. 6. siRNA knockdown of Iigp1 reverses IFN-γ-mediated inhibition of L2. MECs were transfected with siRNA or equal amounts of nonsilencing control siRNA, treated with IFN-γ, and infected with L2. Columns 1–4 show results of a single experiment performed in quadruplicate. Graph columns correspond to recoverable IFU of siRNA-transfected sample versus control (filled bars, left axis) and mRNA transcript in siRNA knockdown as a percent of control (empty bars, right axis). Data show means and SD of single experiments: Iigp1 siRNA Iigp1-1 (*A*); Gtpi siRNA Gtpi-1 (*B*). *, $P < 0.01$; **, $P < 0.001$.

as supporting information on the PNAS web site) and appeared similar to those present in control chloramphenicol-treated cultures, which readily colocalized with Lamp1 (21) (Fig. 9 and data not shown). However, the inclusions in IFN-γ-treated cells did not acquire Lamp1 (Fig. 9). Interestingly, the majority of inhibited inclusions still located to the perinuclear region, in close proximity to the Golgi apparatus, in IFN-γ-treated cells (data not shown). These results imply that GTPase-mediated inhibition of L2 growth was not the result of phagosome remodeling like that described for *Mycobacterium tuberculosis* in IFN-γ-activated macrophages (22).

Because p47 GTPases disrupt intracellular trafficking, we investigated whether sphingomyelin trafficking to the inclusion was altered by IFN-γ treatment. Fluorescent sphingomyelin, endogenously synthesized from 6-(*N*-[{7-nitrobenzo-2-oxa-1,3-diazol-4-yl}amino]caproyl) sphingosine, traffics from the host trans-Golgi to the chlamydial inclusion early in the developmental cycle (8). Notably, IFN-γ inhibited L2 sphingomyelin acquisition (Fig. 5). Sphingomyelin-positive L2 inclusions were reduced by ≈50% in IFN-γ-treated cells at 16 h p.i. In contrast, >80% of L2 control and IFN-γ-treated or untreated MoPn inclusions acquired sphingomyelin (Fig. 10, which is published as supporting information on the PNAS web site). These results strongly imply that inhibition of L2 growth by IFN-γ was the result of reduced trafficking of host lipids to the inclusion.

Interfering RNA Analysis Identifies Iigp1 as an Antichlamydial Effector. We used a siRNA screen to target the mRNAs of six p47 GTPases (Tgtp, Iigp1, Igtp, Lrg-47, Irg-47, and Gtpi) and a GBP homologue (EST1) to ascertain their possible effector function(s) in the observed inhibition of chlamydial growth. Transcripts for these genes were reduced between 40% and 80% in MECs during the first 12 h of infection in IFN-γ-treated cells. A preliminary screen using siRNA corresponding to each of the GTPases in a L2

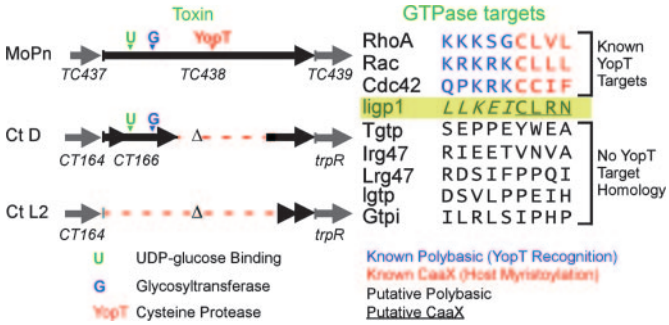

Fig. 7. A proposed mechanism of murine toxin function in the inactivation of the murine p47 GTPase Iigp1. The toxin of the MoPn and *C. trachomatis* serovars D and L2 are shown with the putative GTPase-inactivating domains. Only the mouse toxin possesses the YopT cysteine protease domain that is known to cleave the GTPase targets RhoA, Rac, and Cdc42 (26). The targets for cleavage and myristoylation are identified. At its N terminus Iigp1 possesses a putative polybasic cleavage and myristoylation site. Other murine p47 GTPases lack similar protease and myristoylation sites within their primary sequences.

growth rescue assay indicated that only Iigp1 was capable of reversing the IFN-γ inhibitory effect (data not shown). A comparison of Iigp1 and Gtpi siRNAs in the knockdown rescue assay is shown in Fig. 6. Iigp1 siRNAs, but not Gtpi siRNA, consistently rescued L2 growth in IFN-γ-treated cells ($P < 0.02$–0.05). These findings, together with those observed for sphingomylein trafficking (Fig. 5), are consistent with the proposed function of Iigp1 as an inhibitor of Golgi lipid trafficking (23, 24). Although indirect, the data collectively argue that Iigp1 is the primary IFN-γ-inducible GTPase responsible for the inhibition of L2 growth in MECs.

