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IL-23 induces IL-22 and IL-17 production in response to *Chlamydia muridarum* genital tract infection, but the absence of these cytokines does not influence disease pathogenesis

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Abstract

OBJECTIVE—*Chlamydia trachomatis* Infections are a significant cause of reproductive tract pathology. Protective and pathologic immune mediators must be differentiated in order to design a safe and effective vaccine.

METHODS—Wild-type mice and mice deficient in IL-22 and IL-23 were infected intravaginally with *Chlamydia muridarum* and their course of infection and oviduct pathology were compared. Local genital tract and draining lymph node immune responses were also examined in IL-23 deficient mice.

RESULTS—IL-22 and IL-23 deficient mice exhibited normal susceptibility to infection and oviduct pathology. IL-23 was required for development of a *Chlamydia*-specific Th17 response in the lymph nodes and for production of IL-22 and IL-17 in the genital tract. However, influx of Th1 and innate immune cells was not compromised in the absence of IL-23.

CONCLUSIONS—IL-22 and IL-23 play either redundant or minimal roles in the pathogenesis of *Chlamydia* infection in the mouse model. Induction of Th17-associated cytokines by a *Chlamydia* vaccine should be avoided since these responses are not central to resolution of infection and have pathologic potential.

Keywords

Th17; immunopathology; intracellular bacteria

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INTRODUCTION

Chlamydia trachomatis infections of the female reproductive tract can lead to the development of pelvic inflammatory disease, chronic pelvic pain, infertility, and ectopic pregnancy. These sequelae result from the host inflammatory response, which is dually responsible for resolution of infection and the development of genital tract pathology. Differentiation of protective immune mediators from those that are primarily pathologic is critical for vaccine development. Studies in animal models of *Chlamydia* genital tract infection have repeatedly demonstrated a central role for IFNγ production by CD4+ T cells in controlling infection and preventing the development of oviduct disease.[1–5] This protective Th1 response is counterbalanced by the anti-inflammatory cytokine IL-10, which inhibits Th1 activation and delays clearance of *C. muridarum* infection from the genital tract.[6]

IL-22 is member of the IL-10 family of cytokines that exhibits complex protective and pathologic effects depending on the disease model examined. Although IL-10 and IL-22 have limited homology, their heterodimeric receptor complexes share a common chain, IL-10 receptor beta, and predominately induce STAT3 activation.[7–10] The unique subunit of the IL-22 receptor, IL-22 receptor alpha-1, is expressed exclusively by non-hematopoietic cells including epithelial cells, while the IL-10-specific receptor subunit, IL-10 receptor alpha, is widely expressed by both hematopoietic and non-hematopoietic cells.[8, 11] The expression pattern of the IL-22 receptor explains the localization of IL-22-induced responses to environmental interfaces including the skin, lungs, and gastrointestinal tract.[11–14] In addition, IL-22 receptor mRNA has been detected in the female reproductive tract including the ovaries, cervix, and placenta.[11, 15, 16]

IL-22 promotes mucosal immunity by enhancing epithelial barrier integrity, expression of anti-microbial molecules, and mucin production.[12, 13, 17, 18] The importance of IL-22 in mucosal host defense was first documented in models of infection with extracellular bacteria including *Klebsiella pneumoniae* pulmonary infection and *Citrobacter rodentium* intestinal infection, where mice succumbed to infection when IL-22 was inhibited or absent.[12, 13] In contrast, IL-22 induces immunopathology in the small intestine in response to peroral infection with the intracellular parasite *Toxoplasma gondii*.[1920] The reduced pathology observed upon neutralization of IL-22 in this model was associated with significant decreases in proinflammatory cytokine and chemokine production in the draining lymph nodes and ileum.[19] Indeed, IL-22 has been demonstrated to induce production of several neutrophil chemokines (CXCL1, -2, -3, -5, -6, -8) in addition to up regulating expression of matrix metalloproteases (MMP1, -3, -10).[13, 15, 21, 22] Enhanced neutrophil influx and MMP production are clearly associated with oviduct damage in response to *Chlamydia* infection in the mouse model.[23–27]. Thus, IL-22 induces responses in other models that are linked with disease development during chlamydial genital infection.

There are a limited number of studies examining the role of IL-22 in the female reproductive tract under both physiologic conditions and in the context of infection. IL-22-producing immature NK cells have been detected in the human uterus, where they have been proposed to play a role in tissue regeneration after cyclic shedding.[28] In the context of infectious diseases, mouse models of vaginal infection with *Candida albicans* and *Neisseria gonorrheoae* failed to show a requirement for IL-22 in infection control.[29, 30] We previously reported significantly increased levels of IL-22 in genital tract secretions from *C. muridarum*-infected IFNγ-deficient mice, which were associated with a heightened Th17 response, increased neutrophil infiltration, and the development of severe oviduct pathology.[2] IL-22 and IL-17 production have also been observed by CD4+ T cells isolated

from the cervical washes of women infected with *C. trachomatis*.[31] These data indicate that IL-22 may be involved in the pathogenesis of *Chlamydia* genital tract infection.

