

IL-10 Gene Knockout Mice Show Enhanced Th1-Like Protective Immunity and Absent Granuloma Formation Following *Chlamydia trachomatis* Lung Infection¹

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We previously reported that higher IL-10 production is correlated with lower IFN- γ production, weaker delayed hypersensitivity (DTH), and slower organism clearance following chlamydial infection in mice. To assess more directly the role of IL-10, we examined protective immunity and pathological reaction in C57BL/6 IL-10 gene knockout (KO) and wild-type mice. The results showed that in the absence of endogenous IL-10, mice had significantly accelerated chlamydial clearance and developed significantly stronger DTH responses, which could be inhibited by local delivery of rIL-10. Consistent with the enhancement of DTH responses, IL-10 KO mice showed stronger and more persistent CD4 T cell-dependent IFN- γ production and significant elevation of IL-12 and TNF- α production. Additionally, wild-type, but not IL-10 KO, mice showed granuloma formation that was correlated with higher levels of Th2 cytokine (IL-5) production at the later stages of infection. Moreover, chlamydial infection, unlike parasitic protozoan infection, did not induce significant acute toxicity in IL-10 KO mice, which may be due to the low (undetectable) levels of systemic release of proinflammatory cytokines. These results suggest that IL-10 inhibits the priming and expansion of Th1-like T cell responses and that IL-10 plays a role in the fibrotic reaction seen with chlamydial infection. *The Journal of Immunology*, 1999, 162: 1010–1017.

The immune response to microbial infection is determined by complex interactions between immunocompetent cells and soluble factors, including cytokines (1–4). IL-10, a cytokine produced by Th2-like CD4 T cells, macrophages, and B cells, is a potent component in the regulating mechanism (5, 6). In vitro studies initially demonstrated that IL-10 suppresses both the proliferative response and IFN- γ production in Th1 CD4 T clones and mixed spleen cells (7–10). This suppression is correlated with the inhibitory effect of IL-10 on cytokine production and costimulatory molecule expression on APCs. IL-10 has also been shown to inhibit the antimicrobial activity of macrophages, possibly through altering TNF- α production (11–13). The recent development of IL-10 knockout (KO)³ mice has provided a powerful tool for studying the role of IL-10 in vivo (14, 15). Several studies using various protozoan infection models have shown that IL-10 plays an important role in balancing the protective and pathological immune responses during intracellular parasitic infection (16–18). In particular, these studies have demonstrated that while IL-10 inhibits protective immune responses to parasitic infection, the absence of endogenous IL-10 due to targeted gene disruption paradoxically

results in lethal pathological reactions, characterized by systemic overproduction of proinflammatory cytokines. To date, however, no data are available regarding the alteration of protective and pathological immune responses to intracellular bacterial infection in IL-10 KO mice.

Chlamydia trachomatis is an obligate intracellular bacterium that causes a variety of human and animal diseases, affecting principally mucosal epithelial surfaces in the eye and genital tract. Persistent or multiple reinfection that induces immunopathological reactions sometimes results in chronic inflammation with dense lymphocytic and plasma cell infiltration, occasional granuloma formation, and fibrotic scarring of the mucosa, ultimately leading to blindness and fallopian tubal obstruction (19–21). Recent studies in both the mouse and human have shown that IFN- γ production and Th1-like T cell responses, including delayed-type hypersensitivity (DTH), are highly correlated with host resistance to chlamydial infection (20–33). Paradoxically, immunopathological responses (mucosal scarring) are also thought to be mediated by DTH responses, as suggested by studies using local delivery of soluble chlamydial Ags in guinea pig and monkey models (34, 35).

Using a murine model of *C. trachomatis* mouse pneumonitis (MoPn) lung infection, we recently found that differences in IL-10 production following chlamydial infection among inbred mouse strains were correlated with differences in the types of immune response and the rate of organism clearance in vivo (24, 33). Specifically, BALB/c mice that showed higher Ab (IgG1) responses and greater susceptible to chlamydial infection produced significantly higher levels of IL-10 than did C57BL/6 mice. The latter mounted stronger DTH responses, had higher IFN- γ production, and were more resistant to the infection. The data suggest that IL-10 inhibits host clearance of chlamydial infection and that IL-10 plays a negative regulatory role in the immune responses to chlamydial infection. To more directly assess the effect of IL-10 in immunity and immunopathologic responses to chlamydia, we examined organism clearance and histopathology in C57BL/6 IL-10 KO mice. We also examined the immune responses and cytokine

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³ Abbreviations used in this paper: KO, knockout; DTH, delayed-type hypersensitivity; MoPn, *Chlamydia trachomatis* mouse pneumonitis; EB, elementary body; SPG, sucrose phosphate glutamic acid buffer; IFU, inclusion-forming units.

production in IL-10 KO mice during chlamydial infection and tested the effect of supplemental exogenous rIL-10 on the immune responses and cytokine production in the gene-targeted mice. Our data show that IL-10 KO mice mount strong and persistent Th1-like T cell responses (IFN- γ production and DTH) and produce significantly lower Th2-like (IL-5) cytokine. The alteration of immune responses and cytokine patterns was correlated with a faster clearance of organisms and the absence of granuloma formation following chlamydial infection. Moreover, chlamydial infection, unlike parasitic protozoan infection, did not induce significant acute toxicity in the absence of endogenous IL-10.

Materials and Methods

Mice

Female homozygous IL-10 KO mice (IL-10^{-/-}; C57BL/6-II10tm1Cgn) were purchased from The Jackson Laboratories (Bar Harbor, ME). Age- and sex-matched wild-type C57BL/6 mice were purchased from Charles River Canada (St. Constant, Canada). Animals were maintained in the central animal facilities of the University of Manitoba (Manitoba, Canada). IL-10 KO mice were kept in a specific pathogen-free facility with filtered air flow and autoclaved cage, food, and water. Mice of 8–12 wk of age were used for the study.