Chlamydial Toxin as a Potential Inactivator of Murine Iigp1. The murine strain is indisputably resistant to IFN-γ host defense mechanisms in MECs, the natural target cell of *in vivo* infection. How does the mouse-adapted strain, but not human strains, circumvent the proposed lipid trafficking function of Iigp1? A schematic diagram describing a mechanism for this activity is shown in Fig. 7. A major difference between MoPn and *C. trachomatis* strains maps to a cytotoxin loci located in the plasticity zone (12, 25). MoPn encodes three copies of a large (≈350 kDa) protein, each containing domains with homology to the UDP-glycosyltransferase portion of the large clostridial toxin (LCT) and the type III secreted *Yersinia pestis* cysteine protease YopT (12, 25). LCT and YopT inactivate host cell GTPases *in vivo* (26, 27). *C. trachomatis* strains retain a single copy of this gene in which the region of YopT homology either has been deleted or accumulated frame-shift mutations. Yersinial YopT targets small GTPases by binding a polybasic sequence located immediately N terminal to the C-terminal CaaX (cysteine, aliphatic residue, aliphatic residue, any amino acid) isoprenylation sequence (26, 28), then cleaves the lipidic moiety from the cysteine residue, releasing the target protein from membrane association (29). Iigp1 is the only p47 GTPase that retains apparent homology with described polybasic sequences or CaaX motifs (CLRN) (28).

Here, we describe IFN-γ-mediated host-specific innate immune factors that dictate infection tropism of human and murine chlamydial strains in epithelial cells. Both *in vivo* and *in vitro* models of epithelial cell infection show that IFN-γ-induced antichlamydial activity against human and murine strains is mediated by distinct host-specific mechanisms. Human- and mouse-adapted chlamydiae differ in a small subset of genes that appear to have specifically coevolved with their respective hosts to circumvent the effects of IFN-γ. Human genital strains encode a functional tryptophan synthase enzyme (*trp*RBA), which possibly uses exogenous indole supplied by cocolonizing microbes of the human genital tract (17), to evade IFN-γ-mediated IDO expression. The mouse strain lacks the *trp*RBA genes and cannot replicate in IFN-γ-treated human epithelial cells, likely limiting the pathogen's ability to naturally infect humans. However, IFN-γ treatment does not induce IDO expression in mouse epithelial cells, explaining why the mouse strain lacks tryptophan synthase. In contrast, IFN-γ-treated mouse cells are highly permissive for murine strains but nonpermissive for human strains. Our data suggest Iigp1, possibly by inhibition of sphingomyelin trafficking from the Golgi to the inclusion, is responsible for inhibition of human chlamydiae.

Multiple observations suggest that Iigp1 is the primary effector molecule in IFN-γ-mediated control of L2 in MECs. First, Iigp1 transcripts were highly differentially regulated between L2-permissive and L2-nonpermissive growth conditions (41-fold at 6 h posttreatment) (Fig. 4). Second, in the siRNA screen of p47 GTPases, knockdown of Iigp1, but no other GTPase, rescued IFN-γ-mediated inhibition of L2 in MECs. Third, Iigp1 is suspected to modify exocytic traffic from the Golgi (23, 24), a pathway with which chlamydiae must interact to acquire host lipids. Finally, IFN-γ-mediated inhibition of L2 was reversible, arguing against a lethal fusion of the inclusion with lysosomes. However, the role of Iigp1 remains indirect, and more definitive experiments, such as *in vitro* transfection and animal studies using KO mice, are needed to further define the role of this and other p47 GTPases.

Conclusions

In summary, this study shows that differences in murine and human IFN-γ-mediated immunity have influenced the evolution of host-specific chlamydial virulence genes. These simple, yet critical, differences complicate modeling of human chlamydial infection and disease in mice. This is particularly germane to those findings obtained by using the murine model and human-challenge strains to evaluate experimental vaccines caused by the exquisite, yet likely irrelevant, sensitivity of these strains to IFN-γ-inducible murine-specific p47 GTPases. However, our results suggest a direct means for improving the murine model. It should be feasible to engineer a transgenic mouse that expresses IDO in columnar epithelial cells, under control of an IFN-γ-regulated response element, in an Iigp1 KO background. We suspect this combination transgenic-KO strain would be a more relevant small animal model for pathogenesis studies and vaccine development.

