

A Role for Interleukin-6 in Host Defense against Murine *Chlamydia trachomatis* Infection

DWIGHT M. WILLIAMS,^{1,2*} BARRY G. GRUBBS,^{1,2} TONI DARVILLE,³
KATHLEEN KELLY,³ AND ROGER G. RANK³

Division of Infectious Diseases, Audie L. Murphy Memorial Veterans Hospital,¹ and Department of Medicine, University of Texas Health Sciences Center at San Antonio,² San Antonio, Texas 78284, and Department of Microbiology and Immunology, University of Arkansas for Medical Sciences, Little Rock, Arkansas 72205³

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Interleukin-6-deficient (IL-6^{-/-}) knockout mice had significantly increased *Chlamydia trachomatis* levels in lung tissue and increased mortality compared to B6129F₂/J controls early after intranasal infection. Gamma interferon production and chlamydia-specific antibody levels were consistent with a decreased but reversible Th1-like response in IL-6^{-/-} mice. IL-6 is needed for an optimal early host response to this infection.

In our model of murine *Chlamydia trachomatis* pneumonia due to mouse pneumonitis agent (MoPn), significant amounts of interleukin-6 (IL-6) are produced in the lung in response to infection (10). The role of this cytokine in this infection is unclear. IL-6 is known to play a critical role in the differentiation of B cells into antibody-producing plasma cells (1, 19). Plasma cells are a prominent component of the host response to chlamydial infection, may be a histologic marker for IL-6 production in this infection, and tend to correlate with resistance to MoPn (4). This is consistent with the concept that IL-6 and its immunologic effects may be important in host resistance to chlamydia. To investigate this, we have employed IL-6-deficient IL-6^{-/-} mice. It is known that IL-6^{-/-} mice are deficient in a variety of immunologic functions, including Th1 antibody production, cytotoxic lymphocyte function, very early neutrophil production, and Th1 cytokine production (correctable by neutralization of IL-10) (6, 9, 16). These defects make IL-6^{-/-} mice more susceptible than controls to a variety of pathogens (6, 9, 16, 21). The same host defense mechanisms that are deficient in IL-6^{-/-} mice are also of known or potential importance with MoPn (2, 3, 7, 8, 17, 22, 24). Of particular interest, it is known that a Th1 host response is important for successful resistance to MoPn and that a Th2-biased response, including overproduction of IL-10 by a mouse strain, can increase susceptibility (8, 29).

The MoPn biovar of *C. trachomatis* was maintained in HeLa cell culture and was Renografin density purified (24). The HeLa cell material without MoPn contained less than 0.1 ng of endotoxin per ml by Limulus assay. MoPn was harvested and frozen at -70°C until use. Mice at 6 to 8 weeks of age were infected in groups of five each intranasally following sodium pentobarbital anesthesia with 1 × 10² to 5 × 10³ inclusion-forming units (IFU) of MoPn diluted in McCoy's modified 5A medium in a volume of 0.05 ml. The IL-6^{-/-} mice employed in these studies had a disruption in the second exon (first coding exon) of the IL-6 gene by insertion of a Neo^r cassette (9). The genetic background is mixed 129J×C57BL/6 and is not homogeneous. Controls are B6129F₂/J mice. The MoPn susceptibilities of the parental strains (C57BL/6J and 129/J) are similar

(13). All mice were obtained from Jackson Laboratories (Bar Harbor, Maine). Quantitative culture of infected lung tissue was performed with McCoy cell monolayers and is reported as IFU per lung as in our previous studies (28). MoPn antigen levels were determined by enzyme-linked immunosorbent assay (ELISA) detection of chlamydial lipopolysaccharide (Ortho Diagnostics, Inc., Raritan, N.J.) as in our previous publications (11, 26). Assays of minced and filtered whole lung material for gamma interferon (IFN-γ) were performed by ELISA as in our previous studies (24). The ELISAs for IL-6 and IL-10 (Genzyme, Cambridge, Mass.) were performed with the same material by commercial ELISA according to the manufacturer's instructions. Plasma samples were collected at the times specified below, and specific MoPn antibody was determined by ELISA as previously described (24). Rat monoclonal antibody to murine IL-10 (2A5) was purchased from Genzyme (Cambridge, Mass.). Mice were given 200 μg intravenously every other day, starting on day 0 of infection with MoPn. Comparison of groups was performed by Mann-Whitney test with significance reported as two tailed. For multiple comparisons, the Mann-Whitney test was performed after analysis by nonparametric analysis of variance, with the alpha level adjusted for the number of comparisons.