Organism and infection

The mouse pneumonitis biovar of *C. trachomatis* (MoPn) was grown in HeLa 229 cells and purified by discontinuous density gradient centrifugation. Infectivity of the purified chlamydial elementary bodies (EBs) was titrated by infection of HeLa cell monolayers for 48 h followed by methanol fixation of cells and enumeration of inclusions that were stained by a genus-specific mAb conjugated with horseradish peroxidase. The procedure of MoPn purification and infectivity determination was previously described (33). Mice were inoculated intranasally with various doses of MoPn in a volume of 40 μ l. The mice were monitored daily for body weight changes and, as defined in particular experiments, were sacrificed on selected days after infection. To determine the *in vivo* growth of the organism, the lungs from each mouse were aseptically isolated and homogenized in 4 ml of sucrose phosphate glutamic acid (SPG) buffer. Tissue homogenates were spun down at 500 \times *g* for 10 min at 4°C, and the supernatants were divided into aliquots (1 ml/vial) and kept at -70°C until tested. All samples from the same experiment were tested in the same titration assay, and the leftover thawed samples were not used again for testing of chlamydial infectivity. Repeated tests always used a fresh aliquot of the lung sample.

Serum Ab analysis

Serum IgM, IgG1, IgG2a, and IgA Abs to MoPn EBs were determined by ELISA as previously described (33). Results are expressed as ELISA titers at 60 min using the end point (cutoff at OD₄₀₅, 0.5) of the titration curves. The results represent the mean \pm SEM of the titers of all the sera in the same mouse group.

Determination of DTH responses

MoPn-specific DTH was measured as previously described (33). Mice were injected in the hind footpad with 25 μ l of heat-inactivated MoPn EBs (5×10^4 inclusion-forming units (IFU)) in one side and the same volume of SPG buffer in the other side. The difference in thickness between the two footpads at 24, 48, and 72 h was used as a measure of the DTH response. No measurable difference was found between the two footpads injected with heat-inactivated EBs or SPG buffer in uninfected control mice. In experiments examining the effect of local delivery of rIL-10 on DTH responses, heat-inactivated MoPn EBs were injected into footpads in one side with rIL-10 (5 ng/ml) and in the other side without rIL-10. The thicknesses of the footpads were measured before and after MoPn injection. The difference in thickness of each footpad before (0 h) and after (24, 48, and 72 h) heat-inactivated EB (in the presence or the absence of rIL-10) injection was used as a measure of DTH responses.

Histopathological analysis

The lung and footpad injection sites (for DTH analysis) of MoPn-infected mice were examined histopathologically. Lungs were collected on various days following MoPn infection. One lung was used to prepare lung homogenate, and the other was used for histopathological analysis. Footpad injection sites were collected by amputation of the foot at 72 h following

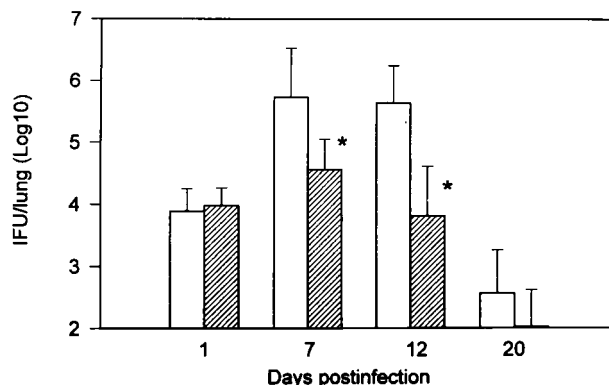


FIGURE 1. Faster clearance of chlamydial infection (MoPn) by IL-10 KO mice compared with wild-type mice. Wild-type (\square) and IL-10 KO (hatched) mice were intranasally infected with MoPn (2000 IFU) and sacrificed on various days postinfection. The lung tissues were analyzed for *in vivo* chlamydial growth as described in *Materials and Methods*. Each point represents the mean \pm SD of IFU (log10) for five or six mice. *, $p < 0.05$

heat-inactivated MoPn EB or SPG only (control) injection. All tissues were fixed in 10% neutral buffered formalin, and the footpads were decalcified in 10% formic acid for 5–8 h. Tissues were embedded in paraffin. Five-micron sections were cut, stained with hematoxylin and eosin, and examined by light microscopy. The examination was blinded as to which samples were derived from IL-10 KO and wild-type mice.

Spleen cell culture and cytokine detection

Spleen cells were cultured as previously described (33). Mice were sacrificed at various days following infection, and spleen cells were cultured at 7.5×10^6 cells/ml with heat-inactivated MoPn EBs (1×10^5 IFU/ml) in the presence or the absence of anti-CD4 (YTS-191) mAb (provided by Dr. Waldmann, University of Oxford, Oxford, U.K.) at 5 μ g/ml or with rIL-10 (2.5 ng/ml). Culture supernatants were harvested at 72 and 96 h for cytokine determination. IL-12 (p70) was measured using InterTest-12x, an ELISA kit purchased from Genzyme (Cambridge, MA). The kit can detect IL-12 at ≥ 10 pg/ml. The determination of other cytokines was performed using ELISA as previously described (33). Purified capture and biotinylated detection Abs were purchased from PharMingen (San Diego, CA). IFN- γ levels in 72-h culture supernatants were tested by a two-mAb sandwich ELISA (XMG1.2 for capture and R4-6A2 for detection). IL-5 levels in 96-h culture supernatants were tested using mAb TRFK as capture Ab and mAb TRFK4 as detector Ab. The TNF- α (72-h supernatants) assay used mAb MP6-XT22 as the capture Ab and biotinylated rabbit anti-mouse TNF- α as the detector Ab. Recombinant cytokine proteins (PharMingen)