IL-22 is produced by Th17 cells, Th22 cells, γδ T cells, lymphoid tissue inducer cells, and NK22 cells.[14, 18, 32–36] Release of both IL-17 and IL-22 from the aforementioned cells is enhanced by IL-23.[1418, 32, 36–39] IL-22 and IL-17 can cooperatively induce the production of proinflammatory cytokines, neutrophil chemokines, and anti-microbial molecules.[13, 18, 22] For example, IL-22 and IL-17 enhance production of S100A8 and S100A9, which form a heterodimeric complex known as calprotectin.[18] Calprotectin induces neutrophil chemotaxis, and acts as an alarmin, potently amplifying inflammation.[40, 41] The interplay between IL-17 and IL-22 has been shown to dictate whether IL-22 exhibits a tissue-protective or damaging role.[42] Herein, we explored the possible cooperative effects of IL-17 and IL-22, by examining the course and outcome of chlamydial infection in mice deficient in IL-23.

The intracellular life cycle of *Chlamydiae* dictates that resolution of infection from the genital tract is dependent on the influx of CD4+ T cells. Even in the presence of a robust innate inflammatory cell influx, such as observed in MHC class II deficient mice, infection is sustained at high levels indefinitely.[43] Thus, we hypothesized that IL-22-mediated induction of antimicrobial molecules was unlikely to be significantly beneficial in infection control in this model, which would be in accordance with findings in other models of intracellular bacterial infection including *Mycobacterium tuberculosis* and *Listeria monocytogenes*.[19, 44, 45] However, given the importance of neutrophil activation and MMP production in development of chlamydial-induced oviduct damage,[23–25, 27, 46] we hypothesized that IL-22-mediated induction of these processes would contribute to pathology, and this cytokine either independently, or in conjunction with IL-17, would play a detrimental rather than protective role during chlamydial genital tract infection. We tested this hypothesis by comparing the course and outcome of *C. muridarum* genital tract infection in mice genetically deficient for IL-22 with immunologically normal mice. In addition, we utilized mice genetically deficient for IL-23 to determine if reductions in both IL-22 and IL-17 would ameliorate oviduct pathology.

MATERIALS AND METHODS

Strains, cell lines, and culture conditions

Plaque-purified *C. muridarum* Nigg was used for all experiments and was isolated as previously described.[47, 48] All chlamydial strains were propagated in L929 cells.[49] Bacteria were titrated by plaque assay [48] or as inclusion forming units (IFU) using fluorescently tagged anti-chlamydial lipopolysaccharde monoclonal antibody (Bio-Rad, Hercules, CA).[50]

Animals

Female C57BL/6 mice were obtained from The Jackson Laboratory (Bar Harbor, ME). The IL-22 knockout (IL-22 KO) mice were kindly provided by Dr. Wenjung Ouyang[14] and the IL-23p19 knockout (IL-23p19 KO) mice by Dr. Nico Ghilardi,[51] both at Genentech. IL-23p19 heterozygous mice used in these studies were the F1 progeny of a C57BL/6 and IL-23p19 KO mouse. Mice were at least 7 weeks of age at the time of infection. Mice were given food and water ad libitum in an environmentally controlled room with a cycle of 12 hours of light and 12 hours of darkness. All animal experiments were approved by the University Institutional Animal Care and Use Committee.

Immunohistochemical analysis of murine genital tract tissues for the IL-22 receptor

Analysis of IL-22 receptor alpha-1 (IL-22R1) expression was conducted as previously described.[52] Genital tract tissues were harvested from C57BL/6 mice, and tissue sections were made as described below (See section **Microscopic histopathological assessment).** Sections were deparaffinized in xylene (3 × 10 minutes) and rehydrated through sequential washings with 100%, 95% and 75% ethanol (2 × 10 minutes). Antigen retrieval was performed by boiling for 10 minutes in 10mM citrate buffer followed by incubation for 30 minutes at room temperature. After peroxidase blocking (3% hydrogen peroxide for 10 minutes), slides were blocked following the Vectastain® ABC blocking protocol for Rat IgG (Vector Laboratories, Burlingame, CA). Receptor expression was visualized using Rat anti-mouse IL-22R1 (R&D Systems, Clone: 496514) at a dilution of 1:50. Additional tissues were incubated with Rat IgG2a (R&D Systems, Clone: 54447) as a control for nonspecific staining.

Murine infection and monitoring

Five to seven days prior to infection mice were subcutaneously injected with 2.5 mg of medroxyprogesterone (Depo-Provera®; Upjohn, Kalamazoo, MI) to induce a state of anestrous.[53] Mice were intravaginally inoculated with 1×10^5 IFU of *C. muridarum* Nigg diluted in 30 μl of sucrose-sodium phosphate-glutamic acid (SPG) buffer unless otherwise indicated. Mice were monitored for cervicovaginal shedding via endocervical swabs,[54] and IFU were calculated as previously described.[50] Bacterial burden was measured in the oviducts by plaque assay.[48] Lower genital tract (LGT) bacterial burden was enumerated for IL-22 KO and C57BL/6 mice in two independent experiments with 5–6 mice per strain per experiment. The course of infection in the LGT of IL-23p19 KO and C57BL/6 was compared in three independent experiments with 4–5 mice per strain. A group of five IL-23p19 heterozygous mice was added for one of these experiments. Bacteria were titrated from the oviducts of IL-23p19 KO and C57BL/6 mice in a single experiment with 3–4 mice per strain per day of analysis.

