

Differential Sensitivity of Distinct *Chlamydia trachomatis* Isolates to IFN- γ -Mediated Inhibition

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Resistance to the mouse pneumonitis (MoPn) strain of *Chlamydia trachomatis* has been mapped to MHC class II-restricted, IL-12-dependent CD4⁺ T cells that secrete a type 1 profile of proinflammatory cytokines, which includes IFN- γ and TNF- α . The relative contribution of IFN- γ is controversial, however, due to variation in results presented by different laboratories. To determine whether *C. trachomatis* strain differences contributed to this apparent conflict, the relative resistance of IFN- γ -deficient mice to murine and human strains of *C. trachomatis* was compared. All human serovars were much more sensitive to the direct inhibitory actions of IFN- γ than the MoPn strain. Furthermore, genital clearance of human serovar D in the C57BL/6 mouse was mediated by class II-independent mechanisms that probably involved local production of IFN- γ by cells of the innate immune system. TNF- α also contributed indirectly to host resistance against all strains tested. The differential susceptibility of distinct *C. trachomatis* strains to effector cytokines such as IFN- γ could not have been predicted by interstrain biologic variation or by the profile of cytokines stimulated during infection. These findings indicate that strain variation should be considered in situations where related isolates of a given parasite produce conflicting data in models of infection and immunity. They also suggest that stimulation of mucosal IFN- γ activity is a relevant goal for a human chlamydial vaccine. *The Journal of Immunology*, 1999, 162: 3541–3548.

The intracellular bacterium, *Chlamydia trachomatis*, is represented by 19 serologically distinguishable, stable variants that are classified into three major strains: *C. trachomatis* lymphogranuloma venereum (LGV)³, *C. trachomatis* serotypes A through K, and *C. trachomatis* mouse pneumonitis (MoPn) (1). All strains display tropism for mucosal epithelial cells and, to varying degrees, macrophages (2–4), and together they account for transmissible diseases of the conjunctival, pulmonary, genital, and/or intestinal mucosae (2, 5). Both murine and nonhuman primate models of infection have been developed to analyze immunological mechanisms of host resistance and evaluate potential chlamydial vaccines. Results from all systems, as well as from human patient populations, point to CD4⁺ T cells as the primary mediators of immunity (6–12) with debatable contributions of CD8⁺ T cells (13–15) and Ab (16–20). Murine immunity to MoPn was mapped more specifically to MHC class II-restricted, IL-12-dependent, type 1 CD4⁺ T cells secreting IFN- γ , TNF- α , and other proinflammatory cytokines (3, 10).

The potential contributions of IFN- γ and/or TNF- α to chlamydial resistance have been analyzed in vivo and in vitro in an effort to identify the molecular mechanisms of cytokine action. Mono-

clonal Ab-mediated neutralization of IFN- γ enhanced host susceptibility to chlamydial respiratory infection (21–23), although resistance could not consistently be restored by passive administration of recombinant cytokine (21). In vitro, IFN- γ inhibited the growth of *C. psittaci* (24) and *C. trachomatis* LGV (25) by an oxygen-independent process (26) that reduced the replication of intracellular reticulate bodies (27, 28). Two IFN- γ -inducible, oxygen-independent pathways to be considered in this regard are the indoleamine 2,3-dioxygenase (IDO) pathway, which involves IDO-driven conversion of tryptophan to *N*-formylkynurenine to limit tryptophan availability (29, 30), and the inducible nitric oxide synthase (iNOS) pathway for generating toxic nitrogen radicals lethal to a variety of intracellular pathogens (31–35). The IDO-driven, tryptophan reversible pathway was implicated in IFN- γ -mediated inhibition of *C. psittaci* replication in human cells (36, 37), whereas the iNOS effector pathway was credited with IFN- γ -mediated, nonreversible inhibition of *C. trachomatis* L2 growth in murine fibroblasts (38, 39), consistent with the species distribution of these effector pathways. The relevant action of TNF- α has been more difficult to localize, its contribution being more readily demonstrable in vivo (11, 40) than in vitro (41). Further evaluation of its relevance to host resistance is warranted.

The notion that IFN- γ - and/or TNF- α -driven pathways mediated similar levels of resistance to all chlamydial strains was recently challenged by publication of conflicting reports on the recovery profiles of IFN- γ -deficient (42) or IFN- γ receptor-deficient mice (43). Johansson et al. (44) determined that IFN- γ receptor gene knockout mice (IFN- γ R^{-/-}) developed more severe primary genital tract infections than control animals and displayed no acquired resistance to rechallenge. At the same time, Perry et al. (3) and Cotter et al. (4) reported minimal effects of an IFN- γ deficiency on the resolution of genital infections, despite macrophage-mediated dissemination of bacteria to systemic organs. One critical difference between these experimental systems was the use of *C. trachomatis* serovar D by one lab (44) and *C. trachomatis* MoPn

