Critical Role of the Interleukin-17/Interleukin-17 Receptor Axis in Regulating Host Susceptibility to Respiratory Infection with *Chlamydia* Species[▽]

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The specific contribution of interleukin-17/interleukin-17 receptor (IL-17/IL-17R)-mediated responses in regulating host susceptibility against obligatory intracellular *Chlamydia* infection was investigated in C57BL/6 and C3H/HeN mice during *Chlamydia muridarum* respiratory infection. We demonstrated that *Chlamydia* stimulated IL-17/IL-17R-associated responses in both *Chlamydia*-resistant C57BL/6 and *Chlamydia*-susceptible C3H/HeN mice. However, C3H/HeN mice developed a significantly greater IL-17/IL-17R-associated response than C57BL/6 mice did. This was reflected by an increase in IL-17 mRNA expression, a higher recall IL-17 production from splenocytes upon antigen restimulation, and higher production of Th17-related cytokines (IL-23 and IL-6) and chemokines (chemokine [C-X-C motif] ligand 2 [CXCL1]/keratinocyte-derived chemokine [KC] and CXCL2/macrophage inflammatory protein 1 [MIP2]) in C3H/HeN mice than in C57BL/6 mice. Furthermore, C3H/HeN mice displayed a massive accumulation of activated and preactivated neutrophils in the airway and lung parenchyma compared to their C57BL/6 counterparts. We further demonstrated that the skewed IL-17/Th17 profile in C3H/HeN mice was predisposed by a higher basal level of IL-17 receptor C (IL-17RC) expression and then further amplified by a higher inducible IL-17RA expression in lungs. Most importantly, in vivo delivery of IL-17RA antagonist that resulted in a 50% reduction in the neutrophilic infiltration in lungs was able to reverse the susceptible phenotype of C3H/HeN mice to respiratory *Chlamydia* infection. Thus, our data for the first time have demonstrated a critical role for the IL-17/IL-17R axis in regulating host susceptibility to *Chlamydia* infection in mice.

Chlamydia trachomatis is an obligate intracellular gram-negative bacterium that primarily infects epithelial cells lining the ocular, respiratory, and urogenital tract surfaces and causes many human diseases including trachoma, pneumonia, and pelvic inflammatory disease (2). Although effective antibiotics are available, the incidence of *C. trachomatis* infections continues to increase worldwide (41). In the United States alone, it is estimated that there are approximately 2.8 million new cases of urogenital *C. trachomatis* infection each year (58). An effective and safe *Chlamydia* vaccine is needed to address the global *C. trachomatis* epidemic, and a comprehensive understanding of the means of protective immunity and immunopathology of *C. trachomatis* infection is essential for vaccine development.

C. trachomatis infection results in a wide variety of clinical manifestations, ranging from asymptomatic to mild or severe symptoms, acute or chronic inflammatory responses, and a wide range of chronic complications (5, 47, 59). Host genetic factors appear to be important in determining the outcome of *Chlamydia* infections. It has been reported that the increased

incidence of *Chlamydia*-induced chronic diseases, such as tubal infertility and scarring trachoma, is correlated with certain human leukocyte antigen (HLA) haplotypes and polymorphism of genes encoding interleukin-10 (IL-10), CD14, and tumor necrosis factor alpha (6, 7, 16, 25, 44, 54). However, how these specific genes are involved in shaping the specific immune responses during *Chlamydia* infection in humans remains unclear. As in humans, inbred mouse strains, such as C57BL/6 (*H-2^b*), BALB/c (*H-2^d*), C3H/HeN (*H-2^k*), and DBA/2J (*H-2^d*) mice respond to respiratory (1, 39, 40, 63), genital (9–12, 52), and intraperitoneal (i.p.) (36) *Chlamydia* infections differently. C57BL/6 mice are regarded as a resistant strain, whereas BALB/c, DBA/2, and C3H/HeN mice are reported as susceptible strains with higher mortality, more prolonged bacterial burden, more severe tissue inflammatory responses (such as neutrophil infiltration), and higher rates of infertility following *Chlamydia* infection. Thus, these inbred mouse strains have been used extensively for identification of specific host factors that regulate immune responses and immune mechanisms underlying the pathogenesis of *Chlamydia* infection.

Based on animal models (5, 8, 38, 50) and human studies (20, 21), T-cell-mediated immunity is essential in host defense against *Chlamydia* infection. Anti-*Chlamydia* immunity requires induction of potent Th1 cellular immunity that is characterized by production of IL-12 and gamma interferon (IFN- γ) (5, 37, 61). Mice deficient in IL-12 (17), IFN- γ (17,

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23), or IFN- γ receptors (23, 24), or mice treated with anti-IL-12 or anti-IFN- γ antibody all demonstrated a marked inability to control *Chlamydia* infection, highlighting an unequivocal role of Th1 cellular immune response in host defense against *Chlamydia* infection. In contrast, skewed induction of Th2 immunity or a high level of IL-10 production increases susceptibility to *Chlamydia* infection in BALB/c mice by inhibiting IFN- γ production (9, 62, 63). While the theory of Th1/Th2 immune regulation has significantly advanced our understanding about anti-*Chlamydia* immunity, it does not appear to explain all genetically determined susceptibilities to *Chlamydia* infection. Susceptible C3H/HeN mice are able to mount comparable, if not higher, levels of Th1 immunity, or a similar ratio of Th1/Th2 responses as seen in resistant C57BL/6 mice (9, 39, 40). Thus, it is likely that other immune mechanisms are also involved in regulating differential susceptibilities to *Chlamydia* infection in different hosts.