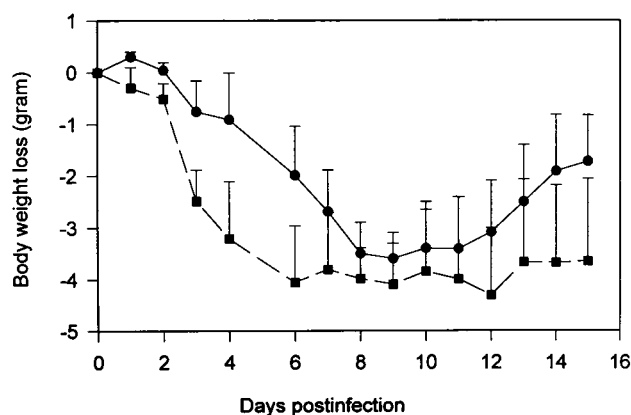


FIGURE 2. Less loss and faster recovery of body weight following chlamydial infection in IL-10 KO mice (\bullet) compared with wild-type (\blacksquare) mice. Mice were intranasally infected with MoPn (2000 IFU) and were monitored daily for body weight change. Each point represents the mean \pm SD of five mice.

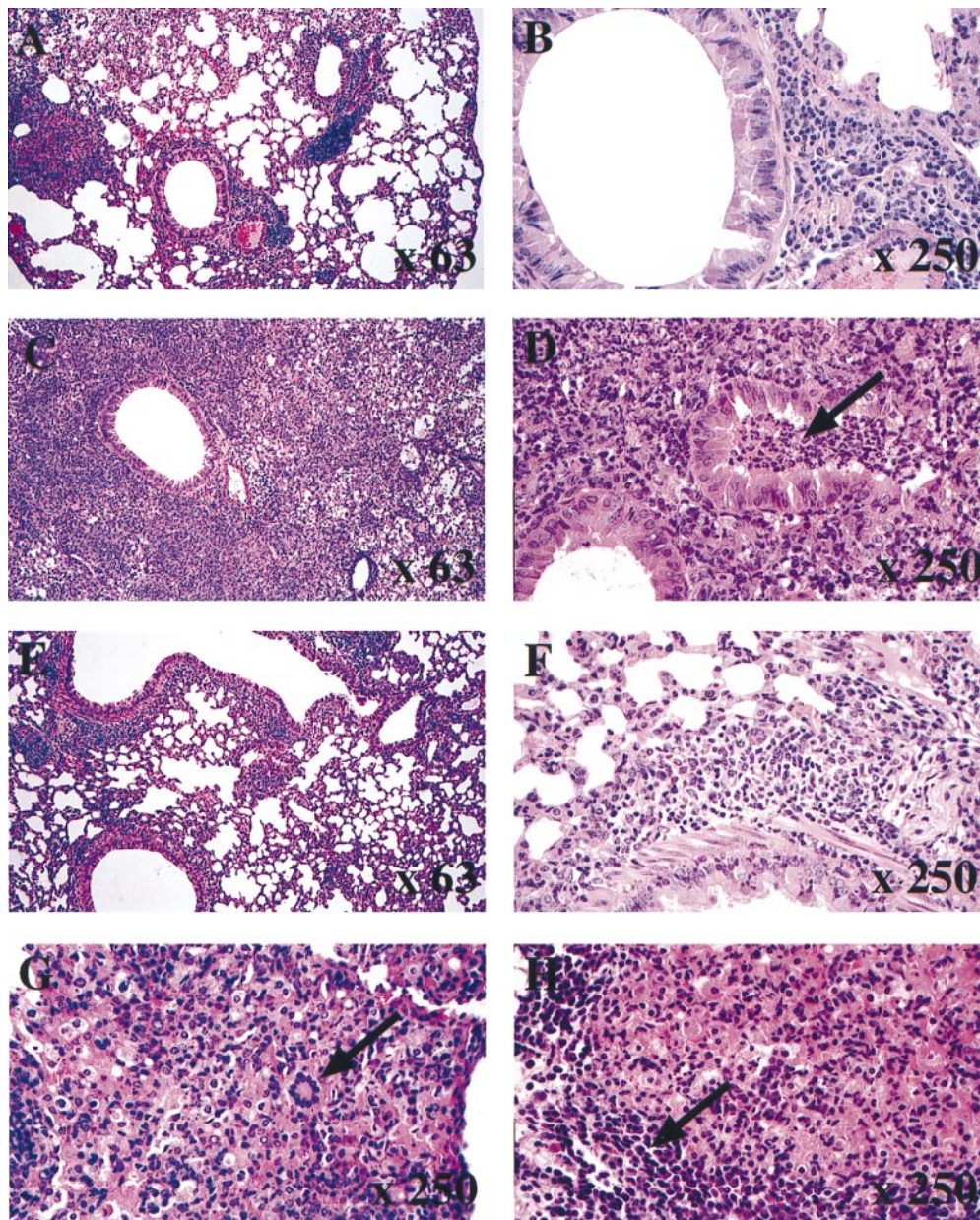


FIGURE 3. Consolidation and granuloma formation in the lung of wild-type, but not IL-10, KO mice following MoPn infection. Photomicrographs of hematoxylin- and eosin-stained sections show the histopathology of MoPn-infected lungs in IL-10 KO and wild-type mice. *A* and *B*, IL-10 KO mice, day 7 postinfection. *C* and *D*, Wild-type mice, day 7 postinfection. The arrow indicates an airway filled with neutrophils. *E* and *F*, IL-10 KO mice, day 20 postinfection. *G* and *H*, Wild-type mice, day 20 postinfection. The arrows indicate a giant cell in an area of granulomatous inflammation (*G*) and plasma cells (*H*).

were used as standards in all the assays. Cytokines in the lung homogenates were also tested using these ELISA.

Statistics

DTH responses and cytokine production were analyzed by Student's *t* test. The IFU of MoPn in the infected lungs and serum Ab titers were transformed to base 10 logarithms and analyzed by Student's *t* test.