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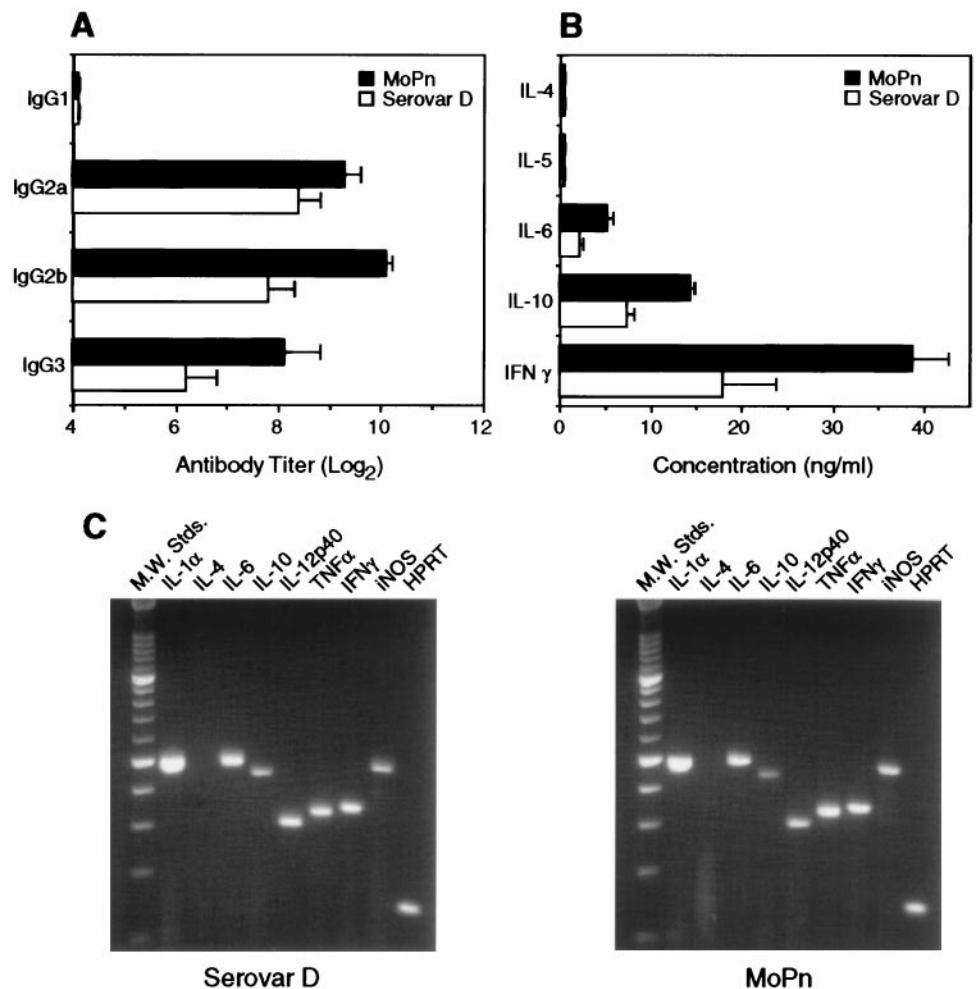
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³ Abbreviations used in this paper: LGV, lymphogranuloma venereum; MoPn, mouse pneumonitis; IDO, indoleamine 2,3-dioxygenase; iNOS, inducible nitric oxide synthase; EB, elementary body; IFU, inclusion-forming unit; MLA, N^G-monomethyl-L-arginine.

FIGURE 1. Ab and cytokine responses of C57BL/6 female mice following genital infection with distinct strains of *C. trachomatis*. The distribution of serum Ig isotypes (A), splenic cytokine production (B), and local cytokine profiles (C) were assessed at 18 days postinfection with the murine MoPn strain or with human serovar D. Ab and spleen cytokine data represent the average of eight mice/group and three mice/group, respectively, and are highly reproducible in repeat experiments. Local cytokine profiles are shown for a single mouse from each group but are representative of results obtained from four mice/group.



by the other two (3, 4). The possibility that distinct chlamydial isolates might exhibit differential sensitivities to IFN- γ or other type 1 cytokines implicated in host resistance formed the basis for the present studies, where strain-specific susceptibilities were directly compared in vivo and in vitro.

Materials and Methods

Animals

Normal C57BL/6 mice were purchased from The Jackson Laboratory (Bar Harbor, ME). MHC class II-deficient (A^b $^{-/-}$) mice on a C57BL/6 background were purchased from Taconic Farms (Germantown, NY). Breeding pairs of iNOS-deficient mice on a 129/J \times C57BL/6 background were obtained from Carl Nathan (Cornell University Medical College, New York, NY). Breeding pairs of IFN- γ -deficient mice (42) were obtained through the courtesy of Tim Stewart (Genentech, San Francisco, CA). TNFRp55-deficient mice ($TNFR$ $^{-/-}$) (45) were obtained through the generosity of Werner Lesslauer (Hoffmann-LaRoche, Basel, Switzerland) and acquired from the colony of David Erle (University of California at San Francisco, San Francisco, CA). IL-12p40-deficient mice (46) were obtained through the kindness of Jeanne Magram (Hoffmann-LaRoche, Nutley, NJ). Animals were bred in an Association for Assessment and Accreditation of Laboratory Animal Care accredited facility in filter-top cages and maintained using standard precautions for immunodeficient mouse strains. Experiments utilizing IFN- γ $^{-/-}$ and $TNFR$ $^{-/-}$ knockout mice were performed twice, and experiments using iNOS $^{-/-}$, A^b $^{-/-}$, and IL-12p40 $^{-/-}$ mice performed once using eight to ten animals per group. Only female mice were used in the reported experiments.

