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Mice Deficient in γδ T Cells Are Resistant to Lethal Infection with *Salmonella choleraesuis*

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Mice deficient in γδ T cells were resistant to the gram-negative bacteremic sepsis caused by infection with a high dose of *Salmonella choleraesuis* accompanied by impaired tumor necrosis factor alpha production in vivo and in vitro. γδ T cells may play a role in the pathogenesis of lethal infection with *S. choleraesuis*.

There is convincing evidence that γδ T cells play important roles in host defense mechanisms against microbial pathogens (1, 5–9, 11, 13, 14, 16). Recently, mice deficient in γδ T cells (T-cell receptor [TCR] $\delta^{-/-}$ mice) because of TCR δ gene mutations have been generated; these mutant mice provide a useful tool for studying the roles of γδ T cells in host defense (17). *Salmonella choleraesuis* is a causative agent for bacteremia not only in swine (original host) but also in humans and mice (15). Therefore, this organism is a good model for investigating host defense mechanisms against bacteremia. In this study to elucidate the roles of γδ T cells in protection and the pathogenesis of lethal infection with *S. choleraesuis*, TCR $\delta^{-/-}$ mice were infected with lethal doses of *S. choleraesuis*.

TCR $\delta^{-/-}$ mice, which are devoid of the gene encoding the δ gene of TCR, and littermate (TCR $\delta^{+/-}$) mice have been described previously (12). *S. choleraesuis* serovar *choleraesuis* 31N-1 was used as the avirulent strain, and strain RF-1 (15) was used as the virulent strain. Nonadherent peritoneal exudate cells (PEC) were stained with fluorescein isothiocyanate-conjugated anti-TCR γδ monoclonal antibody (MAb) GL3 (Phar Mingen, San Diego, Calif.) and phycoerythrin-conjugated anti-Thy1.2 MAb (Caltag Laboratories, Inc., South San Francisco, Calif.). The stained cells were analyzed with a FACScan (Becton Dikinson, San Jose, Calif.).

To confirm the lack of appearance of γδ T cells during salmonellosis in TCR $\delta^{-/-}$ mice, flow cytometric analysis of γδ TCR expression in TCR $\delta^{-/-}$ mice was carried out on day 6 after intraperitoneal infection with a sublethal dose (2.5×10^6 CFU) of *S. choleraesuis* 31N-1. Uninfected control mice injected with phosphate-buffered saline (pH 7.2) were used in parallel. A typical result for five mice is shown in Fig. 1. The proportion of γδ T cells in the peritoneal cavities of TCR $\delta^{+/-}$ mice increased significantly, from <1.5% before infection to 7.2% on day 6 after infection with *S. choleraesuis*. On the other hand, no γδ T cells were detected in the peritoneal cavities of TCR $\delta^{-/-}$ mice during salmonellosis.

There was no significant difference in the numbers of adherent cells for TCR $\delta^{-/-}$ mice and TCR $\delta^{+/-}$ mice. The number

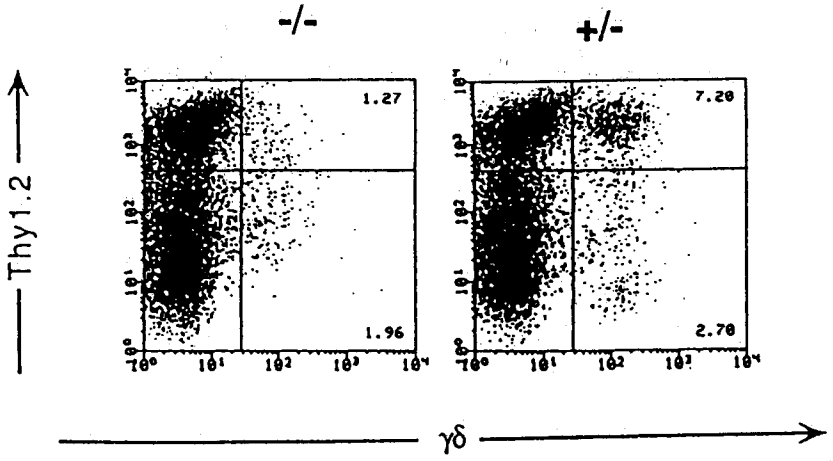

FIG. 1. Expression of γδ TCR on nonadherent PEC in TCR $\delta^{-/-}$ mice and TCR $\delta^{+/-}$ mice on day 6 after infection with *S. choleraesuis*. Mice were inoculated intraperitoneally with *S. choleraesuis* 31N-1 (2.5×10^6 CFU); 6 days later, PEC that did not adhere to culture dishes were counted. Nonadherent PEC in TCR $\delta^{-/-}$ and TCR $\delta^{+/-}$ mice (6 days after infection with *S. choleraesuis*) were stained with fluorescein isothiocyanate-conjugated anti-TCR γδ MAb and phycoerythrin-conjugated anti-Thy1.2 MAb and analyzed with a FACScan. The difference in cell number between TCR $\delta^{-/-}$ mice and TCR $\delta^{+/-}$ mice was statistically significant ($P < 0.01$).

