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NOTES

Chlamydia trachomatis Pneumonia Induces In Vivo Production of Interleukin-1 and -6

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Cytokine induction during *Chlamydia trachomatis* pneumonia may alter the pathogenesis or course of disease. We examined interleukin-1 (IL-1) and IL-6 production by measuring mRNA and bioactivity in murine lungs. mRNA and bioactivity for IL-1α, IL-1β, and IL-6 increased after *Chlamydia* infection. These cytokines may be important in regulating host defenses against *C. trachomatis*.

Cytokine production during infection may play an important role in modulating host defenses to *Chlamydia trachomatis*. Rothermel et al. (26) reported that *C. trachomatis* induced interleukin-1α (IL-1α) production by monocytes. In contrast, Manor and coworkers were not able to confirm IL-1 induction by *C. trachomatis* (17). However, Shemer-Avni et al. (30) reported that recombinant IL-1α could inhibit *C. trachomatis* growth. Thus, this discrepancy concerning induction of IL-1 by *Chlamydia* infection requires further investigation.

IL-1α is a possible host defense mechanism against *Chlamydia* spp. (30) and other infectious agents (4, 18, 22) but also may be a factor in pathogenesis. *C. trachomatis* is a significant cause of blinding trachoma and tubal infertility, both of which may depend on immunologically mediated inflammation and fibrosis (9, 12, 20, 23, 24, 29, 33). IL-1 is an important mediator of inflammation which induces collagen (31) and collagenase production (5, 13) and stimulates fibroblast proliferation (25) and induction of IL-6 gene expression (36). In addition, IL-6 may be responsible for some biologic effects formerly attributed to IL-1α (11, 15, 19, 21, 36).

We have therefore employed our model of murine pneumonia due to murine *C. trachomatis* (MoPn, mouse pneumonitis agent) (38, 39) to determine whether IL-1 and/or IL-6 is produced in vivo during infection with MoPn.

Specific-pathogen-free *nu*/+ mice on a BALB/c background were infected intranasally with a lethal dose of purified elementary bodies (EBs) from MoPn (28, 37–39). Control mice were either unmanipulated mice or mice challenged with a mock-EB preparation. Total RNA isolated from the lungs of both infected and control mice 24 and 48 h postchallenge was analyzed for IL-1α, IL-1β, and IL-6 mRNA (Fig. 1). RNA was extracted, and equal amounts were separated on formaldehyde–1.2% agarose gels (3, 27). EB-challenged mice had mRNA for all three cytokines at 24 h postchallenge. In contrast, RNA from unmanipulated mice contained no detectable mRNA for any of these cytokines. At 48 h postchallenge, the levels of mRNA for all three cytokines increased over the 24-h levels. Densitometry analyses of the autoradiographs from blots in which β-actin and cytokines were run in parallel are presented in Table 1. The data are shown as the ratio of the densitometry values of the cytokine probe to the values of the β-actin probe. Ratios for all three cytokines are clearly increased by 48 h after infection compared with controls, with a smaller increase by 24 h. To ensure that the elevations were due to MoPn infection rather than a nonspecific effect of manipulation of the mice or endotoxin contamination, repeat experiments were performed for IL-1β and IL-6 using mice challenged with a mock-infected HeLa cell control material (Table 1). Ratios for both cytokines were elevated by 24 h, maximized at 48 h, and were declining at 72 h. In contrast, mock challenge of mice did not lead to mRNA induction.

Lung homogenates were harvested at various times postchallenge with purified EBs or control material from mock-infected HeLa cells (Table 2). IL-1 was measured by using the D10.G4.1 murine T-cell line (7). IL-6 activity was determined with the B9 cell line (7). Significant levels of IL-1 were detected in the lungs of EB-challenged mice on both day 1 and day 3 compared with levels in control mice ($P < 0.001$, Student's t test). By day 3, IL-1 levels increased further compared with day 1 values ($P < 0.001$, Student's t test). No IL-1 activity was detected in mock EB-challenged mice on day 3. Additionally, there was no biologically active IL-1 in lungs from normal unchallenged mice (data not shown). Samples from a separate experiment were analyzed for IL-1α by an enzyme-linked immunosorbent assay on lung homogenates on days 1 and 3 postchallenge. On day 1, values were <12 ng/ml for both control and EB-challenged mice. On day 3 in EB-challenged mice there was 126 ± 62 ng of IL-1α (mean ± standard error) per ml compared with control values <12 ng/ml ($P < 0.05$ by Mann-Whitney). By bioassay, there was a trend towards higher IL-6 levels in EB-challenged mice compared with levels in mock EB-challenged mice on day 1. On day 3, however, there were