To determine the susceptibility of C57BL/6 and IL-23p19 KO mice to low doses of infection, 10-fold serial dilutions of *C. muridarum* Nigg ranging from 5×10^1 to 5×10^4 bacteria were resuspended in 30 μl of SPG buffer and intravaginally inoculated into groups of 6–7 mice per dose per strain. On day 6 post-infection, mice were euthanized, and their cervices were immediately processed for detection of infection via IFU.[55]

Processing of oviducts for flow cytometry

Oviducts and cervices were processed for flow cytometric analysis as previously described.[2, 26] Briefly, tissues were harvested and minced with scissors. For measurement of cytokines and bacterial burden, an aliquot of the minced tissue was stored at −80°C until analysis. Cervices were digested with collagenase I (1mg/ml; Sigma-Aldrich, St. Louis, MO), and then cervices and oviducts were repeatedly passed through a 70 μm filter to yield a single cell suspension. Single cell suspensions were incubated with Fc Block (BD Pharmingen; Clone: 2.4G2), and cell surface proteins were subsequently stained with the indicated antibodies. Stimulation of cells for analysis of intracellular cytokines was conducted as below (See section **Detection of *Chlamydia*-specific cytokine production by intracellular flow cytometry)**. Flow cytometry data were acquired using an LSR II Analyzer (BD Biosciences) and analyzed via FlowJo software (Tree Star, Ashland, OR).

Detection of *Chlamydia*-specific cytokine production by intracellular flow cytometry

For analysis of cytokine production, single cell suspensions generated from the cervix or oviducts of individual mice were incubated overnight in complete medium (DMEM

containing 10% FBS, 2 mM glutamine, 100 μM non-essential amino acids, 50 μM β-mercaptoethanol, 100 μg/ml vancomycin and 50 μg/ml gentamicin) with 5 μg/ml of gradient purified UV-inactivated *C. muridarum* elementary bodies (UV-EBs).[56] GolgiPlug™ (1:500 final dilution; BD Biosciences) was added for the last 4 hours of incubation. Cell surface proteins were stained with PerCP-Cy5.5 anti-mouse CD45 (Clone: 30-F11), V450 anti-mouse CD3 (Clone 500A2), and PE anti-mouse CD4 (Clone RM4-5) all from BD Biosciences. After surface staining, cells were fixed, permeabilized, and stained with APC anti-mouse IFNγ (BD ^{2}Biosciences, clone XMG1.2) according to manufacturers instructions (Cytofix/Cytoperm™Kit, BD Biosciences). Cytokine production by WT and IL-23p19 KO cells was analyzed on days 7, 10, and 14 of infection with 3–4 mice per strain per day in two independent experiments.

Detection of cytokines in lower genital tract secretions and oviduct homogenates

LGT secretions were collected via washing the vaginal vault with 100 μl of phosphate-buffered saline with protease inhibitor (Complete EDTA-free protease inhibitor tablets, Roche Diagnostics) during the first ten days of infection as previously described.[57] IL-17, TNFα, and IFNγ were quantified in these lavages and in the homogenized oviducts of C57BL/6 and IL-23p19 KO mice via multiparametric bead array (Millipore, Billerica, MA). IL-22 was measured by ELISA (R&D Systems, Minneapolis, MN). Cytokines were monitored in the LGT secretions of C57BL/6 and IL-23p19 KO mice in two independent experiments with 4–5 mice per group, and cytokines were measured in the oviducts of 3–5 mice per group per day.

Assessment of *Chlamydia*-specific cytokine responses in the iliac nodes

Iliac nodes from IL-23p19 KO and C57BL/6 mice infected intravaginally with *C. muridarum* were harvested on days 0, 7, 14, 21, 28, 35, and 56 post infection. Lymph nodes were processed to a single cell suspension and placed in culture with media alone or UV-EBs (5 μg/well). Supernatants were collected after 96 hours in culture for quantification of cytokines as described above. Cytokine production by iliac lymph node mononuclear cells was evaluated using 5 mice per strain per day.

Microscopic histopathological assessment

Genital tracts were removed en bloc, fixed in 10% buffered formalin, and embedded in paraffin. Longitudinal 4-μm sections were cut and stained with hematoxylin and eosin. Oviduct epithelial cell erosion and oviduct dilatation were assessed for tissues harvested on day 42 using a four-tiered semi-quantitative scoring system by a pathologist blinded to the experimental design.[50, 58] Oviduct pathology for IL-22 KO and C57BL/6 mice was compared on day 42 in two separate experiments with 5–6 mice per group per experiment, and the same comparison was conducted for IL-23p19 KO and C57BL/6 mice.