Results

IL-10 KO mice show accelerated clearance of MoPn lung infection, reduced morbidity, and absence of pulmonary granuloma formation

To examine their susceptibility to chlamydial infection, IL-10 KO and wild-type mice were intranasally infected with a sublethal dose (2×10^3 IFU) of MoPn and sacrificed at various times for

analysis of *in vivo* growth of the organism. As shown in Fig. 1, although it was similar between these two groups shortly postinfection (day 1), chlamydial growth in the lung was about 20-fold (day 7) and 100-fold (day 12) lower in IL-10 KO mice than in wild-type controls at later stages. Notably, unlike the observations of intracellular protozoan infections in which IL-10 KO mice showed more severe disease and higher mortality than wild-type mice (16–18), the fast clearance of MoPn lung infection in IL-10 KO mice was associated with milder disease than that in wild-type mice and without mortality. The body weight loss (an indicator of morbidity) in IL-10 KO mice was less, and weight recovery was faster than those in wild-type mice (Fig. 2). The general condition of IL-10 KO mice (fur ruffling, dehydration, and activity) was also better than that of wild-type controls.

Histopathologic examination of the lung from both wild-type and IL-10 KO mice was performed on days 7 and 20 postinfection. On day 7, all IL-10 KO mice demonstrated patchy peribronchial cellular infiltrates composed mainly of mononuclear cells (Fig. 3, *A* and *B*). Infiltrates were still present on day 20 in 60% of the mice examined (Fig. 3, *E* and *F*). The infiltrates were similar in both character and distribution to those in day 7 group. The remaining 40% showed a lung architecture that was almost completely normal, with only a few small perivascular infiltrates still present.

The histologic appearance of the lung in chlamydia-infected wild-type mice considerably different from that in IL-10 KO mice. On day 7, all the mice showed broad areas of consolidation (Fig. 3*C*). Unlike the infiltrates in infected IL-10 KO mice, polymorphs were especially numerous, and bronchi were often seen filled with an inflammatory exudate rich in neutrophils (Fig. 3*D*). On day 20, the consolidation had resolved, and >60% of wild-type mice showed granuloma formation (Fig. 3*G*) and infiltration by plasma cells (Fig. 3*H*), typical of the immunopathological reactions observed in human chlamydial diseases. Impressively, none of the IL-10 KO mice showed similar immunopathology. In aggregate, the data indicate that clearance of chlamydial infection in IL-10 KO mice was significantly accelerated compared with that in wild-type mice, suggesting that IL-10 may play a role in chlamydia-induced granuloma formation.

IL-10 KO mice acquire rapid and strong DTH responses following MoPn infection

To examine whether the accelerated clearance of chlamydia and the lack of granuloma formation in IL-10 KO mice were correlated with alterations in immune responses, we tested humoral and cell-mediated (DTH) responses in these mice following MoPn infection. MoPn-specific serum IgM, IgG1, IgG2a, and IgA Ab responses in IL-10 KO mice were not significantly different from those in wild-type mice, although a trend for slightly lower Ab responses was observed in IL-10 KO mice (data not shown). In contrast, DTH responses demonstrated by footpad swelling following heat-inactivated EB challenge in IL-10 KO mice appeared earlier and were significantly stronger than those in wild-type mice (Fig. 4, *A* and *B*). Strong DTH responses in IL-10 KO mice were readily measured on day 7 postinfection, when wild-type mice showed marginal reaction (Fig. 4*A*). On day 15 postinfection (Fig. 4*B*), DTH responses were apparent in both IL-10 KO and wild-type mice, but they were significantly stronger in IL-10 KO than in wild-type mice.

Histopathologic analysis of footpad reactions at 72 h after footpad EB injection on day 15 postinfection showed that MoPn-infected IL-10 KO mice had a heavy, mixed inflammatory cell infiltrate involving s.c. connective tissue, skeletal muscle, periosteum, and sometimes epidermis. Wild-type mice, however, showed a much less extensive infiltrate that was limited to s.c. connective tissue and was composed almost exclusively of mononuclear cells (data not shown). The data demonstrate that knockout of the IL-10 gene dramatically enhanced DTH responses against chlamydial infection, indicating an inhibitory role played by IL-10 in T cell-mediated immune responses *in vivo*.

CD4 T cells from IL-10 KO mice show increased Th1, but decreased Th2, cytokine production

To directly examine the organism-specific CD4 T cell cytokine-producing patterns underlining the enhancement of cell-mediated immune responses, we examined the Th1 (IFN- γ) and Th2 (IL-4 and IL-5) cytokine production by splenic CD4 T cells collected from IL-10 KO and wild-type mice. As shown in Fig. 5, IFN- γ production by spleen cells of IL-10 KO mice was significantly

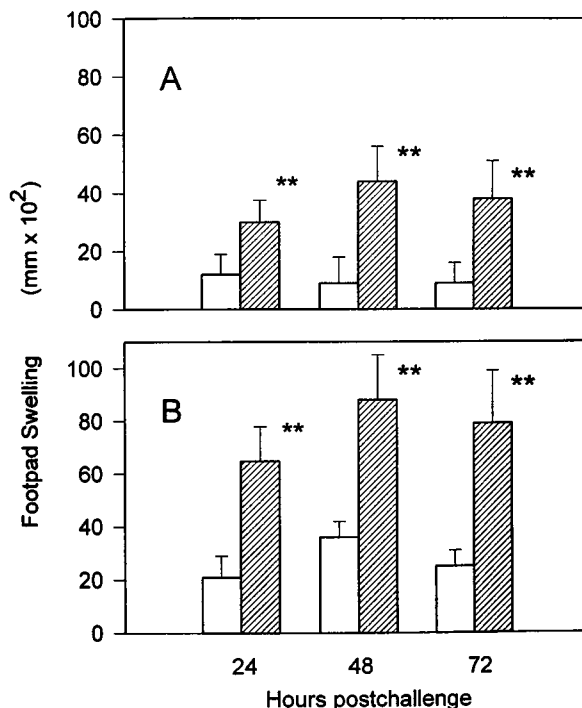


FIGURE 4. Earlier mounting and stronger DTH responses in IL-10 KO mice (▨) compared with wild-type mice (□) as demonstrated by footpad swelling. Mice (five or six mice per group) were challenged with heat-inactivated MoPn in the footpad at 7 (*A*) or 15 (*B*) days postinfection. Footpad reaction was measured at 24, 48, and 72 h following MoPn injection (see *Materials and Methods*). Data show the mean \pm SD of footpad swelling in each group of mice. One of two experiments with similar results is shown. **, $p < 0.01$.

