Legionella pneumophila-Induced Visual Learning Impairment Reversed by Anti-Interleukin-1β (43917)

MICHAEL GIBERTINI,[1] CATHERINE NEWTON, THOMAS W. KLEIN, AND HERMAN FRIEDMAN[2]
Department of Medical Microbiology and Immunology, University of South Florida College of Medicine, Tampa, Florida 33612

Abstract. **Infecting mice with the opportunistic intracellular pathogen *Legionella pneumophila* markedly inhibited place learning of infected C57BL/6 mice as determined by the Morris water maze test. Mice infected with *L. pneumophila* evinced much less ability to learn the position of a hidden platform than did normal noninfected mice, which quickly learned the location of the hidden platform and escaped from the cool water of the pool with increasing efficiency. However, infected mice treated with anti-interleukin-1 (anti-IL-1) neutralizing antibody learned the task with about the same efficiency as the controls. When the animals were tested 1 week after learning, control animals remembered the task well and were able to escape with near maximal efficacy. On the other hand, *L. pneumophila*-infected mice performed as poorly after the 1 week rest as during the training period, indicating that infection blocked learning and not merely performance. Mice infected with *L. pneumophila* and given the antibody treatment were found to be indistinguishable from controls in that they remembered the task and escaped with good efficiency. Thus, the results of this study suggest that the pro-inflammatory cytokine, IL-1β, is involved, at least partly, in the attenuation of spatial navigational learning in mice infected acutely with a sublethal concentration of *L. pneumophila*. These results, therefore, suggest that cognitive impairment of *L. pneumophila*-infected mice may be related to the cytokine IL-1β and, furthermore, that cytokines may be related to learning and memory changes experienced by individuals suffering acute bacterial infections.** [P.S.E.B.M. 1995, Vol 210]

Legionella pneumophila is the etiologic agent of Legionnaire's disease, which was first recognized in 1976 when a number of conventioneers at an American Legion Convention in Philadelphia became ill with a life-threatening pneumonia (1). Infection with *L. pneumophila,* an opportunistic intracellular bacterium, is associated with high fever induction (2). In this regard, increased levels of interleukin-1 (IL-1), a pyrogenic cytokine, are associated with infection by *L. pneumophila* and of other gram-negative bacterial (3–6). Furthermore, lipopolysaccharide, which is present in all gram-negative bacteria, is a strong inducer of IL1 and has been associated with many of the symptoms of gram-negative bacterial infections (4–6). The major mechanisms for resistance to *L. pneumophila* involve cell-mediated immunity, since the organism primarily infects monocytes and macrophages (7–9). These infected cells respond by producing and releasing cytokines including IL-1 and tumor necrosis factor-α (TNF-α) (4, 10). Individuals infected with either viruses or bacteria, especially gram-negative bacteria, have been observed to manifest a set of responses referred to as "sickness behavior," which is characterized by listlessness, fatigue, reduced social exploration, and lack of appetite (11, 12). Slow-term memory loss and confusion leading to learning

[1] Present address: Midwest Research Institute, 425 Volker Boulevard, Kansas City, MO 64110.
[2] To whom requests for reprints should be addressed at Department of Medical Microbiology/Immunology, University of South Florida, College of Medicine, Tampa, FL 33612.

Received June 27, 1994. [P.S.E.B.M. 1995, Vol 210]
Accepted April 5, 1995.

0037-9727/95/2101-0007$10.50/0

deficits may also be involved with these responses (13). Certain aspects of sickness behavior have been attributed, in part, to pyrogenic cytokines including IL-1 and TNF-α (11–14).