In the past few years, significant advances have been made in our understanding of T-cell immunity with the discovery of a unique $\alpha\beta^+$ CD4 $^+$ T helper lineage termed Th17 cells (13, 19) that differ from the classic Th1 and Th2 lineages. Th17 cells produce novel proinflammatory cytokines including IL-17 (also called IL-17A) and IL-17F—two members of the IL-17 cytokine family, which has a total of six family members (IL-17A to IL-17F) and five receptors (IL-17 receptor A [IL-17RA] to IL-17RE) (27). In mice, Th17 lineage differentiation requires transforming growth factor beta and IL-6 for initiation and IL-23 for further expanding and becoming an established population (3, 30, 31, 35, 53). Although IL-17-producing CD4 $^+$ T cells represent one lineage of adaptive immunity, the IL-17/Th17 response functions as a classic innate immune component. Specifically, IL-17 and IL-17F interact with IL-17R, consisting of IL-17RA and IL-17RC subunits, to induce production of other proinflammatory cytokines (e.g., IL-1 and tumor necrosis factor), chemokines (e.g., chemokine [C-X-C motif] ligand 1 [CXCL1], CXCL2, and chemokine [C-C motif] ligand 2 [CCL2]) and growth factors (e.g., IL-6, granulocyte colony-stimulating factor, and granulocyte-macrophage colony-stimulating factor) from tissue structural cells including fibroblasts and epithelial and endothelial cells. As a result, IL-17/Th17 response leads to an accumulation of neutrophils at the sites of infection and inflammation (27, 28). In addition to Th17 cells, $\gamma\delta^+$ T cells and neutrophils are also capable of producing IL-17 in response to infectious agents including *Mycobacterium tuberculosis* (34), *Escherichia coli* (49), *Aspergillus fumigatus* (43), and *Listeria monocytogenes* (18). While a growing body of evidence indicates that IL-17-mediated neutrophilic response plays a critical role in host defense against extracellular bacteria (65, 66), IL-17-mediated responses are also responsible for severe tissue damage in other infection models (15, 45, 46), due largely to their potent effect on neutrophils. To date, however, the specific contribution of the IL-17/Th17 response in host susceptibility to *Chlamydia* infection remains unclear.

In the present study, we investigated the role of the IL-17/Th17 response in host resistance against respiratory infection caused by the mouse pneumonitis biovar of *Chlamydia muridarum* in resistant C57BL/6 and susceptible C3H/HeN mice. The objectives of our study were (i) to characterize the IL-17/Th17 response in conjunction with well-characterized Th1 im-

munity, clinical evaluation, and bacterial clearance during *C. muridarum* infection; (ii) to investigate whether the IL-17/Th17 immune profile had any correlation with *Chlamydia* resistance and/or *Chlamydia* susceptibility, and (iii) if this does occur, to determine the mechanism(s) by which the IL-17/Th17 response modifies host immune responses to *C. muridarum* infection.

MATERIALS AND METHODS

Mice. Six- to 8-week-old female C57BL/6 and C3H/HeN mice were purchased from Charles River Laboratory (Senneville, Quebec, Canada) and maintained under specific-pathogen-free conditions at the InVivo Laboratory of the IWK Health Centre. All animal procedures were approved by the University Committee on Laboratory Animal in accordance with the guidelines of the Canadian Council on Animal Care.

***Chlamydia* strain.** The mouse pneumonitis biovar, *C. muridarum*, was originally obtained from Xi Yang (University of Manitoba, Winnipeg, Manitoba, Canada) and propagated in McCoy cells (ATCC, Manassas, VA) according to procedures described previously (63, 32). Briefly, McCoy cells were infected for 48 to 72 h and harvested with sterile glass beads. The cell suspensions were subjected to brief sonication on ice, and cellular debris was removed by centrifugation at $500 \times g$ for 10 min. The supernatants were collected and spun at $30,000 \times g$ for 30 min to pellet the organism. The elementary bodies (EBs) were then further purified by discontinuous density gradient centrifugation using 30% Isovue-370 (Bracco Diagnostics, Princeton, NJ) and 50% sucrose (Sigma, Oakville, Ontario, Canada) as described previously (32). Purified EB preparations were stored in sucrose-phosphate-glutamic acid (SPG) buffer in small aliquots and frozen at -80°C until needed. To determine the titer of purified EB stocks, McCoy cells grown in 96-well plates were inoculated with serial dilutions of the EB stock. The plates were centrifuged at $1,300 \times g$ for 30 min at 37°C to facilitate the infection. Each well was then washed with Hank's buffer, and 200 μl of minimal essential medium containing 10 $\mu\text{g}/\text{ml}$ gentamicin and 1 $\mu\text{g}/\text{ml}$ cycloheximide was added to each well and incubated for 72 h at 37°C in 5% CO_2 . After the incubation, the cell monolayer was fixed with 100% methanol, stained with a genus-specific rabbit anti-*Chlamydia* antibody (Biodesign International, Saco, ME) and horseradish peroxidase-conjugated anti-rabbit immunoglobulin G (IgG) secondary antibodies (Sigma, Oakville, Ontario, Canada) and developed in 1% 4-chloro-1-naphthol (Sigma, Oakville, Ontario, Canada) (63). The number of inclusion bodies was determined by counting them by looking for their brownish staining using a microscope. The titer of the EB stocks, expressed as the inclusion-forming units (IFU), was calculated according to the number of inclusion bodies of the inoculum and the dilution factor. Aliquots of purified *C. muridarum* were heat inactivated by incubating the organism at 65°C for 30 min and used as heat-killed crude *C. muridarum* antigen for in vitro lymphocyte restimulation assay.

Other reagents. RPMI 1640 medium was supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, 100 mg/ml streptomycin, and 2 mM of L-glutamine (Invitrogen, Oakville, Ontario, Canada). IL-17-containing supernatants were generated by transducing A549 cells with a replication-deficient adenoviral gene transfer vector expressing murine IL-17 (AdIL-17) (64). The concentration of murine IL-17 in the supernatants was determined by an enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, MN). A control virus Addl70-3 without the transgene was used to generate control supernatants as described previously (56, 57). All recombinant adenoviral vectors were amplified, purified, and titrated following protocols described previously (57).

In vivo model of pulmonary *Chlamydia* infection and sample collection. Purified *C. muridarum* EBs were used to establish an in vivo model of pulmonary *Chlamydia* infection following procedures described previously (56, 63). Briefly, *C. muridarum* stocks were diluted in SPG buffer and passed through a 30-gauge needle 10 times to ensure proper dispersion of the organisms. A dose of 2×10^3 IFU *C. muridarum* was diluted in 25 μl of SPG buffer and delivered intranasally into isoflurane-anesthetized mice with a fine pipette tip (55, 56). The mice were monitored daily for body weight changes with 25% loss of original body weight established as the end point. At selected days after infection, groups of mice were sacrificed by exsanguination, and various tissues, including lung and spleen tissues, were isolated and processed aseptically. Bronchoalveolar lavage (BAL) fluid was collected using 500 μl of phosphate-buffered saline (PBS) as described previously (55, 56). BAL fluids were centrifuged at $500 \times g$ for 10 min, and supernatants were stored at -80°C until cytokine/chemokine measurement. The

lung tissue samples were homogenized in 3 ml SPG buffer; half of the lung homogenates were centrifuged at $500 \times g$ at 4°C for 15 min, and the supernatants were collected for determination of the *Chlamydia* load in lungs by counting the number of IFU on McCoy cells or quantitative PCR as described below. The other half of the lung homogenates were centrifuged at $2,000 \times g$ at 4°C for 30 min, and the supernatants were stored at -80°C until cytokine and chemokine measurement. The pellets were combined and resuspended in 0.5% cetyltrimethylammonium chloride (CTAC) (Sigma, Oakville, Ontario, Canada) and stored at -80°C until myeloperoxidase (MPO) assays. Single-cell suspensions were prepared from spleens and restimulated with or without heat-inactivated *C. muridarum* antigen for 72 h at 37°C in 5% CO_2 . The culture supernatants were stored at -80°C until cytokine/chemokine measurement.