Growth of *Chlamydia* and infection protocol

C. trachomatis strains serovar D, serovar L2, and MoPn were grown in HeLa 229 cells, and elementary bodies (EB) were purified by discontinu-

ous density centrifugation as previously described (47). Infection of the uterine mucosa could be readily achieved by either vaginal or intrauterine inoculation with MoPn but only by intrauterine inoculation with human chlamydial strains. Therefore, mice pretreated with progesterone were infected vaginally with 1.5×10^3 inclusion-forming units (IFU) MoPn in 5 μ l of a sucrose-sodium phosphate-glutamic acid buffer (SPG) as previously described (3) or by direct intrauterine inoculation of 10^5 IFU serovar D or serovar L2 in 30 μ l of SPG by midline celiotomy under methoxyflurane anesthesia (Metofane; Mallinckrodt Veterinary, Mundelein, IL), followed by closure with surgical staples (9-mm Autoclips; Roboz, Rockville, MD). Infections were monitored by enumerating IFUs recovered from swabbing the vaginal vault with Calgiswabs (Spectrum Medical Industries, Los Angeles, CA) on HeLa cell monolayers using indirect immunofluorescence as described previously (10).

Ab and cytokine assays

Serum and vaginal washes collected 18 days postinfection were isotyped by ELISA against homologous heat-killed EBs (56°C for 30 min) using alkaline phosphatase-conjugated anti-mouse Ig sera (Southern Biotechnology Associates, Birmingham, AL) as described previously (10). Cytokines produced by splenic mononuclear cells after 72-h in vitro restimulation with homologous heat-killed EBs were also assayed by ELISA using capture and detection mAbs from PharMingen (San Diego, CA) as previously described (3). Local cytokine production was measured by primer-directed RT-PCR amplification of Trizol-extracted (Life Technologies, Grand Island, NY) genital tract RNA followed by agarose gel analysis of PCR products as described previously (3).

Effects of exogenous IFN- γ on chlamydial growth in vitro

Duplicate cultures of murine L-929 fibroblast cells or intestinal epithelial cells (IEC4.1) (48) were established in 96-well plates (5×10^4 cells/well) in Eagle's MEM or modified Ham's medium, respectively, supplemented with 10% FCS and 10 μ g/ml gentamicin sulfate. Cells were cultured for

24 h in varying concentrations of murine rIFN- γ or TNF- α (Genzyme, Cambridge, MA) and then washed and infected with *C. trachomatis* strains MoPn, D, or L2 at multiplicities of infection (MOIs) that resulted in ~20% of the cells being infected (equivalent to an MOI of 0.25). Monolayers were refed with medium containing the homologous cytokine for an additional 24–48 h and then fixed in absolute methanol, stained with chlamydial specific Ab, and IFU determined (49). Alternatively, cell monolayers were removed with trypsin, and the cells were washed, lysed, and assayed for chlamydial growth by titration of IFU from clarified supernatants on HeLa 229 cell monolayers. In certain experiments, the reversibility of IFN- γ -mediated inhibition was examined by washing infected cultures after 24 h of incubation in IFN- γ and either refeeding with IFN- γ -free medium or adding 0.0125–3.2 mM of the iNOS competitive inhibitor *N*^G-monomethyl-L-arginine (MLA) (Calbiochem, San Diego, CA). Cells were fixed and stained 24–72 h later by immunofluorescence for IFU determination. The effect of IFN- γ was considered to be chlamydiaicidal if inhibition was nonreversible or chlamydiastatic if inhibition was reversible.

Statistical analyses

Differences between groups were analyzed for statistical significance using Student's two-tailed *t* test.

Results

Ab and cytokine profiles induced by *C. trachomatis* infections of the genital mucosa

Normal C57BL/6 mice were infected genitally with doses of *C. trachomatis* MoPn or serovar D chosen to maximize the respective infections. Systemic and local immune responses were measured 18 days postinfection. Serum Abs were predominately of the IgG2 and IgG3 isotypes with little to no detectable IgG1, a profile consistent with stimulation of type 1 immune responses (Fig. 1A). Systemic (Fig. 1B) and local (Fig. 1C) cytokine responses were also Th1-biased as reflected by the production of proinflammatory cytokines IFN- γ and TNF- α , but not traditional Th2 cytokines IL-4 and IL-5, consistent with previous reports in MoPn-infected mice (3). IL-10, a Th2-derived regulatory cytokine that is produced simultaneous with IFN- γ in certain parasitic infections (50), was also expressed. Thus, host immune responses induced following infection of the genital mucosa with *C. trachomatis* MoPn or serovar D were markedly similar and reflected a strong bias toward type 1 T cell-mediated immunity.