higher than that of wild-type mice. Notably, the differences in IFN- γ levels between wild-type and IL-10 KO mice were more dramatic in the later stages (10- to 20-fold difference on days 18–20) than in the early stage (5-fold difference on day 7) of infection. CD4 T cells appeared to be the major source of IFN- γ , because addition of anti-CD4 mAb in cell culture blocked most of the IFN- γ production (>80%; data not shown). The results suggest that IL-10 plays an inhibitory role in both the initiation and expansion stages of Th1-like responses to chlamydial infection, and its role is more significant for inhibiting Th1-like T cell expansion. On the other hand, in the early stage of infection (day 7), IL-5 production in IL-10 KO mice was comparable to that in wild-type mice, while in the late stage of infection (day 20) it was significantly lower in IL-10 KO mice than that in wild-type controls (Fig. 6). The data suggest that IL-10 is not essential for Th2-like T cell activation; rather, it may play a role in Th2-like CD4 T cell expansion *in vivo*, possibly via inhibition of Th1-like responses (IFN- γ production).

Increase in proinflammatory cytokine (IL-12 and TNF- α) production in IL-10 KO mice

The elevated IFN- γ production and DTH responses in IL-10 KO mice could imply that these proinflammatory cytokines (TNF- α and IL-12) may also be altered due to the absence of endogenous IL-10. We therefore examined IL-12 and TNF- α production by spleen cells collected from MoPn-infected IL-10 KO and wild-type mice. As shown in Fig. 7*A*, TNF- α production by spleen cells was fourfold higher in IL-10 KO than in wild-type mice at the early stage (day 7) of infection. At the later stages of infection (days 18–20), TNF- α production was readily detectable in IL-10

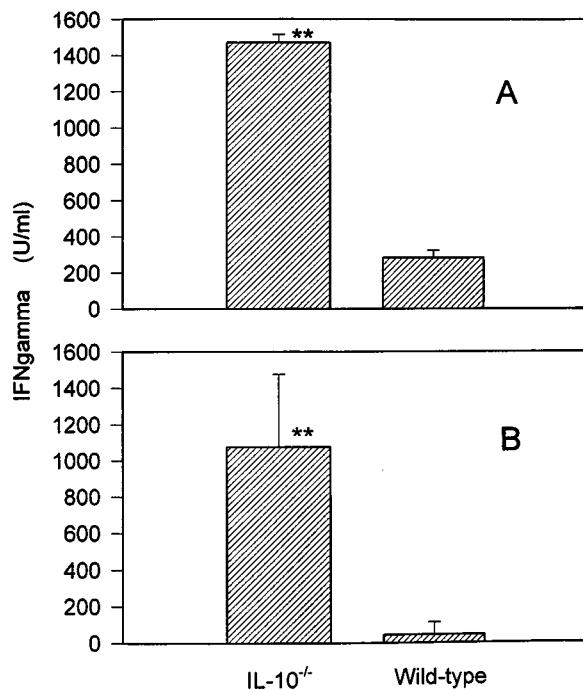


FIGURE 5. Elevation of IFN- γ production by CD4 T cells in IL-10 KO mice. Wild-type and IL-10 KO mice were infected intranasally with MoPn (2000 IFU), and spleen cells were cultured on day 7 (A) or day 20 (B) postinfection. IFN- γ production by spleen cells (72-h supernatants) after organism-specific stimulation was measured by ELISA. Data are shown as the mean \pm SD in each group of mice. One of three experiments with similar results is shown. **, $p < 0.01$

KO mice, although the absolute levels decreased (100–250 pg/ml), while its production in wild-type mice decreased to undetectable levels (<40 pg/ml). Similarly, high levels of IL-12 production by spleen cells collected from IL-10 KO mice were also observed, especially at the early stage of infection (day 7; Fig. 7B). Notably, no detectable IL-12 production by spleen cells was found in MoPn-infected wild-type mice upon MoPn *in vitro* stimulation. The data suggest that IL-10 can inhibit IL-12 and TNF- α production, thereby suppressing Th1-like responses following chlamydial infection.

Since systemic production of proinflammatory cytokines, especially TNF- α , can result in systemic toxicity, we examined serum TNF- α and IL-12 production. The results showed that virtually no measurable IL-12 (<10 pg/ml) or TNF- α (<40 pg/ml) production was present in the sera of either MoPn-infected IL-10 KO or wild-type mice at any of the time points tested (days 1, 3, 7, 12, 18, and 20 postinfection). The data suggest that *C. trachomatis* infection, unlike intracellular protozoan infection, does not induce significant systemic cytokine production.