MPO assay. Lung MPO activity was quantified as described previously (48). Briefly, the lung homogenate pellets in 0.5% CTAC were thawed and heated for 2 h at 60°C and then centrifuged at $10,000 \times g$ for 10 min. The clear supernatants were collected and serially diluted to react with the substrate containing 3 mM tetramethylbenzidine dihydrochloride, 120 μM resorcinol, and 2.2 mM H_2O_2 (Sigma, Oakville, Ontario, Canada). The reaction was stopped by adding 50 μl of 2 M H_2SO_4 , and the absorbance was determined at an optical density at 450 nm. The recombinant human myeloperoxidase (VWR, Mississauga, Ontario, Canada) was used as a standard.

Quantitative PCR analysis to determine in vivo *C. muridarum* burden. To quantify the bacterial burden in the lungs of *C. muridarum*-infected mice, total nucleic acid from 100 μl of each lung homogenate sample was extracted using DNAzol (Invitrogen, Oakville, Ontario, Canada) according to the manufacturer's instructions. The level of bacterial burden in each sample was determined by quantitative PCR with *Chlamydia*-specific primers for 16S rRNA using SYBR green supermix (Invitrogen, Oakville, Ontario, Canada) in a 7900H fast real-time PCR machine (Applied Bioscience, Foster City, CA). The sense primer was 5'-CGCCTGAGGAGTACACTCGC-3', and the antisense primer was 5'-CCAACACCTCACGGCAGGAG-3'. The *Chlamydia* load in each mouse lung was calculated using known copy numbers of *C. muridarum* DNA standards that were extracted from a purified *C. muridarum* preparation using the same procedure and expressed as $\log_{10}$ value of copies of 16S rRNA per ml. This assay is highly consistent with the traditional IFU assay but more sensitive at detecting low antigen loads (60).

RNA isolation for reverse transcription-PCR (RT-PCR) and quantitative RT-PCR. In separate experiments, total RNA was isolated from fresh or frozen lung tissues derived from naïve or *C. muridarum*-infected C3H/HeN and C57BL/6 mice using TRIzol reagent (Invitrogen, Oakville, Ontario, Canada) according to the manufacturer's protocol. Four micrograms of total RNA was then treated with DNase I (Invitrogen, Oakville, Ontario, Canada) to remove trace amounts of genomic DNA contamination. The first strand of cDNA was synthesized using SuperScript III reverse transcriptase and oligo(dT)₂₀ primer (Invitrogen, Oakville, Ontario, Canada). The expression of IL-17 (IL-17A), IL-17F, IL-17RA, and IL-17RC before and during the course of *C. muridarum* infection was quantified using SYBR green supermix (Invitrogen, Oakville, Ontario, Canada) in a 7900H fast real-time PCR machine. The expression levels of the target gene were normalized to the reference gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression level using a standard curve with known copy numbers of plasmid DNA encoding GAPDH. The primer pairs for real-time PCR were as follows: for IL-17, the forward primer was 5'-GCTCCAGAAGGCCCTCAGACT-3' and the reverse primer was 5'-CCAGCTTCCCTCCGATTTGA-3'; for IL-17F, the forward primer was 5'-CTGGAGGATAACACTGTGAGAGT-3' and the reverse primer was 5'-TGCTGAATGGCGACGGAGTTTC-3'; for IL-17RA, the forward primer was 5'-GTGGCGGTTTTCCTTCAGCCACTTTGTG-3' and the reverse primer was 5'-GATGCTGTGTGTCAAGGTCTCCACAGT-3'. All primers except IL-17RC were synthesized by Integrated DNA Technologies, Inc. (Montreal, Quebec, Canada). IL-17RC primer pair was purchased from SA Bioscience Co. (Frederick, MD).

Primary lung fibroblast stimulation assay. Primary lung fibroblasts were isolated from C3H/HeN and C57BL/6 mice according to procedures described previously (26). Briefly, the lungs were removed aseptically from naïve mice, cut into small pieces, and seeded into 100-mm tissue culture dishes with RPMI 1640 medium containing 20% FBS. The lung pieces were removed after 3 to 5 days after the fibroblasts were attached to the dish. Fibroblasts were then passaged as needed and used at passage 3 to 5. On the day before the assay, fibroblasts were seeded into 96-well flat-bottom tissue culture plates at a density of 1×10^5 cells/well overnight. The cells were then stimulated with different concentrations of IL-17-containing A549 cell supernatants or control A549 cell supernatants for 24 h. The culture supernatants were collected and stored at -80°C until cytokine and chemokine measurement. The data were normalized by subtracting the

background level induced by control A549 cell supernatant stimulation for each condition.

In vivo gene transfer of IL-17R antagonist AdIL-17R:Fc. We previously demonstrated that a recombinant adenovirus expressing a fusion protein consisting of the murine IL-17RA extracellular domain with the murine IgG CH2 and CH3 domains (AdIL-17R:Fc) was able to block IL-17 receptor A signaling (65). Groups of C3H/HeN mice were i.p. injected with 1×10^9 PFU of AdIL-17R:Fc or empty control viral vector Add1170-3 or with PBS alone 24 h after *C. muridarum* infection. Mice were monitored daily for body weight changes and sacrificed at 12 days after *C. muridarum* infection. All samples were collected and processed as described above.