Differential influence of IFN- γ and TNF- α on chlamydial clearance in vivo

The relative contributions of two prominent proinflammatory cytokines, IFN- γ and TNF- α , to immune-mediated clearance of murine and human chlamydial strains from the genital mucosa was directly compared in groups of normal and gene-deficient mice. Shedding of MoPn from genital epithelial cells diminished at a similar rate in normal and IFN- γ -deficient mice (Fig. 2), indicating the relative IFN- γ insensitivity of this murine strain. In contrast, genital clearance of the human serovar D strain was severely compromised in IFN- γ -deficient mice (Fig. 2). Normal mice resolved serovar D genital infections within 7–10 days, while IFN- γ -deficient mice required nearly 50 days. Similar results were obtained following infection with the L2 serovar of LGV (data not shown), indicating a critical role for IFN- γ in the clearance of infections with human but not the murine *C. trachomatis* strain. Documentation of such distinct differences in strain susceptibility to IFN- γ -mediated immunity in vivo was unexpected and, to our knowledge, not previously reported in the field of parasite immunity.

The influence of TNF- α on the resolution of genital infections with human and murine chlamydial strains was evaluated in similar experiments using mice bearing targeted mutations in the TNF- α p55 receptor molecule. Infection of these mice or their immunologically competent counterparts with serovar D or MoPn revealed a marginal but statistically significant reduction in the rate of chlamydial clear-

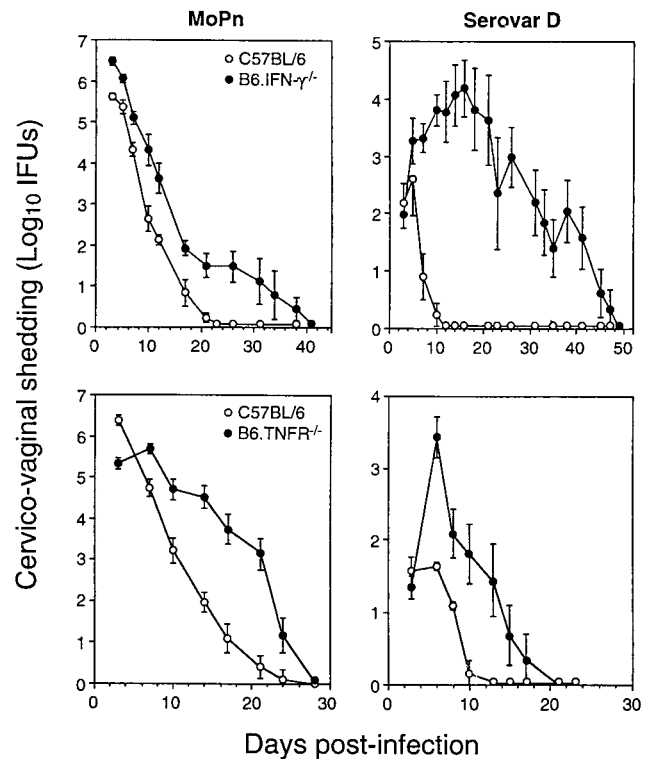


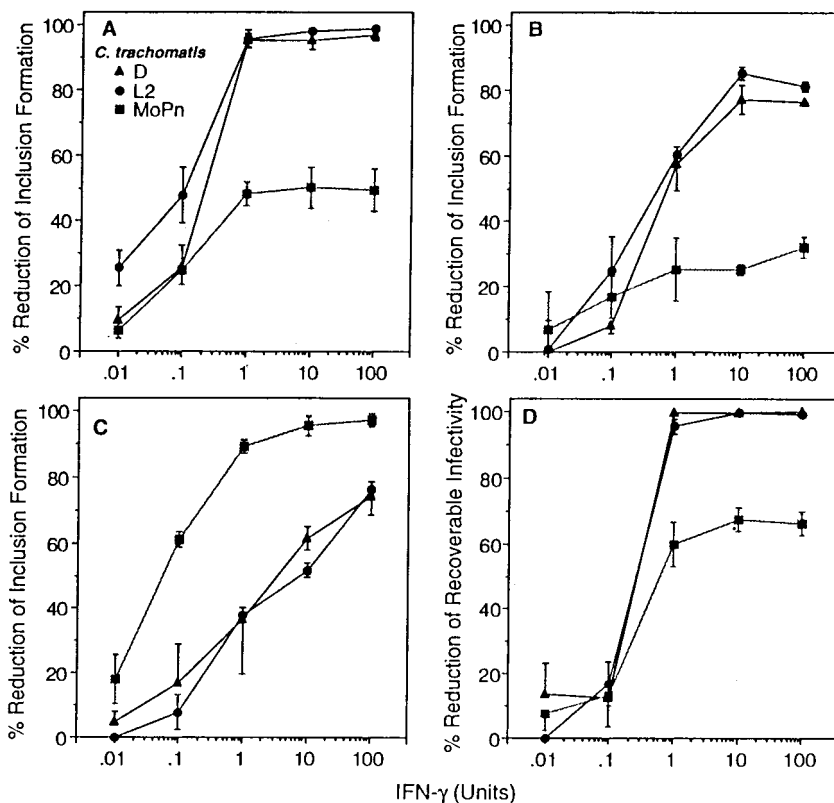
FIGURE 2. Clearance of murine MoPn and serovar D from the genital mucosa of IFN- γ ^{-/-} (upper panels) and TNFRp55^{-/-} (lower panels) mice. Groups of 10 female mice were infected with 1.5×10^3 IFU MoPn or 10^5 IFU serovar D as described in *Materials and Methods* and monitored for chlamydial shedding by enumeration of infectious EBs collected on vaginal swabs. Peak recoveries of infectious serovar D from normal mice were 2 logs lower than peak recoveries of MoPn despite use of a higher infectious dose of the human strain. Results are representative of two separate experiments. Differences between groups were statistically significant ($p < 0.05$) at most time points.