In the present study, we found that mice infected with *L. pneumophila* exhibited sickness behavior as defined above. Furthermore, the infected animals showed marked inhibition of place learning ability as determined by Morris water maze method. Morris water maze is a spatial navigational learning test where swimming animals use distal visual cues to find the position of a platform hidden just below the surface of the water (15–17). Since *L. pneumophila* infection is associated with IL-1 production, IL-1 has been connected to sickness behavior, and IL-1 has been shown to impair spatial navigation learning (18), we examined if exogenous anti-IL-1β antibody could affect the inhibition. The learning impairment was reversed by anti-IL-1β administration, despite the continuation of illness induced by the *L. pneumophila* infection. These results support the view that cognitive impairment may be a component of cytokine mediated sickness behavior, since antibody against a cytokine reversed the learning disability occurring in infected mice.

Methods and Materials

Animals. Female C57Bl/6 mice were used for this study. They were obtained from Harlan Laboratories (Indianapolis, IN) at 7–9 weeks of age and each weighed approximately 15–20 g at the time of experiment. They were housed in groups of 6–10 and fed mouse pellets and water *ad libitum*.

Bacteria and Infection. A virulent strain of *L. pneumophila,* serogroup 1, was cultured on buffered charcoal yeast extract agar (19). The bacteria were suspended in pyrogen-free saline (PFS) and injected intraperitoneally (ip) at a concentration of 8×10^6 bacteria per mouse, which is a sublethal dose (6, 19). A bacteria concentration of $2–4 \times 10^8$ resulted in 100% mortality within 2 days (data not shown). Induction of sickness in these mice was assessed by fever and visual observation of sickness behavior, which was defined as being hunched, less mobile, and less responsive to noise, and eating less. Temperature measurements of the animals were taken rectally with a Fisher NIST telethermometer (Pittsburgh, PA). The probe was inserted 1 cm, and the temperature recorded after 10 sec.

Spatial Learning. The mice were tested in a modified Morris water maze, which consisted of a round tank 100 cm in diameter, with vertical polyvinyl chloride sides 60 cm high (15). The tank was filled to a depth of 15 cm with water (22°C to 25°C) and rendered opaque by powdered milk. A round platform, 10 cm in diameter, with the top positioned 0.5 cm below the surface of the water, was used as a target for all training trials. This platform was positioned in the center of the northwest quadrant of the tank. Mice were trained individually by a standard protocol consistent with those in the literature (15–17). This training consisted of 18 trials performed in six blocks of three 60-sec trials per block. Training was done on two consecutive days with three blocks per day. Performance of each mouse in the trial was measured as the latency in seconds needed to locate, mount, and remain on the platform. The swimming tank was located in a room where sound, lighting, and distal cues on wall and ceiling could be controlled.

Experimental Design. The ability of mice to learn the position of the hidden platform while burdened with *L. pneumophila* was tested in a preliminary dosing experiment and two replicate experiments. Data presented in this paper are from the two replicate experiments. Mice were injected with PFS (control animals; $n = 12$) or infected with *L. pneumophila* ($n = 30$) 24 hr prior to 2 days of training on the water maze. Half of the *L. pneumophila*-infected mice were given hamster monoclonal anti-IL-1β antibody (100 μg/mouse in 0.1 ml of PFS, ip; Genzyme, Cambridge, MA) 2 hr prior to the start of training on both days. This dose had protected mice from septic shock in previous studies in this laboratory (6). The remaining infected animals were given PFS. They were then rested for 7 days prior to being tested for retention of their place learning. Additional control measures were taken during the training. These controls involved the use of a visible platform and the calculating of swimming speed. Two animals per group were sacrificed after the second day of training (56 hr after the infection), and their spleen and liver were examined for the presence of *L. pneumophila* using the colony-forming unit (CFU) assay on agar plates, as described previously (8). Additionally, two mice per group were examined after the retention testing for *L. pneumophila*.

