Compensatory response of IL-1 gene knockout mice after pulmonary infection with *Klebsiella pneumoniae*

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This study was designed to determine the role of interleukin (IL)-1 in the inflammatory response against experimentally induced pneumonia caused by *Klebsiella pneumoniae*. The host immune responses of IL-1 gene knockout (IL-1 KO) mice and immunocompetent wild-type (WT) mice were compared after pulmonary infection with *K. pneumoniae*. There were no significant differences between the survival rates and viable bacterial counts in lungs and blood of IL-1 KO and WT mice after pulmonary infections under different conditions. Histopathological analysis showed a similar inflammatory response in both groups of mice. However, in the early stage of infection, the level of tumour necrosis factor alpha (TNF-α) in homogenized lungs of IL-1 KO mice was significantly higher than in WT mice. To determine the role of endogenous TNF-α in the recovery of the defence mechanism in IL-1 KO mice, mice were treated with an anti-TNF-α mAb before infection with *K. pneumoniae*. The results revealed a significantly lower survival rate of anti-TNF-α mAb-treated IL-1 KO mice than BSA-treated IL-1 KO mice. The data suggest that compensatory production of TNF-α in IL-1 KO mice contributes to the host defence against *K. pneumoniae* infection.

INTRODUCTION

*Klebsiella pneumoniae*, a capsulate Gram-negative bacterium, is one of the most important causative pathogens of respiratory tract infections in humans (Podschun & Ullmann, 1998). Pneumonia caused by this organism is an expansive and voluminous pneumonia characterized by destruction of alveolar septa. This type of pneumonia is often difficult to treat, particularly in debilitated patients, with reported mortality rates of 20 to 40% (Lawrence & Komshian, 1989; Bryan et al., 1983; Edelman et al., 1994; Domenico et al., 1982).

Interleukin (IL)-1 is a potent proinflammatory cytokine that has been identified in numerous physiological processes as well as inflammatory diseases (Dinarello, 1996). IL-1 is an important mediator of pulmonary inflammation induced by bacteria and bacterial products (Ulich et al., 1991a, b). Elevated IL-1 levels have been found in pleural fluids of patients with empyema (Silva-Mejias et al., 1995) and, in patients with unilateral community-acquired pneumonia, significantly higher IL-1 concentrations have been detected in bronchoalveolar lavage fluids (BALFs) from infected lungs, compared with BALFs from non-involved lungs and serum (Dehoux et al., 1994). Effective host defence against *K. pneumoniae* infection depends on a non-specific immunological response by phagocytic cells, including neutrophils and macrophages.

IL-1 and tumour necrosis factor alpha (TNF-α) are produced mainly by macrophages. IL-1 and TNF-α share a wide range of biological activities, including the activation of neutrophils (Dinarello, 1992; Ogle et al., 1992; Sauder et al., 1984; Shalaby et al., 1985; Steinbeck & Roth, 1989). Evidence indicates that the local production of proinflammatory cytokines is crucial for clearance of bacterial infections of the lung. Indeed, passive immunization against TNF impairs host defence during pneumococcal, *Legionella* and *Klebsiella pneumoniae* in mice (Brieland et al., 1995; Laichalk et al., 1998; van der Poll et al., 1997). The role of IL-1 during *Klebsiella pneumonia* is less well defined. The present study was designed to determine the role of IL-1 in the inflammatory process after pulmonary infection with *K. pneumoniae*, by comparing the IL-1-deficient and wild-type (WT) mice.
**METHODS**

**Animals.** Specific pathogen-free, IL-1 gene knockout mice (IL-1 KO mice) on a BALB/c background and corresponding control BALB/c male mice were used. IL-1α/β double-gene knockout mice were produced together with mice deficient in either the IL-1α or the IL-1β genes. The IL-1 KO mice were supported at the Laboratory Animal Experimentation of Toho University School of Medicine.

**Bacteria.** *K. pneumoniae* strain DT-S (capsular type 1), isolated from the sputum of a patient with pneumonia, was kindly provided by Takeda Pharmaceutical, Osaka, Japan. *K. pneumoniae* strain T-113, a clinical isolate from sputum of a patient with pneumonia, was also used. These strains were kept frozen at −80 °C in brain heart infusion (BHI) broth containing 15% (v/v) glycerol.