Fluorescence-activated cell sorting (FACS) staining and ICCS. To phenotype infiltrated cellular components in lung parenchyma, the lungs were removed from mice aseptically on days 4, 6, and 12 postinfection and total lung-derived cells were prepared following collagenase digestion as described previously (55). The cell pellets derived from BAL fluid were used to phenotype cellular components in the airway. Approximately 10^6 cells were first blocked by an anti-mouse CD16/CD32 antibody (clone 2.4G2) for 15 min and then labeled with monoclonal antibodies in a combination of fluorescein isothiocyanate (FITC)-labeled CD11b (FITC-CD11b) (clone M1/70), PerCPy5.5-labeled CD11c (PerCPy5.5-CD11c) (clone N418), Alexa Fluor 647-labeled bone marrow stroma cell antigen 2 (BST2) (also called pDCA-1) (Alexa Fluor 647-labeled BST2) (clone eBio927), and a phycoerythrin (PE)-labeled antibody, such as PE-Gr1 (clone RB6-8C5) or PE-B220 (clone RA3-6B2) or isotype control PE-rat IgG2a (all purchased from eBioscience, San Diego, CA). For standard intracellular cytokine staining (ICCS), splenocytes were isolated from *C. muridarum*-infected C57BL/6 and C3H/HeN mice on day 12 postinfection and restimulated with or without heat-inactivated *C. muridarum* antigen for 16 h and then treated with GolgiPlug (BD Pharmingen, San Diego, CA) for an additional 6 h. The cells were washed with PBS containing 1% FBS, and ICCS was conducted by using a Cytofix/Cytoperm kit (BD Pharmingen, San Diego, CA) according to the manufacturer's instructions as described previously (55). The cells were first surface labeled with PerCPy5.5-CD3 (clone 145-2C11), allophycocyanin-labeled T-cell receptors $\alpha\beta$ (TCR $\alpha\beta$) (clone H57-597), and FITC-CD4 (clone RM4-5) (all purchased from eBioscience), then fixed, permeabilized, and intracellularly labeled with PE-IL-17A (clone TC11-18H10) or PE-rat IgG1 (purchased from BD Pharmingen). For direct ICCS, naïve or *C. muridarum*-infected mice were treated with 500 μl of 0.5 mg/ml of brefeldin A i.p. and sacrificed 6 h later. Splenocytes were quickly processed, surface stained with FITC-TCR $\delta\gamma$ (clone GL3), then fixed, permeabilized, and intracellularly labeled with PE-IL-17A and Alexa Fluor 647-IFN- γ (clone XMG1.2) (33). A total of 100,000 to 300,000 events were collected on a FACScaliber (BD Biosciences, Sunnyvale, CA), and the data were analyzed using WinMDI 2.8 software (Scripps Institute, La Jolla, CA).

Cytokine ELISA. The cytokine contents in BAL fluids and culture supernatants were determined by using antibody pairs specific for mouse IFN- γ , IL-12, IL-17, IL-4, IL-6, IL-13, CXCL1/keratinocyte-derived chemokine (KC), and CXCL2/macrophage inflammatory protein 2 (MIP2) (all purchased from R&D Systems) and IL-23 (purchased from eBioscience).

Statistical analysis. In most cases, the differences between two mouse strains were analyzed using an unpaired, two-tailed Student *t* test. The changes in body weight and the mortality rate over the course of infection in C3H/HeN and C57BL/6 mice or in mice treated with different adenoviral vectors were analyzed using two-way analysis of variance (ANOVA) test or log rank survival test using GraphPad Prism software when applicable. In all cases, a *P* value of <0.05 was considered statistically significant.

RESULTS

Characterization of respiratory *C. muridarum* infection in C3H/HeN and C57BL/6 mice. The *C. muridarum* respiratory infection model was established in C3H/HeN and C57BL/6 mice following intranasal delivery of *C. muridarum* (2,000 IFU/mouse). Both C57BL/6 and C3H/HeN mice remained relatively healthy until day 6 postinfection. C57BL/6 mice showed marginal body weight loss starting on day 7 postinfection (Fig. 1A). Only 7% of C57BL/6 mice reached the end point after this dose of infection (Fig. 1B). In contrast, C3H/HeN mice had significantly more body weight loss than that observed in C57BL/6 mice starting on day 8 postinfection onwards. Consequently, 30 to 46% of C3H/HeN mice reached the end point

FIG. 1. Differential severity of clinical manifestations and mortality rates in C3H/HeN and C57BL/6 mice. Groups of C3H/HeN and C57BL/6 mice were infected with 2,000 IFU of *C. muridarum* intranasally, and their body weight was monitored every day. The mice were sacrificed at the scheduled time points or the end point, which was determined when the mouse lost $\geq 25\%$ of original body weight. (A) The body weight changes were calculated and expressed as percent change from the original body weight. The data are presented as the means minus standard errors of the means (SEMs) (error bars) of pooled three independent experiments with a total of 24 mice per strain for days 0 to 6 (D0 to D6, respectively) and 13 mice per strain for days 7 to 12. The difference between C3H/HeN and C57BL/6 mice in body weight change was analyzed using two-way ANOVA test. Values that were significantly different are indicated as follows: **, $P < 0.01$; ***, $P < 0.001$. (B) The survival rate in each mouse strain over the course of 20 days of infection was monitored, calculated, and expressed as a percentage of a total number of mice (13 mice). The difference between the survival rates of C3H/HeN and C57BL/6 mice was analyzed by using the log rank test and GraphPad Prism software. Values that were significantly different ($P = 0.02$) are indicated by the bracket and asterisk. (C) Infected C3H/HeN and C57BL/6 mice were sacrificed on days 2, 4, 6, 12, and 21 postinfection, and the bacterial burden in each mouse lung was determined based on the copy number of 16S rRNA by quantitative PCR. The data are expressed as means plus SEMs (error bars) for four to nine mice per time point per mouse strain. Values that were significantly different ($P < 0.01$) for the two strains by unpaired, two-tailed Student *t* test are indicated by the short black line and two asterisks. (D) The bacterial burden in mice that reached the end point (with $\geq 25\%$ body weight loss) [C3H/HeN (D)] and the ones that survived (with $< 25\%$ body weight loss) [C3H/HeN (S)] and C57BL/6 mice on day 12 postinfection is plotted. Each symbol shows the value for an individual mouse. The line represents the mean value for each group of mice.

starting day 11 onwards after infection (Fig. 1B), consistent with previous reports (39, 40). We further examined the kinetics and bacterial load in the lungs of these two strains of mice on days 2, 4, 6, 12, and 21 (Fig. 1C). While we observed a statistically significant higher bacterial burden in C3H/HeN mice on days 4 and 6 postinfection than that in C57BL/6 mice, the overall bacterial growth curve was similar in the two mouse strains; bacterial loads increased dramatically as early as day 2 postinfection and reached a plateau around day 6 to 12 postinfection, decreasing by day 21 (Fig. 1C). It is of note that although C3H/HeN mice had reached peak bacterial burden in lungs by day 6, they did not reach the end point until day 11. Furthermore, we found that mice reaching the end point had comparable bacterial loads in lungs with the mice that survived the infection (Fig. 1D), suggesting that the bacterial load per se was not the ultimate cause or the only cause of the severe clinical illness and higher mortality rate observed in C3H/HeN mice.