Statistics

Statistical comparison of flow cytometry data, cytokine levels, or the course of infection was conducted via two-way ANOVA with Bonferroni post-test analysis. A Mann-Whitney U test was used to determine significant differences in pathology scores. A Fisher's exact test was used to determine differences in susceptibility to low dose infection. Comparisons of pathological data, cytokine responses over time, and course of infection over time requires 8–10 tissues or mice per group to yield a power of 0.74–0.90 to detect a 25–30% difference between groups since the variance may approach 20% because of biological variability of the infection and response to infection among individual animals. Prism software (GraphPad Software, LaJolla, CA) was utilized for all statistical analysis. Values of $P < 0.05$ were considered significant.

RESULTS

Murine genital tract epithelial cells express the IL-22 receptor

The IL-22 receptor is a dimeric complex of the IL-10 receptor beta chain (IL-10R2), which is ubiquitously expressed, and the IL-22 receptor alpha-1 chain (IL-22R1), which is only expressed by non-hematopoietic cells.[8, 10, 11] Although a role for IL-22 receptor signaling has been reported at mucosal sites including the pulmonary and gastrointestinal tracts,[12, 13] expression of this protein has not been previously described in the genital tract. Using immunohistochemistry, we detected IL-22R1 expression in the murine ectocervix (Fig. 1A), endocervix (Fig. 1B and 1C), uterine horns (Fig. 1D), and oviducts (Fig. 1E). Receptor expression was localized to the epithelium, and no staining was observed for stromal cells of the genital tract. No staining was observed when sections were incubated with the relevant immunoglobulin isotype (Fig. 1F).

IL-22 deficiency has no effect on bacterial burden or oviduct pathology

Since we detected expression of IL-22R1, and we previously documented IL-22 in murine genital tract secretions during active *C. muridarum* infection,[2] we sought to determine if IL-22 was involved in resolution of infection from the genital tract. Comparison of the course of lower genital tract infection for C57BL/6 and IL-22 deficient mice revealed that infection resolved with normal kinetics in the absence of IL-22 (Fig. 2A). In addition, none of the mice exhibited clinical signs of bacterial dissemination as has been observed in models of infection with extracellular bacteria in the absence of IL-22.[13, 39] We also examined the possibility that IL-22 could influence oviduct pathology in this model. Histological analysis revealed that erosion of the oviduct epithelium was comparable between strains (Fig. 2B). In addition, we detected similar degrees of oviduct dilatation in the presence and absence of IL-22, with 5 of 6 mice in both groups developing severe dilatation (Fig. 2C).

IL-23 induces IL-17 and IL-22 production in response to *C. muridarum* infection in the genital tract and iliac lymph nodes

IL-23 is composed of the shared IL-12p40 subunit and the unique IL-23p19 subunit.[59] IL-23 enhances the release of IL-17 and IL-22 from both innate and adaptive immune cells.[1418, 32, 37–39] In order to determine the role of IL-23 in the cytokine response to *C. muridarum* genital tract infection, we intravaginally infected IL-23p19 deficient mice. Lower genital tract secretions were collected for the first 10 days of *C. muridarum* infection, and oviducts were harvested on day 10 post-infection. These time points represent peak days of cytokine production at both sites.[26] Examination of cytokine levels in the absence of IL-23 revealed significantly reduced IL-17 (Fig. 3A and E) and IL-22 (Fig. 3B and F) but no difference in TNFα (Fig. 3C and G) or IFNγ (Fig. 3D and H) at either of these sites.

IL-23 has been previously shown to promote the stability of the Th17 lineage.[60] In order to determine the role of IL-23 in the adaptive immune response to *Chlamydia*, we harvested the iliac lymph nodes (ILN) from infected mice and stimulated them with *C. muridarum* elementary bodies. In the absence of IL-23, *Chlamydia*-specific release of both IL-17 and IL-22 was significantly reduced (Fig. 4A and B). Similar to our previous findings in IL-17 receptor deficient mice,[2] IFNγ production was reduced on day 7 in the ILNs of IL-23p19 deficient mice, but levels were comparable to those detected for wild-type mice by day 14 (Fig. 4C). Despite early reductions of IFNγ in the ILN, flow cytometry revealed no difference in the frequency of *Chlamydia*-specific IFNγ-producing $CD3^{+}CD4^{+}$ T cells in either the cervix or oviducts on days 7, 10, or 14 post-infection (data not shown). These findings are in accordance with the detection of normal levels of IFNγ at both of these sites in the absence of IL-23 (Fig. 3D and H). These data indicate that IL-23p19 KO mice provide

an appropriate model to examine the contributions of IL-17 and IL-22 to chlamydial pathogenesis without the confounding effects that would result from reductions in the protective cytokine IFNγ.[2–4]

Infection resolves with normal kinetics in the absence of IL-23

Given the role of IL-17 and IL-22 in enhancing mucosal immunity, we sought to determine if the reductions in IL-17 and IL-22 that we observed in the absence of IL-23 impacted the ability of mice to control infection in either the lower or upper genital tract. We followed the course of lower genital tract infection in C57BL/6, IL-23p19 deficient and IL-23p19 heterozygous mice and found no difference in the kinetics of infection between any of the strains (Fig. 5A). We also found no difference in the bacterial burden in the oviducts over the peak days of infection in the absence of IL-23 (Fig. 5B).