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1. World Health Organization (2001) *Global Prevalence and Incidence of Selected Curable Sexually Transmitted Infections: Overview and Estimates* (W.H.O., Geneva), pp. 1–43.
2. Plummer, F., Simonsen, J. N., Cameron, D. W., Ndinya-Achola, J., Kreiss, J. K., Gakinya, M. N., Waiyaki, P., Cheang, M., Piot, P. & Ronald, A. R. (1991) *J. Infect. Dis.* **164,** 1236–1237.
3. Anttila, T., Saikku, P., Koskela, P., Bloigu, A., Dillner, J., Ikaheimo, I., Jellum, E., Lehtinen, M., Lenner, P., Hakulinen, T., *et al.* (2001) *J. Am. Med. Assoc.* **285,** 47–51.
4. Resnikoff, S., Pascolini, D., Etya'ale, D., Kocur, I., Pararajasegaram, R., Pokharel, G. P. & Mariotti, S. P. (2004) *Bull. W. H. O.* **82,** 844–851.
5. Whitcher, J. P., Srinivasan, M. & Upadhyay, M. P. (2001) *Bull. W. H. O.* **79,** 214–221.
6. Communicable Disease Center (2002) *Sexually Transmitted Disease Surveillance 2001* (Communicable Disease Center, Atlanta), pp. 1–20.
7. Moulder, J. W. (1991) *Microbiol. Rev.* **55,** 143–190.
8. Hackstadt, T., Scidmore, M. A. & Rockey, D. D. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 4877–4881.
9. Morrison, R. P. & Caldwell, H. D. (2002) *Infect. Immun.* **70,** 2741–2751.
10. Cotter, T. W. & Byrne, G. I. (1996) *Res. Immunol.* **147,** 587–595.
11. Perry, L. L., Su, H., Feilzer, K., Messer, R., Hughes, S., Whitmire, W. & Caldwell, H. D. (1999) *J. Immunol.* **162,** 3541–3548.
12. Read, T. D., Brunham, R. C., Shen, C., Gill, S. R., Heidelberg, J. F., White, O., Hickey, E. K., Peterson, J., Utterback, T., Berry, K., *et al.* (2000) *Nucleic Acids Res.* **28,** 1397–1406.
13. Johnson, R. M. (2004) *Infect. Immun.* **72,** 3951–3960.
14. Su, H. & Caldwell, H. D. (1995) *Infect. Immun.* **63,** 3302–3308.
15. Caldwell, H. D., Kromhout, J. & Schachter, J. (1981) *Infect. Immun.* **31,** 1161–1176.
16. Mellor, A. L., Baban, B., Chandler, P., Marshall, B., Jhaver, K., Hansen, A., Pandelakis, A. K., Iwashima, M. & Munn, D. J. (2003) *J. Immunol.* 1652–1655.
17. Caldwell, H. D., Wood, H., Crane, D., Bailey, R., Jones, R. B., Mabey, D., Maclean, I., Mohammed, Z., Peeling, R., Roshick, C., *et al.* (2003) *J. Clin. Invest.* **111,** 1757–1769.
18. Fehlner-Gardiner, C., Roshick, C., Carlson, J. H., Hughes, S., Belland, R. J., Caldwell, H. D. & McClarty, G. (2002) *J. Biol. Chem.* **277,** 26893–26903.
19. Taylor, G. A., Feng, C. G. & Sher, A. (2004) *Nat. Rev. Immunol.* **4,** 100–109.
20. MacMicking, J. D. (2005) *Curr. Opin. Microbiol.* **8,** 74–82.
21. Scidmore, M. A., Rockey, D. D., Fischer, E. R., Heinzen, R. A. & Hackstadt, T. (1996) *Infect. Immun.* **64,** 5366–5372.
22. MacMicking, J. D., Taylor, G. A. & McKinney, J. D. (2003) *Science* **302,** 654–659.
23. Kaiser, F., Kaufmann, S. H. & Zerrahn, J. (2004) *J. Cell Sci.* **117,** 1747–1756.
24. Walenta, J. H., Didier, A. J., Liu, X. & Kramer, H. (2001) *J. Cell Biol.* **152,** 923–934.
25. Belland, R. J., Scidmore, M. A., Crane, D. D., Hogan, D. M., Whitmire, W., McClarty, G. & Caldwell, H. D. (2001) *Proc. Natl. Acad. Sci. USA* **98,** 13984–13989.
26. Shao, F., Merritt, P. M., Bao, Z., Innes, R. W. & Dixon, J. E. (2002) *Cell* **109,** 575–588.
27. von Eichel-Streiber, C., Boquet, P., Sauerborn, M. & Thelestam, M. (1996) *Trends Microbiol.* **4,** 375–382.
28. Reid, T. S., Terry, K. L., Casey, P. J. & Beese, L. S. (2004) *J. Mol. Biol.* **343,** 417–433.
29. Zumbihl, R., Aepfelbacher, M., Andor, A., Jacobi, C. A., Ruckdeschel, K., Rouot, B. & Heesemann, J. (1999) *J. Biol. Chem.* **274,** 29289–29293.