ance from TNFR^{-/-} mice (Fig. 2, lower panels). Since IFN- γ and TNF- α exhibit some functional overlap, the ability to detect the loss of either cytokine alone suggests that cytokine-specific as well as shared mechanistic pathways may be operative in mediating chlamydial clearance from the genitourinary tract.

Differential influence of IFN- γ and TNF- α on chlamydial growth in vitro

To determine the extent to which the IFN- γ susceptibility displayed by serovars D and L2 extended to other human isolates, an in vitro system was established to monitor the effects of added cytokines on intracellular replication and inclusion formation. Inclusion formation by human serovars D or L2 was inhibited in a dose-dependent manner by the addition of species-specific IFN- γ to human HeLa 229 cells, murine L-929 fibroblasts, or murine IEC4.1 epithelial cells (Fig. 3, A–C). The remaining human chlamydial serovars (serovars A through K) exhibited similar levels of inhibition (data not shown). This was shown to reflect IFN- γ -mediated inhibition of chlamydial growth, as determined by diminished recovery of infectious EB from clarified lysates of IFN- γ -treated, serovar D-infected cells (Fig. 3D). Failure to reverse this inhibition by removal of cytokine-containing medium indicated that IFN- γ was microbicidal rather than microbistatic after 20 h of exposure (data not shown). Inclusion formation by the murine MoPn strain was highly sensitive to inhibition by human rIFN- γ in

FIGURE 3. The effect of exogenous IFN- γ treatment on chlamydial inclusion formation and growth in murine fibroblasts, murine epithelial cells, and human epithelial cells. Inclusion formation was measured in murine L-929 cells treated with murine rIFN- γ (A), murine IEC4.1 epithelial cells treated with murine rIFN- γ (B), and human HeLa 229 cells treated with human rIFN- γ (C), as determined by direct staining of cells for chlamydial inclusions. D, The recovery of infectious organisms from IFN- γ -treated L-929 cells, where IFN- γ -treated and chlamydial infected cells were harvested, lysed, and chlamydial infectivity contained in clarified supernatants assayed on HeLa 229 cells. Error bars represent variation in results between two separate experiments.



human HeLa 229 cells but was suppressed only 50–60% by murine rIFN- γ in murine fibroblasts or epithelial cells (Fig. 3, A–C). Sensitivity of human but not a murine chlamydial strain to inhibition by murine IFN- γ correlates with the differential dependence of these isolates on IFN- γ -mediated clearance in vivo (Fig. 2). Data also suggest species-specific adaptation of the MoPn strain to the inhibitory effect of IFN- γ .

Exogenous TNF- α had no measurable effect on in vitro growth of MoPn, serovar D, or serovar L2 at concentrations sublethal for the host IEC4.1 cell line (data not shown). While these data argue against a direct inhibitory role for TNF- α on chlamydial growth, they do not

rule out an indirect contribution in vivo by activation of other cells and/or factors present in the environment of the genital mucosa.

Role of nitric oxide effector pathway in IFN- γ -mediated chlamydial inhibition

Resolution of MoPn genital infections was previously shown to occur very efficiently in the absence of IFN- γ -inducible nitric oxide synthase (51, 52), the enzyme required for initiation of the nitric oxide effector pathway (53). Unlike MoPn, however, serovar D displayed a high degree of sensitivity to IFN- γ -induced effector mechanisms, mechanisms that could involve the generation of