**Pulmonary infection with *K. pneumoniae*.** Bacteria that had been cultured on blood agar for 24 h at 37 °C were suspended in sterile saline and adjusted to various densities of *K. pneumoniae*. Each mouse was anesthetized with a mixture of xylazine, ketamine/HCl and saline by intradermal administration, followed by intranasal inoculation of 20 μl of the bacterial suspension. Survival was recorded every 24 h until 14 days after inoculation.

**Determination of viable bacterial counts in blood and lung tissues.** Mice were killed by ether inhalation and cardiac blood samples were collected under sterile conditions. Lungs were removed aseptically and homogenized in sterile saline using a tissue homogenizer (Omni EZ Connect Homogenizers; OMNI International). Blood and lung homogenates were serially diluted with sterile saline and added onto blood agar plates. After incubation of the medium for 24 h at 37 °C, the number of bacterial colonies was counted and the number of viable bacteria in the organs was calculated. The remaining blood samples were allowed to clot at 4 °C and then centrifuged at 14 000 g for 5 min. Serum samples were preserved at −80 °C until measurements of cytokines were taken.

**Histopathological examination.** Mice were killed at 6, 12, 24, 48 or 72 h after inoculation with *K. pneumoniae*. The lungs of mice were obtained and fixed with 4% buffer formalin, dehydrated and embedded in paraffin. Sections were cut at 3 μm thickness and stained with haematoxylin and eosin using a standard staining procedure, then examined under a light microscope.

**Determination of cytokine concentrations.** The concentrations of IL-10 and IL-12, gamma interferon (IFN-γ) and TNF-α in serum and homogenized lung tissues were determined with ELISA kits purchased from Gendex. Assays were performed according to the protocols recommended by the manufacturers.

**Anti-TNF-α antibody.** Hybridoma cell lines (MP6-XT22.11; rat IgG1) secreting mAb against mouse TNF-α were used. MP6-XT22.11 cells were kindly provided by J. S. Abrams, DNAX Research Institute of Cellular and Molecular Biology, Palo Alto, CA, USA. The anti-TNF-α mAbs found in the ascites fluid were partially purified by 50% (NH₄)₂SO₄ precipitation (Nakane et al., 1988).

**Statistical analyses.** Data were expressed as the mean ± standard error mean (SEM). Differences in survival rates were analysed by using the log rank test. Differences in the number of bacteria and cytokine levels were analysed by using the Mann–Whitney U-test. Differences were considered statistically significant if *P* values were less than 0.05.

**RESULTS AND DISCUSSION**

IL-1α and IL-1β are cytokines, primarily produced by activated macrophages, with central roles in the initiation and coordination of the host response to infection and injury (Dinarello, 1991). van der Meer (1988) and van der Meer et al. (1988) reported that pretreatment with recombinant human IL-1β (rhIL-1β) 24 h before a lethal Gram-negative infectious challenge with *K. pneumoniae* or *Pseudomonas aeruginosa* enhanced the survival of normal and neutropenic mice, respectively. We studied the effects of pulmonary administration of IL-1 before pulmonary infection with *K. pneumoniae*. The results revealed that IL-1-pretreated mice survived significantly longer than saline-pretreated control mice (T. Matsumoto, M. Tanabe, K. Yoshida, K. Tateda & K. Yamaguchi, unpublished data). These data suggest that IL-1 plays an important role in pulmonary infection with *K. pneumoniae*.