Table 1 shows the results of four separate experiments in which B6129F₂/J and IL-6^{-/-} mice were infected with 1 × 10² (experiments 1 to 3) or 3 × 10² (experiment 4) IFU of MoPn intranasally. Groups contained five mice each. Statistical analysis was by Mann-Whitney test. In experiment 1, MoPn antigen levels were determined in lung on day 5 postinfection. Mice were given either rat antibody to IL-10 or control rat normal immunoglobulin (Ig). In this experiment, a *P* value of <0.01 was considered significant. IL-6^{-/-} mice given normal rat Ig were significantly more susceptible than B6129F₂/J mice given the same Ig (*P* < 0.008). Treatment with antibody to IL-10 significantly reduced the susceptibility of IL-6^{-/-} mice compared to that of IL-6^{-/-} control mice (*P* < 0.008). The same antibody treatment also tended to make B6129F₂/J more resistant, but this was to a lesser degree and did not reach statistical significance (*P* < 0.08). Because the B6129F₂/J mice are not homogeneous, a parental C57BL/6J group was also included and was not statistically different from the B6129F₂/J mice. Experiment 2 shows a repeat experiment performed with only B6129F₂/J and IL-6^{-/-} mice infected with the same MoPn dosage but not given Ig. Quantitative cultures were performed

* Corresponding author. Mailing address: Division of Infectious Diseases, Audie L. Murphy Memorial Veterans Hospital, 7400 Merton Minter Blvd., San Antonio, TX 78284. Phone: (210) 617-5109. Fax: (210) 949-3303. E-mail: dwight.williams@med.

TABLE 1. Determination of MoPn levels in lung by ELISA or culture at days 5 and 15 after infection

Mouse group	MoPn level detected by ^a :	
	ELISA (ng/lung)	Culture (IFU/lung)
Day 5		
Expt 1		
B6129F ₂ /J		
Treated with rat IgG	2,409 ± 214	ND ^b
Treated with anti-IL-10 antibody	1,682 ± 219†	ND
IL-6 ^{-/-}		
Treated with rat IgG	5,782 ± 717*	ND
Treated with anti-IL-10 antibody	1,778 ± 215**	ND
C57BL/6J	1,642 ± 172††	ND
Expt 2		
B6129F ₂ /J	ND	(6.0 ± 1.4) × 10 ³
IL-6 ^{-/-}	ND	(27.5 ± 8.3) × 10 ^{3***}
Day 15		
Expt 3		
B6129F ₂ /J	32,230 ± 6,040	(12.6 ± 4.6) × 10 ⁴
IL-6 ^{-/-}		
Treated with rat IgG	62,520 ± 6,320§	(39.4 ± 18) × 10 ^{4#}
Treated with anti-IL-10 antibody	35,689 ± 7,467	(17.8 ± 6.4) × 10 ^{4‡}
Expt 4		
B6129F ₂ /J	10,300 ± 4,700	ND
IL-6 ^{-/-}	89,400 ± 24,700‡‡	ND

^a Data are expressed as means ± standard errors. All statistics were calculated according to the Mann-Whitney test. *, $P < 0.008$ versus B6129F₂/J treated with rat IgG (two-tailed test); †, $P < 0.08$ versus B6129F₂/J treated with rat IgG (two-tailed test); **, $P < 0.008$ versus IL-6^{-/-} treated with rat IgG (two-tailed test); ††, $P > 0.2$ versus B6129F₂/J treated with rat IgG; ***, $P = 0.56$ versus B6129F₂/J; §, $P < 0.004$ versus B6129F₂/J (two-tailed test); ||, $P > 0.99$ versus B6129F₂/J (two-tailed test); #, $P < 0.06$ versus B6129F₂/J (two-tailed test); ‡, $P > 0.4$ versus B6129F₂/J (two-tailed test); ‡‡, $P < 0.008$ versus B6129F₂/J.