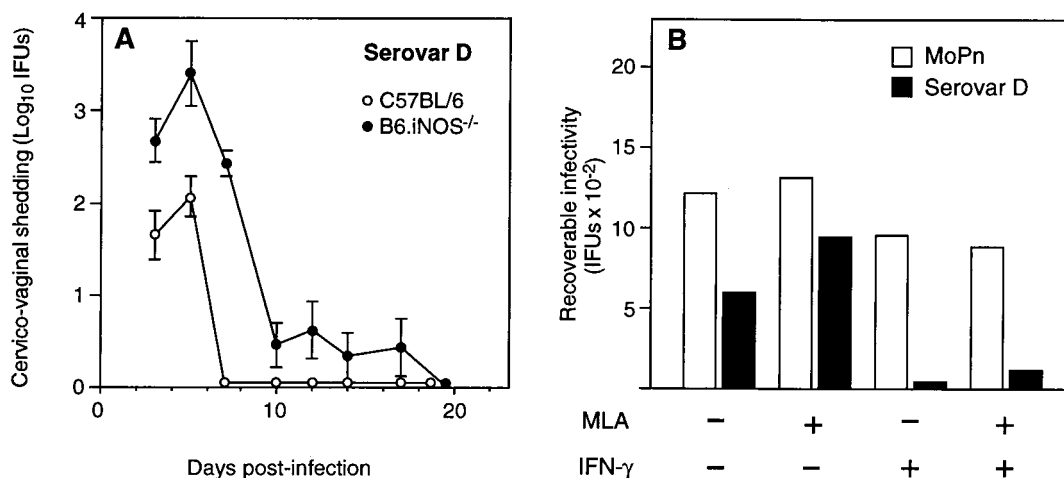


FIGURE 4. Effect of iNOS deficiency on growth of *C. trachomatis* serovar D in vivo and in vitro. A, In vivo clearance of serovar D from the genital mucosa of normal or iNOS-deficient mice (single experiment utilizing 10 animals/group). B, MLA reversibility of IFN- γ -mediated inhibition of MoPn and serovar D growth in IEC4.1 murine epithelial cells. MLA (0.8 mM) was used to reverse inhibition mediated by 100 U murine rIFN- γ . Differences between homologous cultures with IFN- γ alone or IFN- γ plus MLA were not statistically significant.

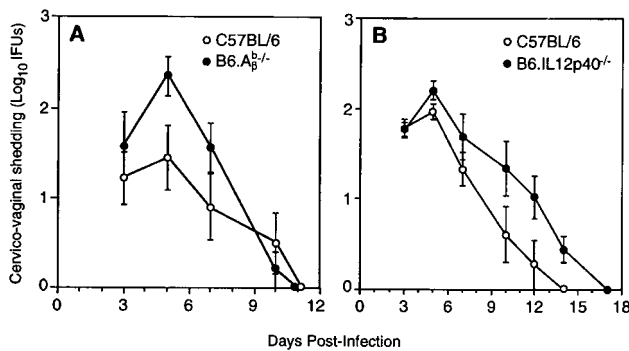


FIGURE 5. Clearance of serovar D from the genital mucosa of MHC class II-deficient ($A^b_\beta^{-/-}$) mice (A) and IL-12p40-deficient mice (B) as compared with normal C57BL/6 mice (single experiments utilizing eight animals/group).

toxic nitrogen radicals within the cytoplasm of infected cells. To test this possibility, *in vivo* clearance of serovar D was compared in normal mice and in mice lacking a functional iNOS gene (Fig. 4A). Resolution of infections was only slightly delayed in iNOS^{-/-} mice and only during the final stages of clearance when small numbers of *Chlamydia* persisted. The failure of the L-arginine competitive inhibitor, MLA, to reverse IFN- γ -mediated inhibition of serovar D growth *in vitro* confirmed that activation of the nitric oxide pathway was not the primary mechanism of IFN- γ action (Fig. 4B). Since it could be argued that results obtained using these murine cell lines were not relevant to IFN- γ -induced pathways in human cells, the capacity of human IFN- γ to induce transcription of iNOS in HeLa cells was evaluated by RT-PCR. No signal was detected (data not shown), however, indicating that IFN- γ -mediated inhibition of chlamydial growth in human cells also occurred through an iNOS-independent mechanism.

Source of IFN- γ required for inhibition of human chlamydial strains

The rapid clearance of serovar D from the normal genital mucosa, occurring often within 1 wk of infection, afforded minimal opportunities for induction, expansion, and recruitment of protective CD4⁺ T cells to the genital mucosa. Given strain differences in chlamydial sensitivity to IFN- γ , the potential for differential dependence on MHC class II-restricted, IL-12-driven type 1 T cells as the relevant source of effector cytokines also had to be consid-

ered. The relative role of this pathway was evaluated by comparing resolution of serovar D infections in normal mice, in MHC class II deficient mice (which, in the C57BL/6 strain, can be accomplished by mutation of the A^b_β gene), or in IL-12p40-deficient mice (Fig. 5). While slight enhancement of infection was observed in either of these gene knockout mice as compared with control animals, effects were much less dramatic than were previously noted with the MoPn strain. MHC class II knockout mice maintained high level genital tract infections with MoPn for the entire 70-day observation period (10), and IL-12p40-deficient mice displayed extended MoPn infections lasting up to 50 days (results obtained by mAb-mediated cytokine depletion (3) were similar to those obtained using these IL-12p40 knockout mice (data not shown)). Yet the effect of these deletions on clearance of serovar D was marginal. The absence of class II-associated functions in $A^b_\beta^{-/-}$ mice was confirmed by the failure to detect T cell-dependent switching of serum Ig isotypes and the absence of systemic IFN- γ responses following infection. Nevertheless, $A^b_\beta^{-/-}$ mice generated a local IFN- γ response that was detected by RT-PCR amplification of genital tract RNA (Fig. 6) and that presumably mediated host resistance to epithelial infection. The local cellular source of IFN- γ in these mice has not yet been identified.