In the present study, we used IL-1 KO mice to investigate the specific role of IL-1 after pulmonary infection with *K. pneumoniae*. We evaluated the influence of IL-1 deficiency on the survival of mice after pulmonary infection with *K. pneumoniae*. The survival rates of IL-1 KO mice and WT mice were not significantly different 14 days after inoculation with *K. pneumoniae* strain T-113 (1.76 × 10⁸ c.f.u. per mouse) (Fig. 1a). The use of a higher dose (3.6 × 10⁸ c.f.u. per mouse) of *K. pneumoniae* strain T-113 for inoculation did not result in a significant change in the survival between the groups (Fig. 1b). We could not find any significant difference in the survival between the groups even when mice were infected with another highly virulent strain of *K. pneumoniae*, strain DT-S (at 23 c.f.u. per mouse) (Fig. 1c), and at a higher dose (3.6 × 10⁹ c.f.u. per mouse) (Fig. 1d). In the preliminary study, we also evaluated the mortality rates of IL-1 KO mice and WT mice after infection with lower doses of *K. pneumoniae* strain DT-S. However, we could not find a significant difference in the mortality between these two mice strains (data not shown).

We assessed the viable number of *K. pneumoniae* in the lungs of IL-1 KO mice and WT mice at 6, 12, 24, 48 and 72 h after intranasal inoculation with *K. pneumoniae* strain DT-S. The results revealed that the number of c.f.u. in the lungs and serum was similar in the two groups at the above-mentioned times points (Fig. 2). We also evaluated the pathological changes in the lungs of WT mice and IL-1 KO mice after pulmonary infection with *K. pneumoniae*. Macropathological observation of the lungs of both WT mice and IL-1 KO mice showed severe pneumonia and similar inflammatory changes at 72 h after *K. pneumoniae* infection (Fig. 3). Viable bacterial counts in the lungs and serum showed similar kinetics in both IL-1 KO mice and WT mice. There was no apparent difference in the micropathological findings of the lungs between IL-1 KO and WT mice (Fig. 4). Inoculation with *K. pneumoniae* strain DT-S resulted in a partial...
**Fig. 1.** Survival rates of mice after pulmonary infection caused by *K. pneumoniae*. Each mouse was inoculated intranasally with 20 μl of bacterial suspension. Inoculated bacterial doses and bacterial strains were as follows: (a) $1.7 \times 10^2$ c.f.u. per mouse, strain T-113; (b) $3.6 \times 10^3$ c.f.u. per mouse, strain T-113; (c) 23 c.f.u. per mouse, strain DT-S; and (d) $3.6 \times 10^3$ c.f.u. per mouse, strain DT-S. ●, IL-1 KO mice; ○, WT mice; n = 12 in each group.

**Fig. 2.** Viable bacterial number in lungs (a) and in blood (b) of mice after pulmonary infection with *K. pneumoniae*. Each mouse was inoculated intranasally with $2.0 \times 10^3$ c.f.u. of *K. pneumoniae* strain DT-S. Solid bars, IL-1 KO mice; open bars, WT mice; n = 12 in each group.
infiltration of inflammatory cells after 24 h infection. Thereafter, expansive and voluminous pneumonia characterized by the destruction of alveolar spaces was detected at 48 h after infection. Pathological findings also showed similar inflammatory responses in IL-1 KO and WT mice against pulmonary infection with *K. pneumoniae*. Although we hypothesized that IL-1 KO mice may develop more severe infection compared with WT mice after intranasal inoculation of *K. pneumoniae*, our findings suggest that IL-1 KO mice possess the same level of resistance against *K. pneumoniae* infection as present in WT mice.

To determine whether deficiency of IL-1 could influence the production of other cytokines, we measured the concentrations of various cytokines in the lung homogenates of IL-1 KO mice and WT mice. The concentrations of TNF-α in the lungs of IL-1 KO mice were significantly higher than those of WT mice (Fig. 5). However, the concentrations of other cytokines (IL-10, IL-12 and IFN-γ) showed similar kinetics in these two groups (data not shown). Interestingly, TNF-α levels in the lungs of IL-1 KO mice were significantly higher than those of WT mice at 24 h after inoculation. Therefore, we think that these higher concentrations of TNF-α observed in IL-1 KO mice may assist the defence mechanism against *K. pneumoniae* pneumonia.