^b ND, not done.

on lung at day 5 in IL-6^{-/-} and B6129F₂/J mice and were consistent with the increased susceptibility of IL-6^{-/-} mice seen in the prior experiment ($P < 0.05$ is significant). In a similar experiment performed at day 15 (experiment 3), $P < 0.017$ was considered significant. By ELISA, IL-6^{-/-} mice were again significantly more susceptible than controls to MoPn ($P < 0.004$), and treatment with antibody to IL-10 reversed the increased susceptibility. Quantitative culture data followed the same pattern as the antigen data but did not reach statistical significance. An additional control group was examined in which five B6129F₂/J mice were given normal rat IgG. This group did not differ significantly from the control group not given IgG, and the two could be combined for statistical purposes. A repeat experiment on day 15 is shown in experiment 4. Antigen levels were significantly elevated in IL-6^{-/-} mice compared to those in controls ($P < 0.008$ [$P < 0.05$ is significant]). MoPn antigen determinations were also performed at day 32 in groups of six mice each infected with 10² IFU of MoPn. The infection had resolved in both mouse groups at that time with antigen levels of less than 50 ng/lung (not shown in Table 1). Finally, two mortality experiments were per-

formed, each with groups of five mice at a MoPn dose of 5 × 10³ IFU in which mortality was monitored for 20 days. The data are combined. For IL-6^{-/-} mice, single mice died on days 7, 9, 10, 16, 17, and 18 and two mice each died on days 8 and 13, while B6129F₂/J mice died on days 9, 10, 13, 14, 15, and 18, with four survivors ($P = 0.036$ [$P < 0.05$ is significant]).

To determine if the increased susceptibility of IL-6^{-/-} mice correlated with a shift toward a Th2 response in the IL-6^{-/-} animals reversible by antibody to IL-10, relevant cytokine levels were measured in lung tissue on days 5 and 15 with five mice per group (Table 2). A significant decrease in IFN- γ levels was observed in IL-6^{-/-} mice at days 5 and 15 ($P < 0.015$ [$P < 0.017$ is significant]). The decrease in IFN- γ was corrected by treatment with anti-IL-10 antibody. IL-6 levels were significantly reduced at both days. No significant changes were observed in tumor necrosis factor alpha (TNF- α) levels. Paradoxically, significant differences were also not observed in IL-10 on the days examined, but we did not examine very early periods.

Antibody levels to MoPn were also measured for IL-6^{-/-} mice and controls at day 15 postinfection. The IgG response in B6129F₂/J mice was largely directed toward a Th1-like response, with mean IgG1 levels of 14 ± 1 and IgG2a levels of 2,163 ± 1. IL-6^{-/-} mice had a mixed Th1-Th2 pattern, with mean IgG1 levels of 321 ± 3 and IgG2a levels of 527 ± 2. These IgG1 levels were significantly different from those in the B6129F₂/J mice. Treatment with antibody to IL-10 led to a return to a Th1-like response, with mean IgG1 levels of 17 ± 2 and IgG2a levels of 643 ± 2. The groups had 5 to 13 mice each. A repeat experiment showed similar results (not shown).

The data presented here are consistent with a beneficial role for IL-6 in host defense against *C. trachomatis* in our model of MoPn pneumonia. The most likely mechanism involved is a role in initiating or maintaining a Th1 response similar to that which has previously been observed with *Candida albicans* (16). It is well recognized that a Th1 response is necessary for optimal host defense against MoPn (8, 23, 24, 29). IFN- γ , TNF- α , and IL-12 are all involved (8, 12, 23, 26) in Th1-mediated host defense against MoPn in vivo. Host defense mechanisms may also be IFN- γ independent (5, 12, 24) and