Although we did not detect a difference in the kinetics of infection when mice were infected with 100,000 bacteria, we recognized that with such a high dose of infection, innate defense mechanisms induced by IL-22 and IL-17 may be overwhelmed. To explore this possibility, we infected C57BL/6 and IL-23p19 deficient mice with doses of *C. muridarum* Nigg ranging from 50 to 50,000 microorganisms. All of the mice from both strains established an active infection upon inoculation with as few as 500 IFU (data not shown). When the innoculum was decreased to 50 IFU, 6 of 7 C57BL/6 mice and 2 of 6 IL-23p19 deficient mice developed an active infection, but these differences were not statistically significant ($P > 0.05$ Fisher's exact test).

IL-23 is not required for influx of innate immune cells into the oviduct or the development of oviduct pathology

Innate immune responses are key for the development of chlamydia-induced immunopathology,[24–27, 61, 62] and IL-17 and IL-22 have been shown to cooperate in inducing release of neutrophil chemokines and promoting innate inflammation[13, 18, 22] Thus, we examined the influx of innate inflammatory cells into the oviducts of wild-type and IL-23p19 deficient mice on day 10 post-infection. Flow cytometry revealed no differences in the frequency of neutrophils, inflammatory monocytes, or macrophages between the strains at this time point (Fig. 6A). In accordance with these data, no improvement in the severity of oviduct dilatation was found in IL-23p19 deficient mice (Fig. 6B).

DISCUSSION

In the studies outlined in this manuscript, we explored the role of Th17 cells and the associated cytokines IL-22, IL-17, and IL-23 in the mouse model of *Chlamydia* genital tract infection. We show for the first time that the epithelial cells of the murine genital tract express the unique subunit of the IL-22 receptor, IL-22R1. We also demonstrate that IL-23 is required for IL-17 and IL-22 production in response to *C. muridarum* infection and is necessary for the maintenance of a *Chlamydia*-specific Th17 response in the draining lymph nodes. However, we were unable to detect a requirement for any of these cytokines in resolution of infection, susceptibility to low dose infection, or the development of oviduct pathology. The normal resolution of infection observed for IL-23p19 deficient mice could be predicted given these mice developed a Th1 response comparable to wild-type mice. Similarly, no compromise was seen in the ability of innate inflammatory cells to migrate into the oviducts of IL-23p19 deficient mice, and this influx was associated with oviduct pathology similar to that observed for C57BL/6 mice.

The IL-22 receptor is a heterodimer of IL-22R1 and IL-10R2, with expression of IL-22R1 limited to non-hematopoietic cells.[11] IL-22R1 expression was previously detected in the

human cervix and ovary by microarray,[15] but protein expression in vivo has not been previously demonstrated. We detected IL-22R1 expression localized to the epithelium of the ectocervix, endocervix, uterine horns, and oviducts of uninfected C57BL/6 mice. Inflammatory stimuli including LPS, IFNγ, TNFα, and IL-1β may induce higher levels of IL-22R1 expression during acute chlamydial infection.[11, 63] Detection of this receptor on the epithelium of the genital tract is in accordance with reports of receptor expression by epithelial cells at environmental interfaces including the skin, lung, and gastrointestinal tract.[11–14] Columnar epithelial cells of the genital tract are the site of chlamydial replication and are highly susceptible to infection-induced damage. Thus, the IL-22 receptor is appropriately located to play a role during chlamydial infection. Despite detection of this receptor, we determined that *C. muridarum* infection resolved normally in IL-22 deficient mice. Resolution of infection was also normal for IL-23p19 deficient mice despite nearly undetectable levels of both IL-17 and IL-22 in the lower and upper genital tract. These findings are not unique to *C. muridarum*, as pulmonary infection with the intracellular bacterium *Mycobacterium tuberculosis* resolved normally in the absence of IL-17 and IL-22.[19, 64]

There are several possible explanations for why we observed normal control of chlamydial infection in IL-22 and IL-23p19 deficient mice. It is likely that the intracellular replicative niche of chlamydiae hinders the potential protective capacity of anti-microbial proteins induced by these cytokines, which include S100A proteins, β-defensins, Reg proteins, and lipocalins.[13, 15, 18] Chlamydiae are susceptible to anti-microbial peptides in vitro,[65, 66] but innate defense mechanisms have a limited ability to resolve *C. muridarum* infection in vivo independently of the adaptive immune response.[43] It is also possible that the ability of chlamydiae to directly stimulate pattern recognition receptors [67] on epithelial cells and tissue resident immune cells obviates the requirement for epithelial-targeting cytokines peripheral to the Th1 response.[62, 67, 68] Although IL-17 and IL-22 can induce the production of Th1 chemokines including CXCL9,[13] we observed no deficit in IFNγ production or Th1 migration to the genital tract of IL-23p19 deficient mice. PRR stimulation by *Chlamydia* induces the production of many proinflammatory cytokines that can activate the same pathways as IL-22 and IL-17 [62, 67]. These cytokines, in combination with pathways induced directly by PRR stimulation, likely augment production of chemokines necessary for innate and adaptive inflammatory cell influx into the genital tract, thus obviating the requirement for IL-22 and IL-17.