Results

Infection of mice with a sublethal dose of *L. pneumophila* resulted in morbidity (illness) but no death similar to previous results (19). *L. pneumophila* were readily recovered from the spleens and livers of the infected animals 56 hr after infection (data not shown). The rectal temperature of the infected animals (36.5° ± 0.46°C) and of the anti-IL-1β-infected mice (36.8° ± 0.38°C) was approximately 1°C higher than that of the control PFS mice (35.8° ± 0.44°C). The elevated temperatures returned to normal levels by the second day after infection.

Compared with PFS-only injected controls, *L. pneumophila*-infected mice showed slowed place learning as determined by the Morris water maze test. Figure 1 presents the means for these mice after each six blocks of learning trials for each experimental

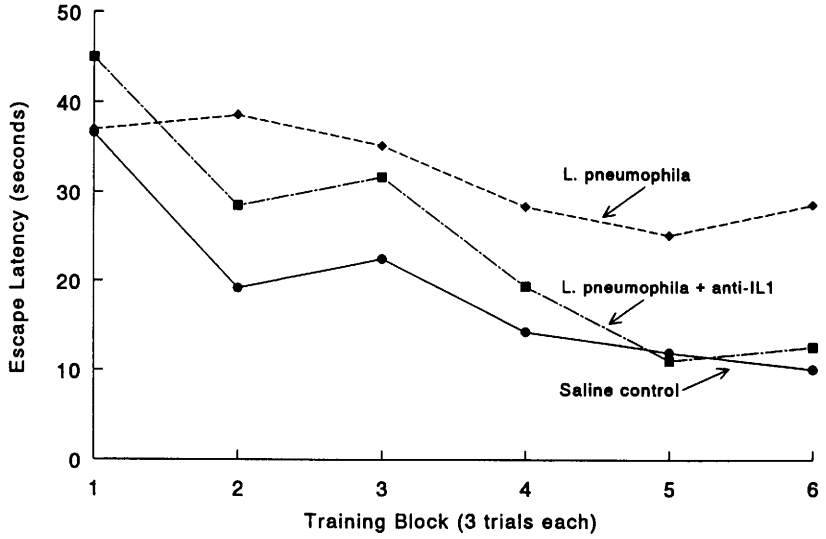

Figure 1. Learning curves for experimental groups of mice over 2 days of training. Saline controls (n = 12) and *L. pneumophila*-infected mice (n = 15) received saline injections prior to blocks one and four. Infected mice also given monoclonal anti-IL-1β antibody treatment (100 μg/mouse, ip; n = 15) at these same time points during training. Standard errors range from 1.1 to 3.9 and are omitted for clarity.

group. It is apparent in this figure that control noninfected mice quickly learned the location of the hidden platform and escaped from the water with increasing efficiency over the training period. Mice infected with *L. pneumophila,* on the other hand, showed some learning after Block 4, but the overall trend was muted compared with controls. Infected mice treated with the anti-IL-1β antibody learned the task with about the same efficiency as the control PFS-injected mice.

Analysis of variance of the learning curves revealed that (i) the noninfected control mice learned the place learning faster than did the *L. pneumophila*-infected mice (F[5,125] = 2.38, $P < 0.05$); (ii) the *L. pneumophila*-infected mice given anti-IL-1β antibody learned faster than did infected mice not treated with antibody (F[5,140] = 4.01; $P < 0.01$); and (iii) the *L. pneumophila*-infected mice given anti-IL-1β antibody learned at the same rate as control mice (F[5,125] = 1.19, P = 0.32). Our preliminary studies and the literature indicate that maximal performance on the Morris water maze for female C57BL/6 mice is around 10 to 15 sec latency to find the platform (16). *L. pneumophila*-infected mice were unable to reach this level of performance.