Elevation of local (lung) IFN- γ and TNF- α production in IL-10 KO mice

In line with the elevated IFN- γ production by their splenic CD4 T cells, IFN- γ levels in the lung homogenates of the IL-10 KO mice were also higher than those in wild-type mice (Fig. 8). The difference was more obvious in the later stages of infection (day 20). Marginal levels of IL-5 were detected at a marginal level in the lung homogenates, and no significant difference was observed between IL-10 KO and wild-type mice (data not shown). Local (lung) TNF- α levels in IL-10 KO mice were significantly higher than those in wild-type mice (Fig. 8). In general, the differences between IL-10 KO and wild-type

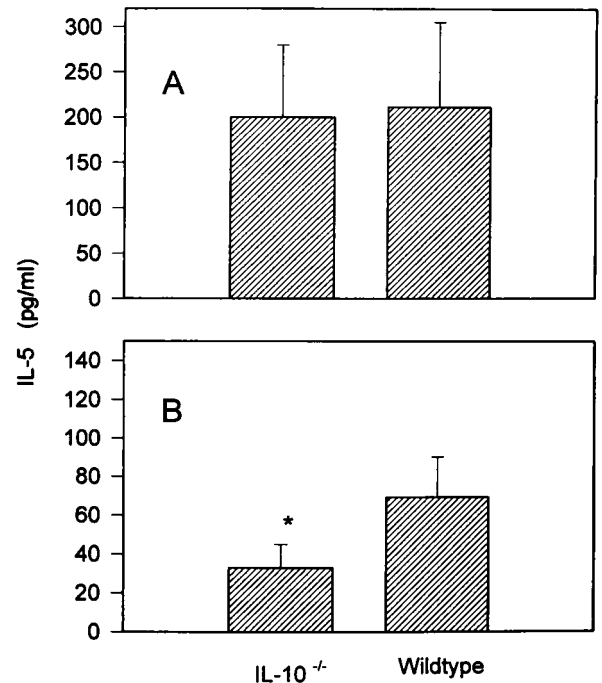


FIGURE 6. Decreased Th2 cytokine (IL-5) production by CD4 T cells in IL-10 KO mice. *Chlamydia*-derived production of IL-5 in the 96-h supernatants of spleen cell culture of the experiment described in Fig. 6 was measured by ELISA. Mice were sacrificed on day 7 (A) or day 20 (B) postinfection. Data are shown as the mean \pm SD. One of three experiments with similar results is shown. *, $p < 0.05$.

mice in cytokine levels in the lung homogenates were not as dramatic as those determined by organism-specific stimulation of spleen cells. Nevertheless, the data demonstrate that both intrapulmonary IFN- γ production and Ag-specific production of IFN- γ by splenic lymphocytes were significantly higher in IL-10 KO than in wild-type mice, suggesting an enhanced Th1-like response in IL-10 KO mice following chlamydial infection.

Exogenous IL-10 inhibits IFN- γ and proinflammatory cytokine production and DTH responses

To further confirm the inhibitory role of IL-10 in Th1-like responses during chlamydial infection, we tested the effect of rIL-10 on IFN- γ and proinflammatory cytokine (TNF- α and IL-12) production by primed spleen cells and on MoPn-specific DTH responses. Addition of rIL-10 significantly decreased IFN- γ production by spleen cells collected from MoPn-infected IL-10 KO mice (Fig. 9A). Exogenous IL-10 also decreased TNF- α (Fig. 9B) and IL-12 production (Fig. 9C). Moreover, injection of rIL-10 into the mouse footpads suppressed the DTH responses in the MoPn-infected IL-10 KO mice, as demonstrated by the significantly milder footpad swelling in the side injected with MoPn plus rIL-10 (5 ng/ml) compared with that in the side injected with MoPn alone (Fig. 10). Overall, the data demonstrate that IL-10 inhibits IFN- γ and proinflammatory cytokine production, suppressing both *in vivo* and *in vitro* cell-mediated immune responses, delays chlamydial clearance, and is associated with local tissue immunopathology.

Discussion

Our previous studies have demonstrated that C57BL/6 and BALB/c mice are different with respect to IL-10 production and resistance to chlamydial infection, with BALB/c mice showing

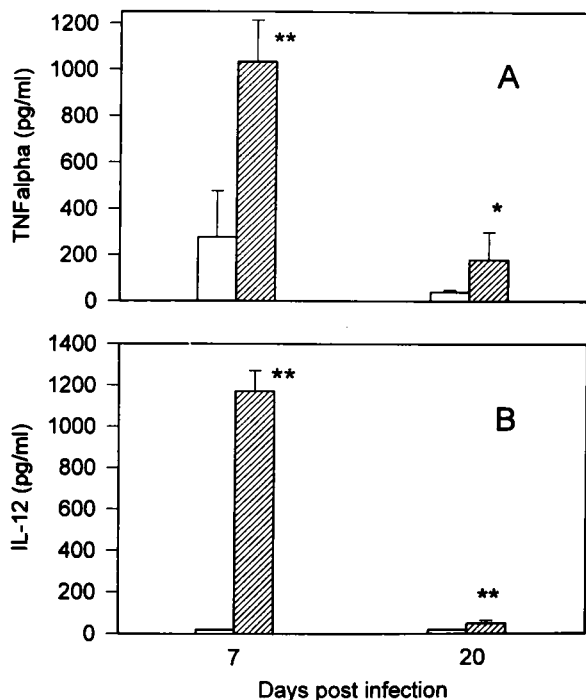


FIGURE 7. Elevation of proinflammatory cytokine (IL-12 and TNF- α) production in IL-10 KO mice. TNF- α (A) and IL-12 (B) production by spleen cell following heat-inactivated MoPn stimulation in IL-10 KO (▨) and wild-type (□) mice were measured at 3, 7, and 20 days following MoPn infection (three to six mice per group at each time point). Data are shown as the mean \pm SD of each group. **, $p < 0.01$; *, $p < 0.05$.