Differential Th17, but not Th1, response in *Chlamydia*-susceptible and *Chlamydia*-resistant mice during respiratory *Chlamydia* infection. To understand whether IL-17/Th17-mediated response is involved in regulating differential host re-

sponses to *Chlamydia* infection, we characterized IL-17/Th17 response in conjunction with well-characterized protective Th1 immunity in lungs and lymphoid organs of C3H/HeN and C57BL/6 mice at multiple time points postinfection. Quantitative RT-PCR was first used to examine the kinetics of IL-17 and IL-17F mRNA expression in lungs after *C. muridarum* infection. We found that C3H/HeN mice had higher IL-17 mRNA expression in lungs than the C57BL/6 mice did at multiple time points and that this difference reached statistical significance on day 6 (Fig. 2A). In contrast, IL-17F expression was comparable in both strains of mice throughout the entire course of infection (Fig. 2B). Therefore, we focused on IL-17 rather than IL-17F in our study. Subsequently, the levels of IL-17 and IFN- γ in BAL fluids were measured by ELISAs. To our surprise, we did not detect any difference in IL-17 levels from C3H/HeN and C57BL/6 mice on days 0, 2, 4, 6, and 12 postinfection (Fig. 2C). Similarly, IFN- γ production in the BAL fluids from the two strains was also comparable (Fig. 2D). However, it was observed that larger amounts of IL-17 were produced from splenocytes upon in vitro restimulation with heat-inactivated *C. muridarum* antigen in C3H/HeN mice on

FIG. 2. Skewed IL-17/IFN- γ responses in C3H/HeN mice following respiratory *C. muridarum* infection. (A and B) C3H/HeN and C57BL/6 mice were sacrificed on days 2, 4, 6, and 12 (D2, D4, D6, and D12, respectively) after *C. muridarum* infection. Total RNA samples were isolated from mouse lungs at various time points. The expression of IL-17 mRNA (A) and IL-17F mRNA (B) was quantified by quantitative RT-PCR from two to five mice per time point per mouse strain and normalized against expression of the housekeeping gene GAPDH (Rel, relative). The data (number of copies per 10^4 copies of the GAPDH gene) are expressed as the mean value of each time point per strain. Values that were significantly different ($P < 0.05$) for the two strains by the Student *t* test are indicated (*). (C and D) At each sacrifice, BAL fluid was collected as described in Materials and Methods. The levels of IL-17 (C) and IFN- γ (D) in BAL fluid from each mouse were measured by ELISAs. The results are pooled from two experiments and expressed as means plus standard errors of the means (SEMs) (error bars) for 8 to 10 mice per time point per mouse strain. (E and F) Single-cell suspensions were prepared from the spleens of C3H/HeN and C57BL/6 mice on days 4, 6, and 12 postinfection and restimulated with heat-inactivated *C. muridarum* antigen (CmAg) or medium alone for 72 h. The levels of IFN- γ (E) and IL-17 (F) in culture supernatants were measured by ELISAs. The data are expressed as the mean plus SEMs (error bars) for three mice for each mouse strain. A similar trend was observed in at least one additional set of experiments. Values that were significantly different ($P < 0.05$) for the two strains are indicated by the short black line and one asterisk. (G) Direct ICCS in naive and infected mice. Two naive and two *C. muridarum*-infected C57BL/6 mice were injected with 500 μ l of 0.5 mg/ml of brefeldin A i.p. and sacrificed 6 h later. The splenocytes were isolated and used for direct ICCS as described in Materials and Methods. IFN- γ - and IL-17-positive cells were first gated and analyzed for their $\gamma\delta$ -TCR expression. The relative percentages of $\gamma\delta^+$ and $\gamma\delta^-$ of IFN- γ -expressing or IL-17-expressing cells are indicated in each panel. p.i., postinfection; SSC, side scatter. (H) Standard ICCS in *C. muridarum*-infected C3H/HeN and C57BL/6 mice on day 12 postinfection. Splenocytes were isolated, restimulated with heat-inactivated *C. muridarum* antigen or control buffer for 16 h, and then cultured in the presence of GolgiPlug for an additional 6 h. IL-17 production was examined by intracellular cytokine staining. The analysis was done by first gating on CD3 $^+$ TCR $\alpha\beta^+$ cells. The percentage of IL-17-producing CD4 $^+$ TCR β^+ and CD4 $^-$ TCR β^+ cells in the spleen of each mouse strain is indicated in each panel.

FIG. 3. Differential Th17-associated cytokine/chemokine responses induced in C3H/HeN and C57BL/6 mice following respiratory MoPn infection. C3H/HeN and C57BL/6 mice were sacrificed on days 6 and 12 after MoPn infection, and BAL fluid samples were collected as described in Materials and Methods. The amounts of IL-23 (A), IL-6 (B), CXCL1/KC (C), and CXCL2/MIP2 (D) in the BAL fluid samples were measured by ELISAs. The data are pooled from two independent experiments that showed similar trends and expressed as the means plus standard errors of the means (error bars) for 10 mice per time point per mouse strain. Values that were significantly different for the two strains by the unpaired, two-tailed Student *t* test are indicated by short lines and asterisks as follows: *, *P* < 0.05; **, *P* < 0.01.

days 4, 6, and 12, while the level of IFN- γ production from the same culture remained similar for the two mouse strains (Fig. 2E and F). This trend, as expressed by the ratio of IL-17/IFN- γ , was consistent in all experiments carried out at different time points postinfection (data not shown). To identify whether $\gamma\delta^+$ T cells are the major cellular source of IL-17 production as observed in many other infection models (18, 34, 43, 49), direct intracellular cytokine staining was carried out with splenocytes isolated from naïve or *C. muridarum*-infected mice for 12 days (33). As demonstrated in Fig. 2G, *C. muridarum* infection resulted in 17.78% and 3.47% of splenocytes producing IFN- γ and IL-17, respectively, compared to 2.65% basal level of IFN- γ -producing cells and 2.05% basal level of IL-17-producing splenocytes observed in naïve mice. While the majority of IFN- γ -producing cells were found to be non- $\gamma\delta^+$ T cells (78% in naïve mice and nearly 92% in *C. muridarum*-infected mice), approximately 50% of IL-17-producing cells in naïve mice were $\delta\gamma^+$ T cells. However, only 23% of IL-17-producing cells in *C. muridarum*-infected mice were $\delta\gamma^+$ T cells, suggesting that *C. muridarum* infection stimulated more non- $\gamma\delta^+$ T cells producing IL-17. Further analysis using standard intracellular cytokine staining indicated that there were more *Chlamydia*-specific Th17 cells (CD4 $^+$ TCR β^+) and CD4 $^-$ TCR β^+ cells in the spleens of *C. muridarum*-infected C3H/HeN mice than in the spleens of *C. muridarum*-infected C57BL/6 mice (Fig. 2H). Taken together, our results demonstrate a differential induction of IL-17/Th17, but not Th1, responses in C3H/HeN and C57BL/6 mice, which is vigorous in the former and moderate in the latter, during respiratory *C. muridarum* infection. Further supporting this skewed IL-17/Th17 profile in C3H/HeN