Discussion

The close phylogenetic relatedness between human chlamydial strains and MoPn has led to their use almost interchangeably to investigate immunity and vaccine development under the assumption that these *C. trachomatis* strains were susceptible to the same molecular mechanisms of immunity. The present studies demonstrate quite clearly that this assumption was invalid and that the susceptibilities of murine and human strains to the inhibitory effects of IFN- γ are distinct; human *C. trachomatis* serovars D and L2 were highly sensitive and the mouse MoPn relatively resistant to IFN- γ -mediated inhibition *in vitro* and *in vivo* in the murine host. The IFN- γ -sensitivity defined for the D and L2 strains extended to all human serovars, rendering MoPn somewhat unique among *C. trachomatis* isolates in its diminished sensitivity to this pleiotropic mediator. Much less variation was observed in strain sensitivity to TNF- α since genital clearance was marginally delayed in TNF- α -deficient mice, regardless of the infecting strain. TNF- α -mediated inhibition of chlamydial growth and/or clearance could not be demonstrated *in vitro*, however, suggesting that the relevant action of this cytokine was indirect and required additional cells or mediators not present in the *in vitro* culture system.

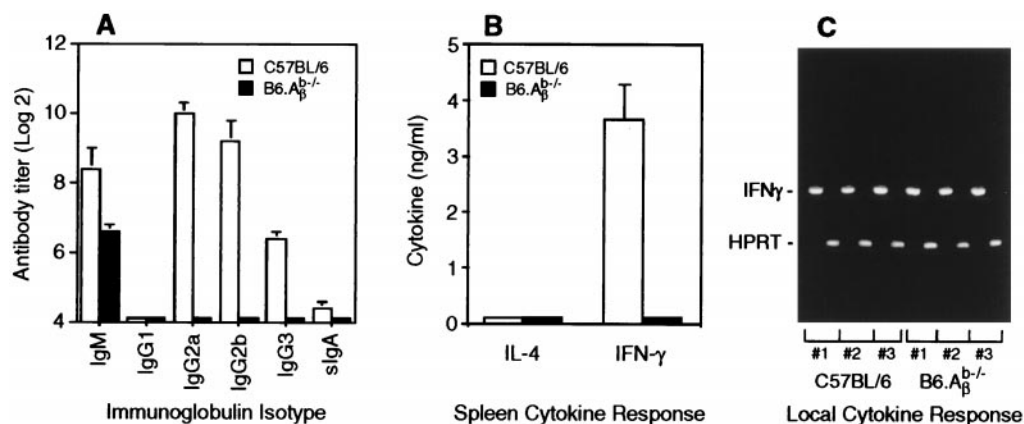


FIGURE 6. Parameters of the host response to serovar D infection of the genital mucosa in normal and MHC class II-deficient ($A^b_\beta^{-/-}$) mice. Production of serum (IgM and IgG isotypes) and secretory (sIgA from vaginal washes) anti-serovar D Abs at day 18 postinfection (A), splenic production of type 1 (IFN- γ) and type 2 (IL-4) cytokines (B), and local production of IFN- γ (C) are shown. All assays were repeated at least twice to verify results.

Collectively, it appeared that both IFN- γ and TNF- α contribute to chlamydial resistance in vivo with the relative contribution of IFN- γ being strain-dependent.

Differential susceptibilities of human vs murine *C. trachomatis* strains to inhibition by murine IFN- γ could not have been predicted from the spectrum of immunological responses evoked during infection with either organism. In both cases, the distribution of serum Ig isotypes and the panel of cytokines produced locally and systemically were similar and differed only in magnitude, most likely as a reflection of the abbreviated course of human *C. trachomatis* infections in the murine host. The capacity of *Chlamydia* or any pathogen to induce a wide array of T and B cell responses does not necessarily imply equal susceptibility to their action, however. This can now be appreciated in the *Chlamydia* system where human but not murine isolates are highly sensitive to murine IFN- γ , a finding that resolves the conflict between the results of Johansson et al. (44) and those of Perry et al. (3) and Cotter et al. (4).

The sensitivity of *C. trachomatis* serovar D to IFN- γ -mediated inhibition may explain the rapid clearance of this strain from the urogenital mucosa of normal as well as immunodeficient mice. Animals carrying targeted mutations in MHC class II or IL-12p40 genes resolved serovar D infections at nearly the same rate as intact control mice, presumably due to IFN- γ produced locally by NK or other cells of the innate immune system. This contrasts sharply with the clearance of MoPn, which is strictly CD4⁺ T cell-dependent (10). The implications of these differences for continued use of the MoPn murine system as a model for human urogenital infection are unclear since the immunological parameters of human infections have not been defined. The fact that human *C. trachomatis* strains have retained sensitivity to human IFN- γ suggests that IFN- γ may participate in the resolution of human urogenital infections, but whether it is derived from cells of the innate or acquired immune systems is unknown. Given these uncertainties, the murine MoPn model of urogenital infection may be appropriate for analyses of cellular events leading to induction and expression of a type 1 T cell response, but it is poorly suited to investigations of IFN- γ -driven effector pathway(s). Similarly, MoPn would be a poor choice of strains to test the efficacy of vaccines designed to stimulate a protective IFN- γ response.