We studied the levels of macrophage inflammatory protein-2 (MIP-2) in the lungs 6, 12, 24, 48 and 72 h after inoculation with *K. pneumoniae*. The results suggested that there was no significant difference in the production of MIP-2 between WT mice and IL-1 KO mice (data not shown). We also evaluated the leukocyte counts in the BALFs after inoculation with *K. pneumoniae* and found that the numbers of leukocytes in the BALFs of WT mice tended to be higher than those of IL-1 KO mice at a very early time point (3 h after inoculation). However, we could not find any significant difference in the leukocyte counts in the BALFs of WT mice and IL-1 KO mice at later time points (data not shown).

Rijneveld et al. (2001) reported that the survival rate of IL-1 receptor type 1 gene-deficient (R⁻/⁻) mice with *Streptococcus pneumoniae* pneumonia was not significantly different from that of WT mice. The survival rate of IL-1 R⁻/⁻ mice pretreated with anti-TNF-α antibody was significantly lower than that of IL-1 R⁻/⁻ mice without anti-TNF-α pretreat-
ment. They reported that TNF-α was more important in the defence mechanism against S. pneumoniae than IL-1. These studies suggested that compensatory production of TNF-α plays an important role in IL-1-deficient mice against S. pneumoniae pneumonia.

Laichalk et al. (1996) reported that administration of a TNF antagonist resulted in a significant reduction of neutrophils in BALFs, increased K. pneumoniae counts in BALFs and shortened the survival time of mice. On the other hand, administration of a TNF agonist resulted in a significant increase of neutrophils in BALFs, reduction of K. pneumoniae counts in BALFs and prolonged survival times of mice.

Since IL-1 and TNF-α can share similar proinflammatory effects in vivo, we determined the role of endogenous TNF-α on the defence mechanism in IL-1 KO mice by using a mAb against TNF-α. The results revealed that the survival rate of IL-1 KO mice pretreated with anti-TNF-α mAb was significantly lower than that of BSA-treated IL-1 KO mice. The survival rate of WT mice pretreated with anti-TNF-α mAb was lower than that of BSA-treated IL-1 KO mice (Fig. 6). There was no significant difference in survival between IL-1 KO and WT mice both treated with anti-TNF-α mAb, thus showing the limited role of IL-1 in the host response to K. pneumoniae infection and confirming the previously described results. These data suggest that TNF-α plays a more important role in the defence mechanism against K. pneumoniae pneumonia than IL-1.

Yamada et al. (2000) studied the role of IL-1 in mice infected with Mycobacterium tuberculosis by using the same strain of IL-1 KO mice used in the present study. They demonstrated significantly higher numbers of M. tuberculosis in the lungs of IL-1 KO mice than in WT mice and impaired nitric oxide production in IL-1 KO mice. Based on these data, they

Fig. 4. Histopathological findings from lungs of IL-1 KO mice and WT mice after infection with K. pneumoniae. Each mouse was inoculated intranasally with $2 \times 10^5$ c.f.u. of K. pneumoniae strain DT-S. Lungs of WT mice (a) and IL-1 KO mice (b) 24 h after inoculation with K. pneumoniae strain DT-S showed similar inflammatory changes, i.e. bronchopneumonia and the same level of inflammatory-cell infiltration. Lungs from WT mice (c) and IL-1 KO mice (d) 48 h after infection also showed similar histopathological findings, such as expansive and voluminous pneumonia associated with destruction of alveolar spaces. Haematoxylin and eosin stain was used; n = 2 in each group. Bars, 1 cm.
concluded that IL-1 is important for the generation of early phase protective immunity against mycobacterial infection. However, they also pointed out that TNF-α mRNA expression was maintained within the normal range in IL-1 KO mice and suggested that TNF-α production may increase to compensate for the lack of IL-1 in IL-1 KO mice. Considered together with our results, we think that compensatory production of TNF-α in IL-1 KO mice may not always occur but that TNF-α is induced in some specific conditions, such as *K. pneumoniae* infection, as found in this study, and *P. aeruginosa* infection, as reported by Schultz et al. (2002).

In conclusion, our results suggest that IL-1 KO mice exhibit the same level of resistance against *K. pneumoniae* infection as WT mice. The mechanism of this phenomenon may be the compensatory enhanced production of TNF-α in IL-1 KO mice.

**REFERENCES**


Role of IL-1 in pneumonia