mice, we also observed significant differences in the production of Th17-related cytokines and chemokines including IL-23, IL-6, CXCL1/KC, and CXCL2/MIP2 in the BAL fluids of C3H/HeN and C57BL/6 mice on day 6, which remained high in C3H/HeN mice on day 12 postinfection with the exception of IL-23 (Fig. 3A to D).

Differential IL-17RA and IL-17RC expression in naïve and MoPn-infected C57BL/6 and C3H/HeN mice. Since we did not detect any difference in the level of IL-17 in the BAL fluid in the two strains of mice by ELISA whereas a greater IL-17/Th17 response in C3H/HeN mice was evident, we hypothesized that C3H/HeN mice might have a higher level of IL-17 receptor expression, which would bind more IL-17 at the tissue site and lead to low measurement of unbound IL-17 by ELISA. To test this hypothesis, we examined the level of both subunits of IL-17 receptor, IL-17 receptor A and IL-17 receptor C, in the lungs of C57BL/6 and C3H/HeN mice before and after *C. muridarum* infection by quantitative RT-PCR. Interestingly, while IL-17RA was expressed at similar levels in naïve C57BL/6 mice and C3H/HeN mice (Fig. 4A), IL-17RC was found to be expressed at a significantly higher level in naïve C3H/HeN mice than in C57BL/6 mice (approximately 1.7-fold higher) (Fig. 4A). A similar observation was also seen in primary lung fibroblasts isolated from naïve C57BL/6 and C3H/HeN mice and subcultured in vitro (Fig. 4A). To investigate whether a higher IL-17RC gene expression profile also correlates with greater biological function of the IL-17R complex, these fibroblasts were used to compare their responsiveness to IL-17 stimulation. Consistent with the IL-17RC gene expression profile, lung fibroblasts derived from C3H/HeN mice pro-

FIG. 4. Differential IL-17RC expression and differential IL-17 responsiveness of primary lung fibroblasts of naïve C3H/HeN and C57BL/6 mice. (A) Total RNA samples were prepared from the lungs or cultured primary fibroblasts for naïve C3H/HeN and C57BL/6, and the level of IL-17RA and IL-17RC expression was determined by quantitative RT-PCR and normalized against the housekeeping gene GAPDH (Rel, relative). Lung data are expressed as the means plus standard errors of the means (SEMs) (error bars) from five mice per strain. Fibroblast data were done in triplicate samples per strain. (B to D) Primary lung fibroblasts were isolated from C3H/HeN and C57BL/6 mice and stimulated with different concentrations of IL-17 or control medium for 24 h. The culture supernatants were used to measure the production of IL-6 (C), CXCL1/KC (B), and CXCL2/MIP2 (D). The data are representative of two independent experiments, and the data are expressed as the means plus SEMs (error bars) of triplicate wells for each condition. Values that were significantly different in the two strains by the unpaired, two-tailed Student *t* test are indicated by short lines and asterisks as follows: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

duced significantly larger amounts of CXCL1/KC, CXCL2/MIP2, and IL-6 than cells from C57BL/6 mice upon IL-17 stimulation (Fig. 4B to D). These results suggest that a higher basal level of IL-17RC predisposes C3H/HeN mice to mount a vigorous proinflammatory response in the early phase of IL-17 production in vivo.

When the expression profiles of IL-17RA and IL-17RC during the course of infection were examined, we found that

IL-17RA was consistently induced at higher levels in C3H/HeN mice throughout the course of infection than in C57BL/6 mice, which showed only marginal changes of IL-17RA following *C. muridarum* infection (Fig. 5A). In contrast, IL-17RC expression levels fluctuated in both mouse strains following *C. muridarum* infection (Fig. 5B). Nevertheless, the overall level of IL-17RC in C3H/HeN mice appeared to be greater than or at least equal to that in C57BL/6 mice (Fig. 5B). Collectively,

FIG. 5. Differential IL-17RA and IL-17RC induction during MoPn infection in C3H/HeN and C57BL/6 mice. Total RNA samples were prepared from lungs of C3H/HeN and C57BL/6 mice on days 0, 2 (D2), 4, 6, and 12 postinfection. (A and B) The levels of IL-17RA (A) and IL-17RC (B) expressed during the course of infection were determined by quantitative RT-PCR. The data are normalized against expression of the housekeeping gene GAPDH (Rel, relative) and expressed as means plus standard errors of the means (error bars) for three mice per time point per strain. Values that were significantly different ($P < 0.05$) for the two strains are indicated by short lines and an asterisk. Values that were significantly different ($P < 0.001$) when the values for naïve mice were compared to the values for C3H/HeN mice by two-way ANOVA test are indicated by short lines and the pound symbol.

our in vitro and in vivo data demonstrate that both IL-17 and IL-17 receptors are involved in regulating differential immune responses in C3H/HeN and C57BL/6 mice.

Differential accumulation of activated and preactivated neutrophils in the lungs of C3H/HeN and C57BL/6 mice in the early and late phases of respiratory *C. muridarum* infection.