These data predict that IFN- γ may play a major role in the elimination of *C. trachomatis* from the human genital mucosa in vivo. If so, the relative ability of each individual to mount an efficacious IFN- γ response through either innate or acquired pathways could have a profound influence on the clinical outcome of infection. For example, NK cell-derived IFN- γ may provide a rapid defense against primary infection that dramatically decreases the infectious burden of *Chlamydia*, but the associated reduction in Ag load could also terminate prematurely the induction of an acquired memory T cell response capable of protecting the host against reinfection. In this regard, repeated or persistent chlamydial infections have been implicated as possible bases for the development of uterine pathology that leads to infertility and pelvic inflammatory disease (54, 55). On the other hand, when innate IFN- γ sources are insufficient and clearance depends upon stimulation of Ag-specific T cells, genetic and environmental factors that influence the induction of type 1 vs type 2 T cell reactivity may dictate the pathological consequences of infection. Evidence supporting such a dichotomy of clinical responses has already been provided in individuals suffering from trachoma, an ocular disease induced by *C. trachomatis* infection of the conjunctival mucosa. Individuals with persistent infection and severe pathological

scarring displayed increased production of type 2 cytokines, such as IL-4 (56), whereas the type 1 inducing cytokine, IL-12, was detected only in patients who presented with inflammation but no scarring (12). Evaluation of host cytokine profiles associated with acute vs chronic stages of sexually transmitted chlamydial infections may provide similar insights into the immunopathological basis of human genital disease.

The molecular mechanisms ultimately responsible for elimination of MoPn or human *C. trachomatis* strains from the uterine mucosa have yet to be defined. Unlike other commonly studied intracellular parasites, *C. trachomatis* resides within a parasitophorous vacuole in the cytoplasm of mucosal epithelial cells rather than macrophages and is, therefore, eliminated by chlamydia-specific pathways operative in the microenvironment of epithelial cells. To date, only two IFN- γ -inducible pathways have documented relevance to the intracellular killing of *Chlamydia*: induction of nitric oxide synthase (57) and IDO-driven tryptophan depletion (36). While mice infected with either human or murine *C. trachomatis* strains synthesized iNOS, in vivo clearance of either infection proceeded normally in the absence of this pathway (Fig. 4, and Ref. 51). Neither can IFN- γ -mediated inhibition of chlamydial growth be reversed by addition of an iNOS competitive inhibitor. The IDO pathway is also an unlikely candidate for consideration since mice lack a functional IDO gene. Therefore, the possibility that an alternative, as yet undefined, pathway may represent the lethal step in chlamydial replication must be considered.

Data presented herein are compatible with the notion that resistance or susceptibility of *C. trachomatis* strains to IFN- γ -mediated effector pathways is not static but has evolved in a species-specific manner. Thus, the murine strain displays greater sensitivity to human IFN- γ than to endogenous murine IFN- γ , while human strains are more sensitive to murine IFN- γ than to human IFN- γ , when compared on a quantitative basis (compare Fig. 3, A and B, with Fig. 3C). That human strains have retained some level of sensitivity to human IFN- γ may simply reflect the fact that these isolates have had less time to adapt to host IFN- γ -driven effector pathways than their rodent counterparts, but the process is probably ongoing. In preparation for the possible development of IFN- γ -resistant human strains, it may be prudent to investigate the IFN- γ -independent mechanism(s) involved in the clearance of MoPn infections as well as the IFN- γ -dependent mechanism(s) now active against human strains. It should also be considered that immune-mediated selection of IFN- γ -resistant variants may contribute to chlamydial persistence in the human host.

Although this is the first report documenting interstrain variation in parasite susceptibility to the molecular effector mechanisms of cell-mediated immunity in vivo, *Chlamydia* may not be the only parasite that displays this level of diversity. Differential susceptibility of *Leishmania* species to in vitro killing by lymphokine-activated macrophages has been noted by Scott et al. (58). The Neal strain of *L. tropica* but not the Maria strain of *L. mexicana amazonensis* was susceptible to macrophage microbicidal activity following cellular activation by a factor later identified as IFN- γ (P. Scott, unpublished observations). It was postulated that resistance of the Maria strain to IFN- γ -mediated killing may underlie the chronic, nonhealing nature of lesions induced by this organism, while the sensitivity of *L. tropica* to IFN- γ -driven effector mechanisms may provide the molecular basis for the self-healing nature of those lesions (58). Variation in the in vitro sensitivity of different clinical isolates of *Mycobacterium avium* to peroxide-induced killing within human

monocytes has also been reported (59), as well as strain differences, in the induction of IFN- γ by *Plasmodium yoelii* (60). Thus, it may no longer be appropriate to assume that results obtained using a given strain of bacteria or protozoa are relevant to all strains within that species, or that animal isolates will behave similarly to those obtained from human sources in all respects. Analysis of the molecular basis for IFN- γ -induced growth inhibition and for the killing of strains resistant to IFN- γ may also provide new insights into the array of host-defense mechanisms available against human pathogens.

Acknowledgments

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