After 2 days of training, mice in all groups were allowed to rest undisturbed for 7 days. The mice were then tested for their ability to remember the previous training. Figure 2 presents the mean time of escape latencies for the retention test block of trials given 7 days after training. From this figure, it is apparent that the noninfected control animals remembered the task well and were able to escape with near maximal efficiency. The *L. pneumophila*-infected mice, however, performed as poorly as during the training period, indicating that the infection actually blocked learning and not merely performance during training. The mice infected with *L. pneumophila* and given the anti-IL-1β antibody treatment were again indistinguishable from

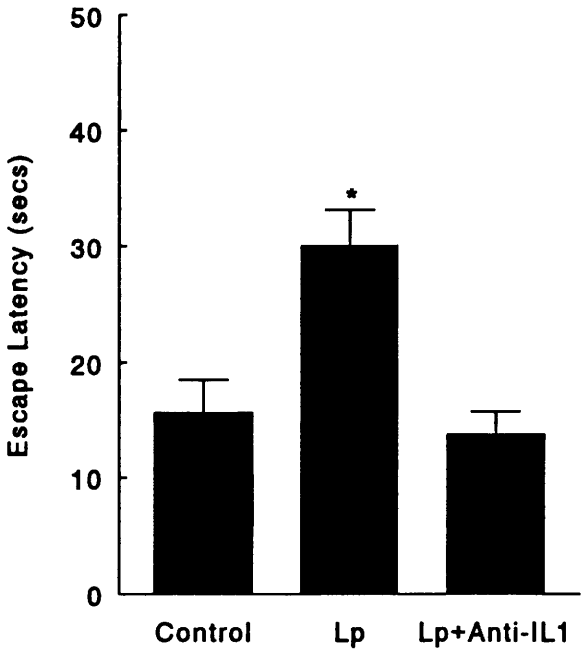

Figure 2. Memory for the water maze 7 days after training. Groups of mice/controls, *L. pneumophila* infected only and infected plus treated with anti-IL-1 serum tested for learning retention after 7-day rest as described in text. *Significantly different from the other two groups ($P < 0.01$).

controls (i.e., they remembered the task and escaped with good efficiency). One way analysis of variance for these means was significant (F[2,34] = 11.3; $P <$ 0.001) and the *L. pneumophila*-infected group was significantly different (Tukey post-hoc comparison; $P <$ 0.01) from both of the other two groups. After the retention test, mice were sacrificed and the spleen and liver examined for viable *L. pneumophila* by the CFU assay. No bacteria were found in any animal, indicating that by 9 days all mice had recovered from the infection (data not shown).

In order to ensure that performance decrements on the maze were due to cognitive (i.e., learning) def-

icits and not to motor, motivational, fatigue, or visual acuity problems caused by the treatments, control measures described above were examined. All groups were similar in terms of swimming ability and speed, ability to locate a visible platform, and vigor in mounting a visible platform (data not shown). The results of these tests showed that the treatments did not affect these noncognitive aspects of place learning performance and may be ruled out as explanations of the results.

Discussion

The place learning in the Morris water maze is considered a test of visual-spatial learning in which all proximal visual, auditory, or olfactory cues are noninformative. The mouse uses distal visual cues from the ceiling or the walls of the room to learn the position of the platform which is hidden just under the surface of the water. This navigational or place learning is reinforced when the mouse finds the platform and is rewarded by removal from the water (15–17). The procedure allows for assessment of learning independently of motor, motivational, fatigue, or visual acuity problems that may be associated with treatments. These noncognitive effects are controlled for by the use of a visible platform and swimming speed as described by McNamara *et al.* (17).

The results of this study indicate that *L. pneumophila* infections interfered with spatial navigational learning in infected mice. Furthermore, the data suggest that IL-1β may be involved in this impaired effect on learning. These results also indicate that the *L. pneumophila*-infected animals neither acquired nor retained memory of the hidden target unlike the controls. It seems likely from these results that *L. pneumophila* infection of mice impaired acquisition of learning.