One of the major biological activities of IL-17 in the host immune system is to stimulate neutrophil-mobilizing chemokine production through IL-17RA or the IL-17RA/IL-17RC heterodimeric complex, which, in turn, recruits neutrophils into inflammatory sites (51, 65). Having examined the kinetic changes of IL-17 and IL-17R expression and their potential contribution to host responses to respiratory *C. muridarum* infection, we further examined the profile of neutrophil infiltration in these two mouse strains. The level of MPO activity was first used as an indirect measurement of neutrophil infiltration in C57BL/6 and C3H/HeN mice on days 2, 4, 6, and 12 postinfection. Consistent with a higher profile of IL-17/Th17 response, C3H/HeN mice had significantly higher MPO activities in BAL fluid and lung homogenate samples than C57BL/6 mice did on days 4, 6, and 12 postinfection (Fig. 6A and B). Of note, both C3H/HeN and C57BL/6 mice demonstrated a biphasic neutrophil response, with a first peak on day 4 and a second peak on day 12 following *C. muridarum* infection. We then further examined the phenotype of infiltrated neutrophils in lungs by FACS on days 4, 6, and 12 postinfection. In agreement with the MPO data, there was a dramatic increase in a cell population with a granulocyte profile in C3H/HeN mice compared to C57BL/6 mice (an average 24.72% of total lung-derived cells in C3H/HeN mice versus an average 10% in C57BL/6 mice) on day 6 postinfection (Fig. 6C). Further analyses indicated that 65 to 70% of this cell population in C3H/HeN mice possessed a neutrophil phenotype (CD11b⁺ Gr1⁺), whereas only 40 to 44% of these cells possessed a neutrophil phenotype in C57BL/6 mice. A promiscuous granulocyte activation marker, BST2 (also called pDCA-1), was used to examine the relative maturity and activation status of neutrophils in two mouse strains (4). As presented in Fig. 6D, C3H/HeN mice had more neutrophils with an activated phenotype of CD11b⁺ Gr1⁺ BST2⁺ than C57BL/6 mice did. However, there was an even more striking difference in the accumulation of neutrophils with a nonactivated phenotype of CD11b⁺ Gr1⁺ BST2⁻ in the lungs of C3H/HeN mice compared to C57BL/6 mice. Similar results were also obtained on days 4 and 12 postinfection (Fig. 6E). Taken together, our results demonstrate that a skewed induction of IL-17/Th17 response results in a massive infiltration of not only activated but also preactivated neutrophils at the infection site, which is associated with an increased susceptibility to *Chlamydia* infection in C3H/HeN mice.

In vivo delivery of an IL-17RA antagonist ameliorated clinical manifestation of *Chlamydia* infection in C3H/HeN mice. Having demonstrated a close correlation between the IL-17R expression level, a skewed IL-17/Th17 profile, and massive accumulation of neutrophils in lungs during the course of infection in susceptible C3H/HeN mice, we hypothesized that blocking IL-17R signaling would be able to ameliorate the clinical symptoms of *C. muridarum* infection in C3H/HeN mice. To this end, a recombinant adenovirus expressing a fusion protein of the IL-17RA extracellular domain and murine Ig Fc portion (AdIL-17RA:Fc) was used for in vivo delivery

24 h following *C. muridarum* infection. Mice receiving control empty virus Addl70-3 or PBS were used as controls. While Addl70-3 treatment was not able to change the course of *C. muridarum* infection compared to PBS-treated mice, AdIL-17R:Fc delivery significantly prevented the body weight loss in *Chlamydia*-infected C3H/HeN mice (Fig. 7A). An approximately 50% reduction in the number of infiltrating Gr1⁺ neutrophils was observed in mice receiving AdIL-17R:Fc treatment compared to PBS-treated mice using FACS analysis (Fig. 7B). As expected, there was no significant effect on the level of neutrophil infiltration in lungs of mice treated with Addl70-3 (Fig. 7B). Similar results were also obtained using the MPO assay (data not shown). Of note, all mice had comparable bacterial loads (Fig. 7C) and IFN- γ levels produced (Fig. 7D) in the lung. Taken together, our data collectively support a pathological role of an uncontrolled IL-17/Th17-induced neutrophilic response in mediating the severity of clinical manifestation of respiratory *Chlamydia* infection in mice.

DISCUSSION

The IL-17 family is emerging as an important regulator of the cytokine networks that coordinate innate and adaptive immunity under a variety of immune inflammatory conditions. In the present study, the role of IL-17/Th17-mediated host responses during respiratory *C. muridarum* infection was investigated in *Chlamydia*-resistant C57BL/6 and *Chlamydia*-susceptible C3H/HeN mice. While $\gamma\delta^+$ T cells are often found as the major cellular source of IL-17 in response to many infectious agents (18, 34, 43, 49), the non- $\gamma\delta^+$ T cells including typical Th17 cells ($\alpha\beta^+$ CD4⁺ T cells) appear to be the major IL-17-producing cells induced by *C. muridarum* infection in both C57BL/6 and C3H/HeN mice. However, there were substantial differences with regard to the magnitude of IL-17/Th17 response in these two mouse strains. C3H/HeN mice naturally express a higher basal level of IL-17RC, which predisposed C3H/HeN mice to develop a more vigorous IL-17-mediated response during the early phase of *C. muridarum* infection compared to C57BL/6 mice. C3H/HeN mice also expressed a higher inducible level of IL-17RA upon *C. muridarum* infection, which likely further amplified IL-17/Th17 responses in the later phase of *C. muridarum* infection compared to C57BL/6 mice. Such differences were demonstrated by an increase in IL-17 mRNA expression, a higher IL-17 recall response, and higher production of Th17-related cytokines (e.g., IL-23 and IL-6) and chemokines (e.g., CXCL1/KC and CXCL2/MIP2). Furthermore, C3H/HeN mice displayed a massive accumulation of activated and preactivated neutrophils in the airway and lung parenchyma compared to C57BL/6 mice as demonstrated by both the MPO assay and FACS analyses. Most importantly, in vivo delivery of IL-17RA antagonist that resulted in a 50% reduction in the neutrophilic infiltration in lungs was able to reverse the susceptible phenotype of C3H/HeN mice to respiratory *Chlamydia* infection. Thus, our data for the first time demonstrate a critical role of the IL-17/IL-17R axis in regulating host susceptibility to *Chlamydia* infection in mice.