TABLE 2. Cytokine determinations in lung on days 5 and 15 after infection

Mouse group	Concn of cytokine expressed ^a			
	IFN- γ (ng/ml)	IL-10 (pg/ml)	TNF- α (pg/ml)	IL-6 (pg/ml)
Day 5				
B6129F ₂ /J	49 ± 8	309 ± 41	177 ± 13	300 ± 60
IL-6 ^{-/-}				
Untreated	11 ± 1*	283 ± 42	171 ± 10	20 ± 9**
Treated with anti-IL-10 antibody	47 ± 5	ND ^b	167 ± 8	ND
Day 15				
B6129F ₂ /J	12 ± 1	313 ± 56	121 ± 10	760 ± 10
IL-6 ^{-/-}				
Untreated	4 ± 1*	320 ± 25	124 ± 12	<10**
Treated with anti-IL-10 antibody	11.5 ± 1	ND	ND	ND

^a Data are expressed as means ± standard errors for groups of five mice each. All statistics were calculated according to the Mann-Whitney test. *, $P < 0.015$ versus B6129F₂/J (two-tailed test); **, $P < 0.01$ versus B6129F₂/J (two-tailed test).

^b ND, not determined.

may be analogous to that involving IL-12 and TNF- α recently described for leishmaniasis (20, 24). TNF- α and IL-6 have multiple immunologic interactions (discussed in references 1, 15, and 26) which may be of importance in this regard. In addition, IL-1a (produced in our model in vivo [10]) has been shown to play a pivotal role in the induction of the other proinflammatory cytokines, including IL-6 in chlamydia-infected epithelial cells in in vitro studies (15). While the antibody defect of IL-6^{-/-} mice with an increased tendency toward a Th2-like response is also of interest regarding host defense, current data indicate that cell-mediated immunity is probably more important than humoral immunity in primary infection with MoPn (14, 18, 25). The fact that IL-6 is needed for optimal host defense is consistent with the histological findings in our model, in which plasma cells (a probable marker for the presence of IL-6) were part of the successful host defense in the resistant (compared to athymic) immunologically intact BALB/c background mouse but were absent in the host response of the very susceptible athymic mouse on the same background (4, 28).

Additional data regarding cytokine abnormalities have recently been reported for the IL-6^{-/-} mouse during a much more acute pulmonary infection with *Streptococcus pneumoniae* (21). IL-10 levels were significantly elevated compared to those in controls at 40 h after infection in that model, at which time IFN- γ levels tended to be decreased, consistent with a decreased Th1 response in IL-6^{-/-} mice at some periods in that infection as well. With nonimmunocompromised mice, Yang et al. have previously shown that treatment with anti IL-10 can make susceptible mouse strains more resistant to MoPn, consistent with a switch to a more Th1-directed immune response (29).

It is not clear what additional IL-6-dependent immunologic modalities, if any, might be important in host defense against MoPn. Thus, some aspects of the innate immune response to chlamydia (discussed in reference 15) could be affected as well and could be partly responsible for the increased susceptibility of the IL-6^{-/-} animals. We have not fully evaluated the possibility that a relative lack of neutrophils could play a role in the observations noted in our study. Blood neutrophil counts performed on day 5 were not clearly different in the two mouse groups (unpublished data), but we did not investigate earlier periods. Neutrophils are known to play a role in early host defense against MoPn (2). We also did not investigate cytotoxic function in the IL-6^{-/-} animals and controls. It is known that NK cell function is stimulated in lung early in infection in our model (27) and thus could be involved in the cytotoxic host defect known to be present in IL-6^{-/-} mice. In this regard, it is of note that preliminary studies in our laboratory have shown that perforin-deficient mice are more susceptible to MoPn than controls (unpublished data).

While these data were under review, Perry et al. published the results of work with the genital model of MoPn infection showing that MoPn infection was resolved in IL-6^{-/-} mice, which is consistent with what was seen in our study (13). While IFN- γ induced by MoPn antigen from spleen from infected IL-6^{-/-} mice was reduced in their model, elevations in MoPn early in infection did not reach statistical significance. Therefore, the effect of IL-6^{-/-} deficiency is apparently greater in lung than in the genital tract. We would agree, however, that IL-6 does not play the critical role in host defense against MoPn equivalent to CD4⁺ T cells without which the infection does not resolve (8, 28).

In summary, these data show that a deficiency of IL-6 in our model leads to a diminished Th1-like response to chlamydial infection with diminished IFN- γ production and increased sus-

ceptibility. Thus, IL-6 can play a significant role in early host defense against *C. trachomatis*.

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