higher organism-specific production of IL-10 and a greater susceptibility to infection. Logically, IL-10 KO mice with a BALB/c background would be a better candidate for examining the role of IL-10 than those with a C57BL/6 background. However, at least two factors limit their suitability for these studies. Firstly, they are commercially unavailable. Second and more importantly, IL-10 KO BALB/c mice spontaneously develop severe inflammatory colitis at young ages, which obviates their use in infection model systems (36). IL-10 KO C57BL/6 mice do not develop inflammatory bowel diseases when kept under specific pathogen-free conditions (37). This mouse strain is therefore more promising for in vivo study of microbial immunobiology following *C. trachomatis* infection. Moreover, although MoPn-infected C57BL/6 mice produce significantly lower levels of IL-10 than similarly infected BALB/c mice, our previous data show that neutralization of endogenous IL-10 in the spleen cell culture of C57BL/6 mice dramatically increases IFN- γ production (33), suggesting that IL-10 plays a crucial role in regulating immune responses in C57BL/6 mice. In our present study using IL-10 KO mice, we demonstrated that IL-10 is inhibitory for Th1-like immune responses induced by chlamydial infection at both the initiation and expansion stages of organism-specific CD4 T cells, possibly via inhibition of IL-12 and TNF- α production. The inhibitory effect of systemically delivered rIL-10 on cell-mediated responses has been shown in several model systems using soluble Ags (38, 39). By local delivery of rIL-10, we now directly demonstrate that IL-10 can inhibit cell-mediated immune responses (DTH) following an intracellular bacteria, chlamydial infection. The combination of results obtained using in vivo and in vitro approaches clearly demonstrates the role of IL-10 in inhibiting cell-mediated immunity to chlamydial infection, and the absence of an inhibitory effect appears beneficial for host resistance to chlamydial infection. On the other hand, it

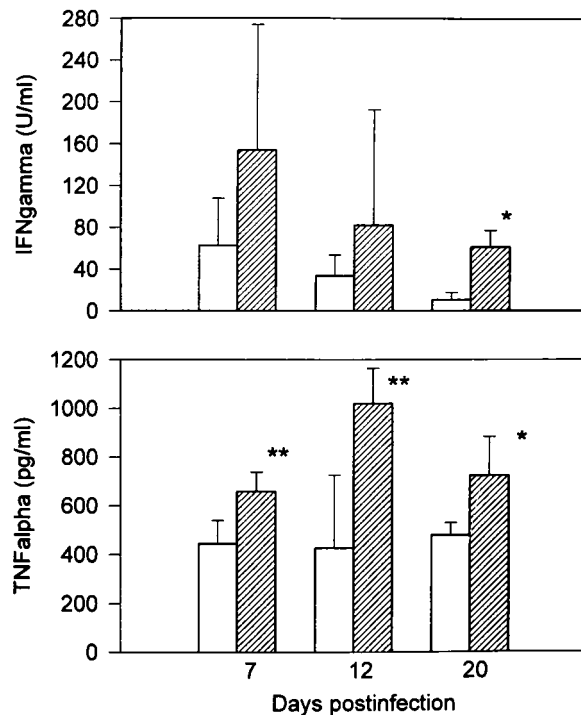


FIGURE 8. Higher IFN- γ and TNF- α levels in the lung homogenates in IL-10 KO (▨) mice compared with wild-type (□) mice. Mice were intranasally infected with MoPn (2000 IFU) and were sacrificed on days 7 and 20 postinfection. The lung homogenates were prepared using SPG (4 ml/mouse) as described in *Materials and Methods*. IFN- γ and TNF- α levels were measured using ELISA. Data are shown as the mean \pm SD. One of three experiments with similar results is shown. **, $p < 0.01$; *, $p < 0.05$.

should be noted that besides its manipulating effect on cytokine responses noted in the study, IL-10 may enhance chlamydial infection directly. Obviously, the question cannot be directly addressed using this gene knockout model because the lack of IL-10 and the increase in the Th1-like response were both present in these mice in the process of chlamydial infection. The fact that IL-10 KO and wild-type mice had similar levels of chlamydial growth on day 1 postinfection (Fig. 1) suggest that mice can be infected equally by chlamydia with or without the presence of IL-10 in vivo. Moreover, although its level was remarkably less than that in IL-10 KO mice, IFN- γ production in wild-type mice was also readily measurable (Fig. 5A). This may be why wild-type mice also resolved chlamydial infection eventually. IL-12 (p70) production was not measurable at the time of determination in wild-type mice. This may suggest that IL-12 was either more transiently expressed or was too low to be detected in wild-type mice. The data may also suggest that IL-12, although capable of enhancing Th1-like responses and thereby accelerating organism clearance, is not necessary for resolution of chlamydial infection.

A novel and unexpected finding in the present study is the difference in the immunopathologic responses (granuloma formation, plasma cell infiltration, and tissue fibrosis) between wild-type and IL-10 KO mice. In the later stages of infection (day 20), when most organisms have been cleared in both wild-type and IL-10 KO mice, wild-type, but not IL-10 KO, mice showed plasma cell infiltration and granuloma formation. Although classically described as a DTH response associated with Th1 cytokine, granuloma formation is currently suggested to be due to Th2-related cytokine responses (40–42) and can be suppressed by Th1-related cytokines (43, 44). Moreover, vaccination with schistosome eggs and

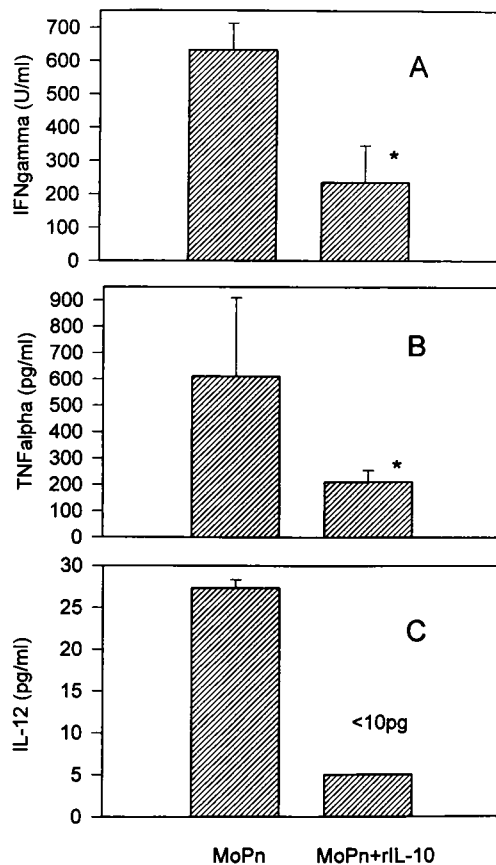


FIGURE 9. Exogenous IL-10 inhibited Th1-related cytokine (IFN- γ and TNF- α) production in IL-10 KO mice. IL-10 KO mice were intranasally infected with MoPn (2000 IFU) and were sacrificed on day 10 postinfection. Spleen cells were cultured in the presence of heat-inactivated MoPn with or without rIL-10 (2500 pg/ml). IFN- γ (A) and TNF- α (B) production in 72-h culture supernatants was measured by ELISA. Data represent the mean \pm SD. *, $p < 0.05$.