It was previously observed that IL-17 played an important role in inducing the Th1 response to *C. muridarum* infection in the lung and was required for normal resolution of pulmonary infection.[69] This contrasts with our findings of only slight reductions in IFNγ and no compromise in resolution of genital tract infection in the absence of IL-17 receptor signaling,[2] or IL-23-dependent induction of the Th17-related cytokines, IL-22 and IL-17 (current work). The genital tract is a mucosal site that must maintain a tolerogenic environment for proper reproductive fitness. Thus, there appears to be a site-specific role for IL-17 in defense against chlamydial infections, and this may hold true for IL-22 as well. Such tissue-specificity is described for *Candida albicans* infection where Th17 cytokines were required for control of oropharyngeal candidiasis [70], but in vulvovaginal candidasis, infection resolved normally in the absence of IL-22, IL-17 and IL-23.[29]

We also determined that *Chlamydia*-induced genital tract pathology was not altered in the absence of either IL-22 or IL-23. It was difficult to predict whether IL-22 would prevent or induce tissue damage in this model given its complex and dual roles in other models. We hypothesized that since IL-17 and IL-22 enhance neutrophil chemokine production and promote MMP release,[13, 15, 21, 22] these cytokines would promote damage. We expected to observe decreased epithelial erosion and oviduct hydrosalpinx in IL-22 and IL-23p19

deficient mice. However, we determined that similar degrees of oviduct pathology developed in both the presence and absence of IL-17,[2] IL-22, and IL-23, which indicates that if these cytokines promote pathologic responses, their role is redundant with other cytokines. On the other hand, IL-22 has been demonstrated to enhance epithelial regeneration after an inflammatory insult.[13, 17, 42, 45, 52, 71] Despite known regenerative effects of this cytokine, we did not observe any difference in the degree of epithelial erosion in the oviducts of IL-22-deficient and wild-type mice. When fully virulent *C. muridarum* are used for infection, enhanced early control of infection and prevention of ascension of *Chlamydia* to the oviduct may be required to prevent oviduct damage.[57, 72] This does not preclude the potential for IL-22 to play a protective and regenerative role in the human genital tract, where *Chlamydia trachomatis* infection is frequently more indolent and chronic in nature.[73]

We previously demonstrated that during *C. muridarum* genital infection, IFNγ-deficient mice exhibited significantly increased bacterial burden, enhanced production of Th17-differentiating cytokines, predominant Th17 and neutrophilic responses, an increased IL-22 response, and enhanced genital tract tissue damage.[2] *Chlamydia trachomatis* has been shown to induce IL-23 by a combination of toll-like receptor stimulation and endoplasmic reticulum stress signals,[74] both of which would be augmented in the presence of a suboptimal Th1 response and increased bacterial burden. $CD4^+$ T cells isolated from the cervix of women actively infected with *C. trachomatis* have been observed to produce IL-17 and IL-22.[31] These cells may play dual roles due to the complex interactions of these cytokines in vivo. [13, 42] However, data in the mouse model indicate a primary role for Th1 cells in host defense and resolution of infection, and our data in mice deficient for the IL-17 receptor,[2] or for Th17 cells and their downstream cytokines reveal that this pathway is dispensable for inducing a robust Th1 response and for resolution of genital tract infection and does not contribute substantially to protection from tissue damage. Given the fragile nature of the female oviduct, and the documented complex and often tissue injurious roles for IL-17 and IL-22, we propose that chlamydial vaccine strategies should avoid induction of these cytokines and focus on selective enhancement of the IFNγ response to chlamydial antigens.

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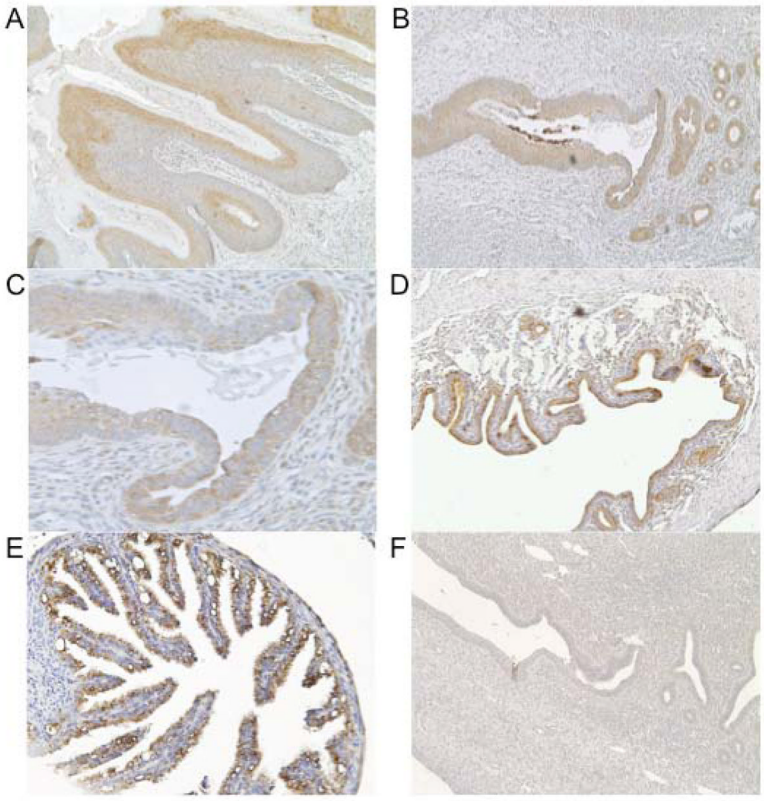