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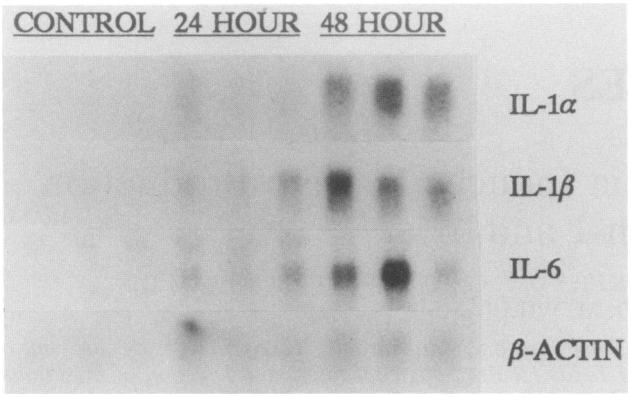

FIG. 1. Northern (RNA) blot analysis of RNA extracted from lungs of either uninfected (control) mice or EB-challenged mice 24 and 48 h postchallenge. Each lane represents the RNA isolated from one mouse.

significant levels of IL-6 detected in challenged mice ($P < 0.001$, Student's t test). The bioactivity in the lung homogenates was confirmed by neutralization of bioactivity with anticytokine antibodies. Dilutions of lung samples from day 3 EB-challenged mice were untreated or treated with polyclonal anti-IL-1α (Advanced Magnetics, Inc., Boston, Mass.) or monoclonal anti-IL-6 (6B4; a gift of Jacques Van Snick) or both antibodies for 1 h before the respective bioassay was performed. Anti-IL-1α antibody inhibited 52% of the IL-1 bioactivity, and anti-IL-6 antibody reduced 74% of the IL-6 bioactivity. Neither antibody had greater than 10% inhibition in the heterologous bioassay. Furthermore, the combination of antibodies did not decrease bioactivity in either assay compared with single-antibody treatment.

In this paper we show that IL-1α, IL-1β, and IL-6 mRNA and bioactivity are induced in murine lungs in response to EB challenge but not by mock-infected HeLa cell control challenge. With this report we confirm the findings of Rothermel et al. (26) that IL-1 is induced by *C. trachomatis* and extend the findings to the in vivo model as well. The specificity of the bioassays is always a matter of concern. The D10.G4.1 assay used to measure IL-1 activity is reported to also detect IL-6 (19). However, anti-IL-6 antibody neutralized very little of the activity in our samples. Specific

TABLE 1. Densitometry ratios for the Northern blot analysis[a]

Expt no.	Group	IL-1α	IL-1β	IL-6
1	Control	0.574 ± 0.173	0.380 ± 0.171	0.232 ± 0.072
	24 h EB	0.668 ± 0.190	1.240 ± 0.711	0.708 ± 0.379
	48 h EB	1.430 ± 0.453	2.160 ± 0.344	1.616 ± 0.468
2	24 h control	ND[b]	0.106 ± 0.014	0
	24 h EB	ND	0.440 ± 0.127	0.506 ± 0.121
	48 h control	ND	0.166 ± 0.078	0
	48 h EB	ND	1.306 ± 0.176	1.034 ± 0.382
	72 h control	ND	0.054 ± 0.059	0
	72 h EB	ND	0.567 ± 0.170	0.413 ± 0.143

[a] Ratios of densitometric values of a Northern blot either of EB-challenged mice at 24, 48, or 72 h postinfection or of unmanipulated (control mice, experiment 1) or mock-EB-challenged (control mice, experiment 2) mice. Each value represents the ratio of the cytokine probe to β-actin probe of three to four separate RNA samples.
[b] ND, not done.