In contrast to other studies in which neutrophils were identified as the major cellular component in mediating IL-17-dependent host resistance against extracellular bacterial infection (49, 64–66), our data suggest that an augmented neutrophil

FIG. 6. Differential neutrophilic responses in the lungs of C3H/HeN and C57BL/6 mice following *C. muridarum* infection. (A and B) C3H/HeN and C57BL/6 mice were sacrificed at various time points postinfection (days) as indicated. The cell pellets derived from BAL fluid (A) and lung homogenates (B) were used to extract MPO, and the levels of MPO activity were determined as described in Materials and Methods. Representative data from at least two repeated experiments are expressed as the means plus standard errors of the means (SEMs) (error bars) for four to eight mice per time point per strain. Values that were significantly different in the two strains by the unpaired, two-tailed Student *t* test are indicated by short lines and asterisks as follows: *, $P < 0.05$; **, $P < 0.01$. (C and D) Total lung-derived cells were isolated from C3H/HeN and C57BL/6 mice on day 6 postinfection. Cells were then surface labeled with anti-CD11b, anti-CD11c, anti-Gr1, and anti-pDCA1 monoclonal antibodies for FACS analysis. Granulocytes were first gated based on the forward scatter (FSC) and side scatter (SSC) profiles and then gated on CD11b⁺ cells. CD11b⁺ cells were then further analyzed for expression of Gr1 and pDCA1. Representative dot plots from C3H/HeN mice and C57BL/6 mice are presented (D). Alexa 647-BST2, Alexa Fluor 647-labeled BST2. (E) The percentage of activated neutrophils (CD11b⁺ Gr1⁺ pDCA1⁺) and nonactivated (CD11b⁺ Gr1⁺ pDCA1⁻) cells in the lung-derived cells is expressed as means minus SEMs (error bars) for three to five mice per strain per time point. Values that were significantly different in the two strains by the unpaired, two-tailed Student *t* test are indicated as follows: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

infiltration as a result of a heightened IL-17/Th17 response and higher IL-17R activity enhances host susceptibility to intracellular *Chlamydia* infection in C3H/HeN mice. The differential IL-17/Th17 responses to *Chlamydia* infection in C57BL/6 and C3H/HeN mice are likely controlled at multiple points. First, C3H/HeN mice naturally express a higher basal level of the IL-17RC subunit than C57BL/6 mice do, leading to a more vigorous IL-17R-mediated immune response upon IL-17 stimulation. Both IL-17RA and IL-17RC are implicated in IL-17-

mediated cytokine and chemokine production via the IL-17RA/IL-17RC heteromeric complex, which is believed to have at least two preformed IL-17RA subunits and one IL-17RC subunit (28, 29, 51). Consistent with this model, our data suggest that IL-17RA may exist in excess and that IL-17RC likely plays a more direct role in controlling the density of functional IL-17R complex on the cell membrane in response to IL-17 stimulation. Although we did not examine how fibroblasts respond to IL-17F stimulation in this study, it is likely

FIG. 7. In vivo delivery of IL-17R antagonist significantly protected C3H/HeN mice from severe body weight loss. Groups of C3H/HeN mice (five mice per group) received 1×10^9 PFU of AdIL-17R:Fc or control viral vector Addl70-3 diluted in 100 μ l of PBS or 100 μ l of PBS alone i.p. 24 h following *C. muridarum* respiratory infection. The mice were monitored daily for body weight changes and sacrificed on day 11 (D11) after *C. muridarum* infection, and various samples were collected and processed as described in the legend to Fig. 1. (A) The percent body weight change in mice treated with AdIL-17R:Fc, or Addl70-3, or PBS 24 h following MoPn infection is shown. Values that were significantly different ($P < 0.05$) for AddIL-17R:Fc-treated mice and Addl70-3-treated or PBS-treated mice on days 9 and 10 postinfection using two-way ANOVA statistical analysis are indicated by an asterisk. (B) The BAL fluid cell pellets were pooled within the group, and the percentage of Gr1⁺ neutrophils in each group was determined by FACS analysis. The data are expressed as the percentage relative to the number of Gr1⁺ cells in the PBS-treated group. (C) The bacterial load in each mouse lung was determined based on the copy number of 16S rRNA by quantitative PCR. (D) The IFN- γ content in the BAL fluid samples were measured by ELISA and expressed as the means plus standard errors of the means (error bars) for each group (five mice per group).

that C3H/HeN mice would also have more robust responses to IL-17F stimulation since IL-17RC alone serves as a high-affinity receptor for IL-17F (29). Such robust cytokine production, particularly IL-6 production in the early phase of *C. muridarum* infection will significantly promote Th17 differentiation in C3H/HeN mice to a much greater extent (3). Second, in addition to the differences under basal conditions, there are substantial differences in the expression profiles of IL-17RA and IL-17RC in C3H/HeN and C57BL/6 mice following *Chlamydia* infection (Fig. 5A and B). Although the precise roles of IL-17RA and IL-17RC in the host responses against *Chlamydia* infection remain unclear, our kinetic results suggest that IL-17RA and IL-17RC have nonredundant roles in regulating IL-17/Th17 response during *Chlamydia* infection. We believe that a higher basal level of IL-17RC and a higher inducible level of IL-17RA may be responsible for the early peak and late peak of neutrophilic responses observed in the lung, respectively (Fig. 6). Finally, the higher bacterial burden observed in C3H/HeN mice (39, 40) may also contribute to the skewed IL-17/Th17 profile at later time points (after day 4) in C3H/HeN mice.

Neutrophils have been reported to promote intracellular growth of *Chlamydia pneumoniae* (41) and *Mycobacterium tuberculosis* (14). Furthermore, IL-17 has been reported to antagonize IFN- γ -induced neutrophil activation against fungal

infection (67) and IFN- γ -induced NK activity (22). Thus, it is possible that an augmented neutrophilic response may be responsible for higher bacterial loads in C3H/HeN mice in our study. However, systemic delivery of IL-17RA antagonist did not have any impact on the bacterial load (Fig. 7C). Therefore, the precise immune mechanism responsible for the impaired ability in controlling *Chlamydia* intracellular growth in C3H/HeN mice remains to be determined. Nevertheless, our data do suggest that the bacterial load does not seem to be the ultimate cause leading to high mortality in C3H/HeN mice. This notion is supported by the comparable level of bacterial burden observed in C3H/HeN mice that either survived or succumbed to infection (Fig. 1D). It is likely that the soluble mediators released from neutrophils at the infection site have more direct impact on body weight changes given the observation that systemic delivery of the IL-17R antagonist was able to attenuate neutrophil infiltration in lungs and weight loss simultaneously (Fig. 7).

We have demonstrated a novel role of the IL-17/IL-17R axis in regulating host susceptibility to *Chlamydia* infection. We believe that a higher basal level of IL-17RC has a critical role in initiating the whole cascade of skewed immune profile and cellular responses in C3H/HeN mice. This may directly or indirectly link to an unidentified immune mechanism that leads to an impaired immune defense and increased bacterial bur-

den in C3H/HeN mice. The high bacterial burden further stimulates immune responses and results in marked induction of IL-17RA, which likely leads to further amplification of skewed neutrophilic response and causes significant tissue damage in the host. To our knowledge, this is the first report demonstrating the IL-17R as a genetic determinant in regulating host responses to intracellular bacterial infection.

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