FIG. 1. IL-22 receptor expression was detected in the murine genital tract

Genital tracts from uninfected C57BL/6 mice were stained with anti-IL-22R1. Ectocervix (A; magnification, x100), Squamocolumnar junction (B; magnification, x100), Squamocolumnar junction (C; magnification, x200), Uterine horn (D; magnification, x100), Oviduct (E; magnification, x200), negative control (Rat IgG2a) uterine horn (F; magnification, x200).

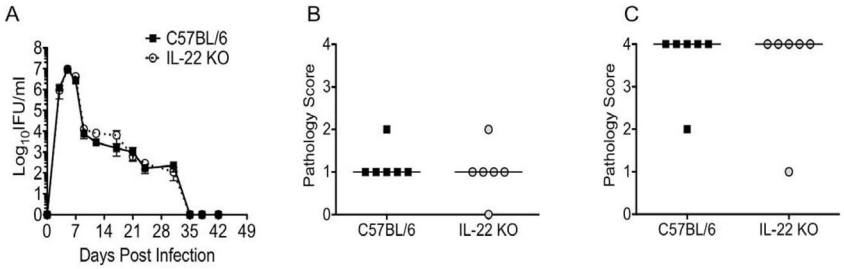

Fig. 2. Resolution of *C. muridarum* infection and the development of oviduct pathology are not influenced by the absence of IL-22

(A) The kinetics of lower genital tract infection for C57BL/6 (black squares) and IL-22 KO mice (open circles, dashed line) does not differ ($P > 0.05$ via two-way repeated measures ANOVA). Data points represent the mean ± SEM of IFU values from 6 mice per strain from a single representative experiment of two. (B,C) Histological analysis of oviduct epithelial cell erosion (B) and oviduct dilatation (C) in genital tracts harvested on day 42 post-infection revealed no difference between the strains. ($P > 0.05$ via Mann-Whitney U-test). Data points represent semi-quantitative scoring of oviduct pathology for individual mice with 6 mice per strain from a single experiment of two. C57BL/6 (black squares) and IL-22 KO mice (open circles). Median indicated by horizontal line.

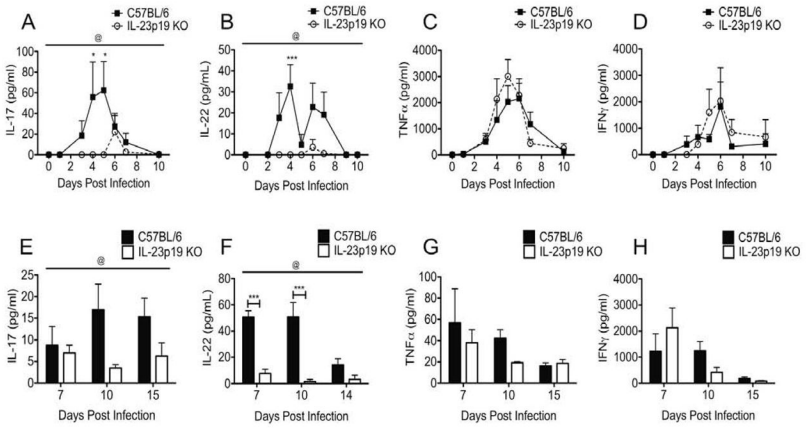

FIG. 3. IL-23 induces production of IL-17 and IL-22 but not TNFα or IFNγ in the genital tract during *C. muridarum* infection

(A–D) Levels of IL-17 (A) and IL-22 (B) in the vaginal lavages of IL-23p19 KO mice (clear circles, dashed line) were significantly reduced over the course of infection compared to C57BL/6 mice (black squares), but no difference was detected for TNFα (C) or IFNγ (D). Data points represent the mean ± SEM for 4–5 mice per strain from one representative experiment of two. @, $P < 0.05$ for IL-17 and IL-22 (by two-way ANOVA over the interval measured). *, $P < 0.05$; ***, $P < 0.001$ on individual days (by two-way ANOVA with Bonferroni post-test analysis). (E–H) Measurement of cytokines in the homogenized oviducts of infected mice revealed significantly reduced levels of IL-17 (E) and IL-22 (F) over the course of infection in the absence of IL-23 but no difference in TNFα (G) and IFNγ (H). Data points represent the mean ± SEM for 3–5 mice strain per day. @, $P < 0.05$ for IL-17 and IL-22 (by two-way ANOVA over the interval measured). ***, $P < 0.001$ for IL-22 on individual days by (two-way ANOVA with Bonferroni post-test analysis).