The spatial learning deficit associated with *L. pneumophila* infection seemed most likely due to the presence of circulating IL-1β, since infection with gram-negative bacteria is known to cause a pyrogenic response associated with pro-inflammatory cytokines (5, 6). Infected mice injected with a blocking anti-IL-1β antibody performed as well as noninfected controls throughout most of the acquisition trials. However, the continued existence of illness symptoms, including fever, in these mice was almost identical to that of mice infected with *L. pneumophila* only. Thus, the presence of fever in the mice given *L. pneumophila* and also antibody did not appear to affect their ability to learn similarly to the control mice. These data are consistent with the view there may be a dissociation between pyrogenic and behavioral effects of IL-1 (12, 13, 18, 20). Kent *et al.* (20) observed that there was a different receptor mechanism in pyrogenic effects of IL-1 and its behavioral effects. However, the presence of other pyrogenic cytokines such as TNF-α, IL-1α, or IL-6 could also account for the fever in anti-IL-1 antibody-treated infected animals. The learning retention tests showed that mice infected with *L. pneumophila* and injected with antibody to IL-1 retained their learning ability in a manner generally indistinguishable from the controls.

Although the present study suggests that IL-1 may be a causal factor in cognitive disturbances during infection, the mechanisms involved are not clear. It is unlikely that anti-IL-1 antibodies could cross the blood-brain barrier. This study, therefore, suggests that circulating cytokines, as opposed to centrally produced cytokines, may be involved in the behavioral effects observed. Cytokines such as IL-1 may appear in the brain after infection, but whether this occurs through direct transfer of circulating IL-1 through the blood-brain barrier or indirectly through intermediate steps involving central nervous system (CNS) structures outside of the blood brain-barrier is not known (21, 22). The hippocampus is important in spatial navigational learning (17) and IL-1β appears to have a role in its function. Peripheral IL-1β or LPS can elicit IL-1α and IL-1β in the hippocampus (23). IL-1 receptors are found in many brain regions but are especially dense in the murine hippocampus (22). In the C57BL/6 mouse brain, the highest densities of IL-1 receptors appear in the dentate gyrus of the hippocampus (24). Recent evidence (25–27) suggests that one of the links between the hypathalmic-pituitary-adrenal (HPA) axis and the immune system may involve hippocampal inhibition of the HPA axis under control of corticosteroids and cytokine receptors of hippocampal neurons. Thus, changes in hippocampal function may be an important component of *in vivo* regulation of an immune response via the HPA axis. This study demonstrates that this link may be dependent on signals from circulating IL-1β and that changes in cognition may be a consequence of normal regulation of an acute immune reaction.

This study was supported in part by grants from the National Institutes of Health (NIAID Grant AI16618 and NIDA Grant DAO7245).

1. Winn WC. Legionnaires disease: Historical perspective. Clin Microbiol Rev **1**:60–81, 1988.
2. Edelstein PH. Legionnaires' disease. Clin Infect Dis **16**:741–749, 1993.
3. Di Giovine FS, Duff GW. Interleukin 1: The first interleukin. Immunol Today **11**:13–20, 1990.
4. Widen RH, Klein TW, Newton CA, Friedman H. Induction of interleukin 1 by *Legionella pneumophila* in murine peritoneal macrophage cultures. Proc Soc Exp Biol Med **191**:304–308, 1989.
5. Dinarello CA. Role of interleukin-1 in infectious diseases. Immunol Rev **127**:119–146, 1992.