TABLE 2. Production of IL-1 and IL-6 postchallenge[a]

Day	IL-1 (U/ml)		IL-6 (U/ml)	
	EB	Control	EB	Control
1	466 ± 74[b]	27 ± 8	429 ± 438	50
3	1,106 ± 198[b,c]	<15	8,544 ± 2,274[b,c]	<50

[a] Mice were challenged with either purified EBs or control HeLa material. Lungs were harvested and homogenized, and the filtrate was tested in cytokine bioassays. Values for each time period represent the mean bioactivity ± the standard deviation from groups containing four to five mice each.
[b] $P < 0.001$ compared with control at the same time period.
[c] $P < 0.001$ compared with day 1 values.

neutralization with anti-IL-1α antibody demonstrated that approximately half the observed activity seen in our bioassay was due to IL-1α. The remaining activity may be due to IL-1β but currently remains undefined. The B9 bioassay for IL-6 is relatively specific and sensitive (1, 34). In addition, specificity of the IL-6 bioassay was confirmed by anti-IL-6 neutralization which ablated three-fourths of the bioactivity.

The effects of IL-1 and IL-6 are complex and interactive. IL-6 and IL-1 are both produced not only by macrophages but also under some circumstances by fibroblasts and/or T cells and/or B cells as well (2, 34). These cytokines are induced by viruses, bacteria, lipopolysaccharides, and phorbol esters (2, 6, 34). Furthermore, a variety of other cytokines in addition to IL-1, including tumor necrosis factor alpha (TNF-α) (either by itself or in combination with gamma interferon), platelet-derived growth factor, IL-3, and granulocyte-macrophage colony-stimulating factor can all be involved in the induction of IL-6 (2, 34). We have shown here for the first time that *C. trachomatis* is also an inducer of IL-6. IL-6 may act in synergy with IL-1 for multiple functions, including the activation of T cells and induction of cytotoxic T-cell activity (10, 11, 15, 19, 34).

IL-1 protects against *Listeria monocytogenes* (4) and *Candida albicans* (14); however, the role of IL-6 in host defense is currently unclear. The production of these cytokines in several infections has been observed. IL-1, IL-6, and TNF-α have been measured in the cerebrospinal fluid of both human patients with meningococcal meningitis and an animal model of meningitis (35). Cytokine induction preceded granulocyte infiltration in the animal model. In another report on patients in the advanced stages of human immunodeficiency virus infection, IL-6 levels in the cerebrospinal fluid were detected more frequently and at higher levels than in patients with early human immunodeficiency virus infection or with noninflammatory neurological diseases (16). IL-6 has been shown to be a mediator in lethal *Escherichia coli* infection, probably as a negative modulator of TNF-α (32). We have previously shown that TNF-α can be a positive factor in host defense against *C. trachomatis* (37) and that therefore, the TNF-α–IL-6 interaction could be important in host resistance. In a model of experimental cerebral malaria, antibodies to gamma interferon or TNF-α prevented the IL-6 response to infection which was otherwise seen (8). Antibodies to IL-6 given in vivo did not appear to prevent cerebral malaria but did reduce serum immunoglobulin G levels. Multiple other cytokine interactions have been described previously, including the mutual induction of IL-1 and IL-6 expression by each other, induction of IL-6 by TNF, and IL-6 suppression of TNF-induced IL-1 production (2). Thus, the potential clearly exists for a role for IL-6 in both pathogenesis and host defense through its direct effects

on T cells and macrophages, B-cell differentiation, hemopoiesis, and mesangial cell growth or indirectly by its interaction with other cytokines, including IL-1 (2, 34). Current studies regarding the role of both IL-1 and IL-6 in host defense against MoPn in vivo are in progress in our laboratory.

The reason for the discrepancy in the positive results concerning the induction of IL-1 by *C. trachomatis* reported by Rothermel et al. (26) and this report and the negative findings of Manor et al. (17) are unclear. Since this report concerns in vivo data and work in the other reports was performed in vitro, methodologic comparisons are difficult. Our control material disclosed no significant endotoxin contamination as an explanation for our findings. The conclusion based on our data is that both IL-1 and IL-6 are induced in vivo by *C. trachomatis* and that these cytokines may play a role in the pathogenesis of and host defense to *C. trachomatis* infection.

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