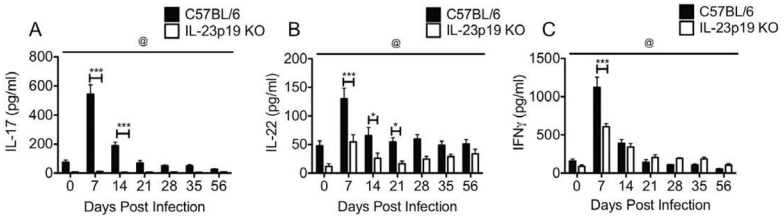

Fig. 4. In the absence of IL-23, *Chlamydia*-specific cytokine production is reduced in the iliac lymph nodes

(A–C) Significantly reduced levels of IL-17 (A), IL-22 (B), and IFNγ (C) were measured in the supernatants of Iliac lymph node mononuclear cells from IL-23p19 KO (white bars) mice restimulated in vitro for 96 hours in the presence of UV-EBs relative to those from C57BL/6 mice (black bars). Bars represent the mean ± SEM for 5 mice/strain. @, $P < 0.0001$ for IL-17 and IL-22 and @, $P < 0.05$ for IFNγ (by two-way ANOVA over the interval measured). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ on individual days (by two-way ANOVA with Bonferroni post-test analysis).

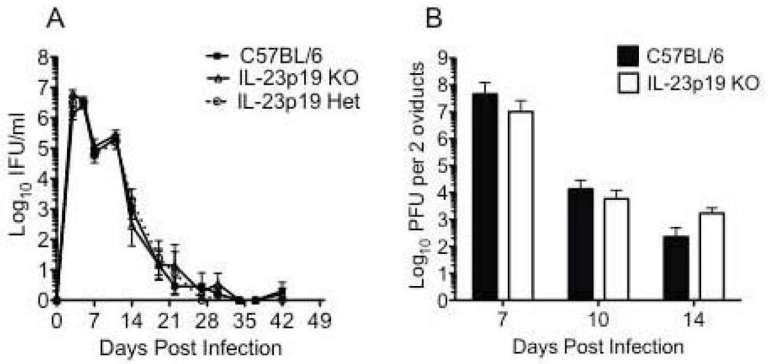

Fig. 5. *C. muridarum* infection resolves with normal kinetics in the absence of IL-23

(A) The kinetics of lower genital tract infection for C57BL/6 (black squares), IL-23p19 KO (open triangles), and IL-23p19 heterozygous mice (open circles) does not differ. Data points represent the mean ± SEM of IFU values from 4–5 mice per strain from a single experiment of three. (B) Analysis of bacterial burden in the oviducts revealed no difference between C57BL/6 (black bars) and IL-23p19 KO mice (white bars). Bars represent the mean ± SEM of PFU for two pooled oviducts of individual mice with 3–4 mice per strain per day.

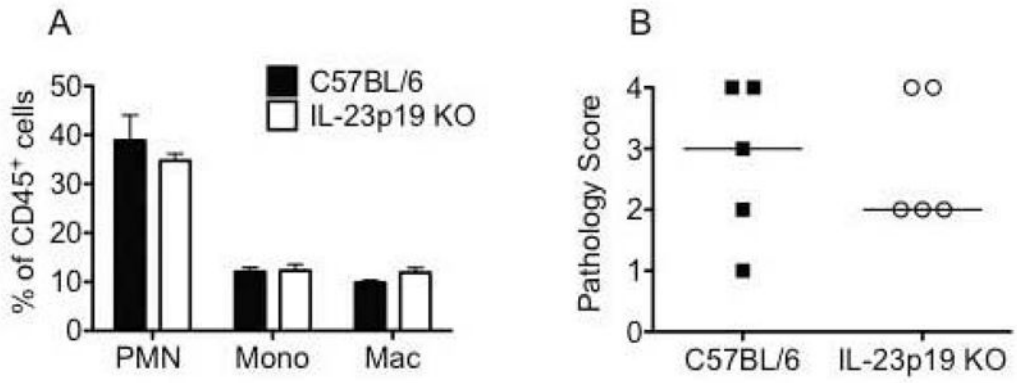

Fig. 6. IL-23 is not required for influx of acute inflammatory cells into the oviducts or the development of oviduct pathology

(A) On day 10 post-infection, flow cytometric analysis revealed no difference in the frequency of innate inflammatory cells in the oviducts of C57BL/6 (black bars) and IL-23p19 KO mice (white bars). Bars represent the mean ± SEM of the frequency of $CD45^{+}$ cells in the oviducts of 4 mice per strain for one representative experiment of two. PMN: $Ly6G/C^{high}$ $F4/80^{neg}CD11c^{neg}$; Mono (inflammatory monocytes): $Ly6G^{med}$ $F4/80^{neg}CD11c^{neg}$; Mac (macrophages): $F4/80^{pos}$ (B) Histological analysis of oviduct dilatation in genital tracts harvested on day 42 post-infection revealed no difference between the strains ($P > 0.05$ via Mann-Whitney U-test). Data points represent semi-quantitative scoring of oviduct dilatation of individual mice for 5 mice per strain from one representative experiment of two. C57BL/6 (black squares) and IL-23p19 KO mice (open circles). Median indicated by horizontal line.