6. Klein TW, Newton C, Widen R, Friedman H. Δ^9-Tetrahydrocannabinol injection induces cytokine-mediated mortality of mice infected with *Legionella pneumophila*. J Pharmacol Exp Ther **267**:635–640, 1993.
7. Horwitz MA. Cell-mediated immunity of Legionnaires disease. J Clin Invest **71**:1686–1697, 1983.
8. Yamamoto Y, Klein TW, Newton CA, Friedman H. Differing and macrophage and lymphocyte roles in resistance to *Legionella pneumophila* infection. J Immunol **148**:584–589, 1992.
9. Horwitz MA, Silverstein SC. The Legionnaires' disease bacterium (*Legionella penumophila*) multiples intracellularly in human monocytes. J Clin Invest **66**:441–450, 1980.
10. Arata S, Newton C, Klein TW, Yamamoto Y, Friedman H. *Legionella pneumophila* induced tumor necrosis factor production in permissive versus nonpermissive macrophages. Proc Soc Exp Biol Med **203**:26–29, 1993.
11. Hart BL. Biological basis of the behavior of sick animals. Neurosci Biobehav Rev **12**:123–127, 1988.
12. Dantzer R, Bluthe R, Kent S, Kelley KW. Behavioral effects of cytokines. In: Rothwell NJ, Dantzer RD, Eds. Interleukin-1 in the Brain. Tarrytown, NY: Pergamon Press, pp68–81, 1992.
13. Bluthe RM, Dantzer R, Kelley KW. Effects of interleukin-1 receptor antagonist on the behavioral effects of lipopolysaccharide in rats. Brain Res **573**:318–320, 1992.
14. Bluthe RM, Dantzer R, Kelley KW. Interleukin-1 mediates behavioral but not metabolic effects of tumor necrosis factor α in mice. Eur J Pharmacol **209**:281–283, 1991.
15. Morris RGM. Spatial localization does not require the presence of local cues. Learning Motivation **12**:239–260, 1981.
16. Upchurch M, Wehner JM. Differences between inbred strains of mice in Morris water maze performance. Behav Genet **18**:55–68, 1988.
17. McNamara RK, Skelton RW. The neuropharmacological and neurochemical basis of place learning in the Morris water maze. Brain Res Rev **18**:33–49, 1993.
18. Oitzl MS, van Oers H, Schobitz B, de Kloet ER. Interleukin-1 beta, but not interleukin-6, impairs spatial navigation learning. Brain Res **613**:160–163, 1993.
19. Newton CA, Klein TW, Friedman H. Secondary immunity to *Legionella pneumophila* and Th1 activity are suppressed by delta-9-tetrahydrocannabinol injection. Infect Immun **62**:4015–4020, 1994.
20. Kent S, Bluthe RM, Dantzer R, Hardwick AJ, Kelley KW, Rothwell NJ, Vannice JL. Different receptor mechanisms mediated the pyrogenic and behavioral effects of interleukin 1. Proc Natl Acad Sci USA **89**:9117–9120, 1992.
21. Banks WA, Kastin AJ. Blood to brain transport of interleukin links the immune and central nervous systems. Life Sci **48**:PL117–PL121, 1991.
22. Cunningham ET, De Souza EB. Interleukin 1 receptors in the brain and endocrine tissues. Immunol Today **14**:171–176, 1993.
23. Ban E, Haour F, Lenstra R. Brain interleukin 1 gene expression induced by peripheral lipopolysaccharide administration. Cytokine **4**:48–54, 1992.
24. Takao T, Tracey DE, Mitchell WM, DeSouza EB. Interleukin-1 receptors in mouse brain: Characterization and neuronal localization. Endocrinology **127**:3070–3078, 1990.
25. Betancur C, Lledo A, Borrell J, Guaza C. Corticosteroid regulation of IL1 receptors in the mouse hippocampus: Effects of glucocorticord treatment, stress, and adrenalectomy. Neuroendocrinology **59**:120–128, 1994.
26. Schobitz B, Sutanto W, Carey MP, Holsboer F, de Kloet ER. Endotoxin and interleukin 1 decrease the affinity of hippocampal mineralocorticoid (type I) receptor in parallel to activation of the hypothalamic-pituitary-adrenal axis. Neuroendocrinology **60**:124–133, 1994.
27. De Kloet ER, Oitzl MS, Schobitz B. Cytokines and the brain corticosteroid receptor balance: Relevance to pathophysiology of neuroendocrine-immune communication. Psychoneuroendocrinology **19**:121–134, 1994.