IL-12 inhibits both granuloma formation and tissue fibrosis induced by natural *Schistosoma mansoni* infection (44, 45). Since the IL-10 KO mice produce significantly higher levels of IL-12 and IFN- γ than wild-type mice and are deficient in granuloma formation, we hypothesize that the increased levels of IL-12 and IFN- γ of these cytokines may be involved in the prevention of immunopathologic (granulomatous) reaction.

The alteration in IL-5 production in IL-10 KO mice suggests that IL-10 may also promote Th2 cytokine production. Variable results have been reported regarding the role of IL-10 in influencing Th2-like cell differentiation (46–49). One study showed that IL-10, similar to IL-4, promoted Th2-like cell differentiation in TCR transgenic mice (46), while another study showed that IL-10 had little effect on skewing Th1- or Th2-like cell differentiation (47). We found that IL-5 production in IL-10 KO and wild-type mice was comparable at the early stage (day 7) of infection, but was different in the later stages, with lower IL-5 levels in IL-10 KO mice. We speculate that the decrease in IL-5 production in the later, but not the early, stages of infection in IL-10 KO mice may reflect an indirect effect mediated by persistent elevation of IFN- γ and/or other Th1-promoting cytokines that are inhibitory for the development of Th2-like cells due to the absence of immunoregulating amounts of IL-10.

The data in the present study are in striking contrast to those obtained from intracellular protozoan infection models in terms of acute toxicity. Although the reason for the difference remains unclear, we speculate that it may reflect the differences in the natural

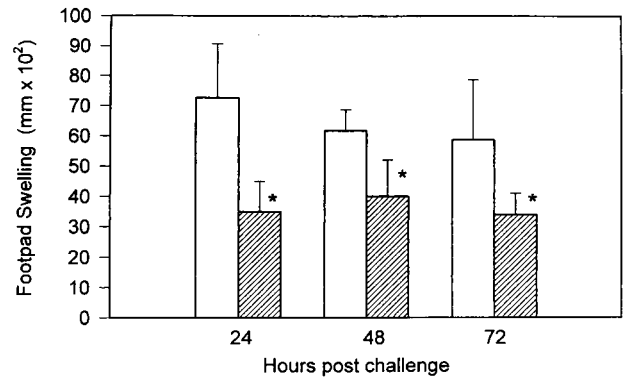


FIGURE 10. Local delivery of rIL-10 inhibited DTH responses in IL-10 KO mice. Mice were intranasally infected with MoPn (2000 IFU), and DTH responses were measured on day 14 postinfection. Heat-inactivated MoPn EBs were injected to footpads in one side with rIL-10 (▨) and on the other side without rIL-10 (□). The thickness of each footpad were measured before and after MoPn injection. The difference in the thickness of each footpad before (0 h) and after (24, 48, and 72 h) MoPn injection was used as a measure of DTH. Data represent the mean \pm SD. *, $p < 0.05$.

pathogenesis among these infections and in the immunoregulating mechanism(s) involved in the pathogenesis. For example, it has been shown that *Toxoplasma gondii* possesses a superantigen that can expand a large percentage of T cells (50), and IL-10 has been shown to play a critical role in inhibiting superantigen-induced T cell activation (51). There is no evidence that chlamydia carries a superantigen. Moreover, intranasal inoculation of MoPn, unlike intracellular protozoan infections, causes mainly local (respiratory) chlamydial growth and inflammatory reaction. Thus, virtually no detectable (<10–40 pg/ml) IL-12 and TNF- α were measured in the sera of wild-type and IL-10 KO mice following chlamydial infection. Indeed, although IL-12 and TNF- α production in spleen cells from IL-10 KO mice was increased in the early stage of chlamydial infection (day 7; Fig. 8), IL-12 and TNF- α production in IL-10 KO mice was transient (decrease to marginal levels on day 20) and was not detectable in the serum above the testing sensitivity level (10 pg/ml). This difference in systemic cytokine production may be critical in explaining the difference in the outcome (death or survival) of IL-10 KO mice following intracellular protozoan and chlamydial infection. This point was particularly supported by the finding that the earlier mortality of IL-10 KO mice following *Trypanosoma cruzi* (18) was associated with overwhelming systemic (serum) release of IL-12 and TNF- α and that it was reversed by neutralizing endogenous IL-12 or TNF- α with anti-IL-12 or anti-TNF- α Abs.

Finally, although no significant acute toxicity has been found in the MoPn-infected IL-10 KO mice, this does not mean that the inhibitory effect of IL-10 on Th1-like responses is not relevant to host homeostasis during chlamydial infection. In fact, one interesting finding in this study was that the difference in IFN- γ production between wild-type and IL-10 KO mice (persistently higher IFN- γ levels in IL-10 KO mice) was more dramatic at the later stages of chlamydial infection when IFN- γ levels had significantly decreased in wild-type mice (Fig. 6). The long term effect of the persistent elevation of IFN- γ was not examined in the present study. Since IL-10 KO mice showed elevated IFN- γ production and an absence of granuloma formation, which is relevant to the sequelae (blindness and infertility) induced by chlamydial infection, the results suggests that modulation of IL-10 production could be an approach to prevent sequelae of chlamydial infection. Obviously, further studies, especially those dealing with the

long term effects of the absence of endogenous IL-10, are required for rational development of chlamydial immunoprophylactic approaches.

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