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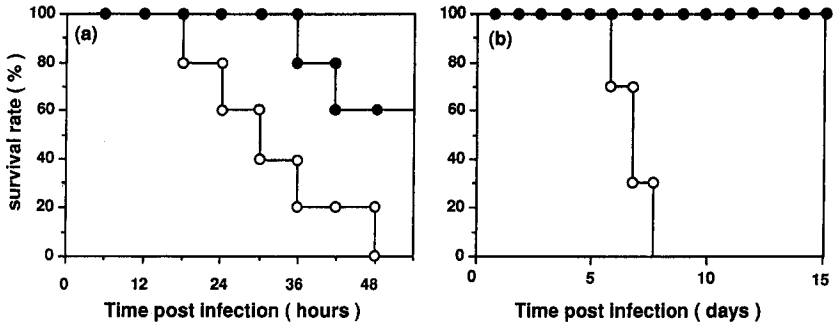

FIG. 2. Survival rates of TCR $\delta^{-/-}$ mice (●) and TCR $\delta^{+/-}$ mice (○) after challenge with lethal doses of *S. choleraesuis*. Groups of 10 mice were challenged intraperitoneally with 10^7 CFU of *S. choleraesuis* 31N-1 (a) or 10^4 CFU of strain RF-1 (b).

of nonadherent PEC in TCR $\delta^{+/-}$ mice ($7.8 \times 10^6 \pm 1.2 \times 10^6$ cells) was significantly higher than that in TCR $\delta^{-/-}$ mice ($3.9 \times 10^6 \pm 0.8 \times 10^6$ cells). No proportional change in CD3-positive and -negative cells was observed among TCR $\delta^{-/-}$ and TCR $\delta^{+/-}$ mice infected with *S. choleraesuis* 31N-1. For CD3-positive cells, the proportion of $CD4^-$ $CD8^-$ cells in the peritoneal cavity was significantly lower in TCR $\delta^{-/-}$ mice (31.0% ± 4.2%) than in TCR $\delta^{+/-}$ mice (42.8% ± 5.5%; $P < 0.05$), while the proportions of $CD4^+$ $CD8^-$ and $CD4^-$ $CD8^+$ cells were significantly higher in TCR $\delta^{-/-}$ mice (52.2% ± 4.3% and 17.2% ± 3.3%, respectively) than those in TCR $\delta^{+/-}$ mice (42.2% ± 4.5% and 10.8% ± 2.6%, respectively; $P < 0.05$) on day 6 after infection.

To compare the resistance against *S. choleraesuis* infection of TCR $\delta^{+/-}$ mice and TCR $\delta^{-/-}$ mice, the survival rates of those mice after intraperitoneal challenge with lethal doses of *S. choleraesuis* 31N-1 were examined. As shown in Fig. 2a, all 10 TCR $\delta^{+/-}$ mice died within 48 h of infection with strain 31N-1 (10^7 CFU or two 50% lethal doses [LD_{50}]), whereas 6 of 10 TCR $\delta^{-/-}$ mice survived beyond 10 days after infection ($P < 0.01$, by generalized Wilcoxon test). We next compared the resistance of TCR $\delta^{-/-}$ mice and TCR $\delta^{+/-}$ mice against infection with a virulent *S. choleraesuis* strain, RF-1. As shown in Fig. 2b, all 10 TCR $\delta^{-/-}$ mice survived, whereas all 10 TCR $\delta^{+/-}$ mice died within 8 days of infection with 10^3 CFU (10 LD_{50}) of RF-1. Thus, TCR $\delta^{-/-}$ mice show resistance to lethal infection with either an avirulent strain (31N-1) or a virulent strain (RF-1) of *S. choleraesuis*.

Infections with lethal doses of gram-negative bacteria induce excessive tumor necrosis factor alpha (TNF-α) production (2, 3), which often causes the immunopathological consequence of inflammatory responses (19). To assess whether death caused by infection with a lethal dose of *S. choleraesuis* 31N-1 is associated with excessive TNF-α production, we compared the TNF-α titers in sera from the TCR $\delta^{+/-}$ mice which died within 48 h of infection, as measured by enzyme-linked immunosorbent assay with a TNF-α kit (Factor-Test mTNF-α; Genzyme, Cambridge, Mass.) at the indicated times after intraperitoneal infection with *S. choleraesuis* 31N-1 (10^7 CFU). As shown in Fig. 3a, the production of TNF-α was significantly increased in the sera of TCR $\delta^{+/-}$ mice by 24 h after infection with *S. choleraesuis*, while no such increase was evident in the sera of TCR $\delta^{-/-}$ mice ($P < 0.01$). Since TNF-α is known to be produced mainly by macrophages (3), the capacity of macrophages to produce TNF-α in vitro in response to heat-killed *S. choleraesuis* 31N-1 (HKS) was compared for TCR $\delta^{-/-}$ and TCR $\delta^{+/-}$ mice. For these experiments, adherent PEC (10^5) obtained from nonprimed TCR $\delta^{-/-}$ and TCR $\delta^{+/-}$ mice were incubated for the indicated times in media containing HKS (corresponding to 2.5×10^5 CFU of viable *S. choleraesuis* 31N-1). Figure 3b presents the kinetics of TNF-α production in vitro in response to HKS by macrophages of each group. The level of TNF-α production by macrophages from TCR $\delta^{+/-}$ mice was significantly higher than that by macrophages from TCR $\delta^{-/-}$ mice at 3 h after stimulation with HKS ($P < 0.01$).

There are several lines of evidence that a significant fraction of γδ T cells are specialized in recognizing heat shock protein 60 derived from microorganisms or mammalian cells (4, 9, 14, 18). We have previously reported that the γδ T cells induced in the peritoneal cavity after infection with *Listeria monocytogenes* produce a significant level of gamma interferon in response to heat shock protein 60 (10). Therefore, we speculate

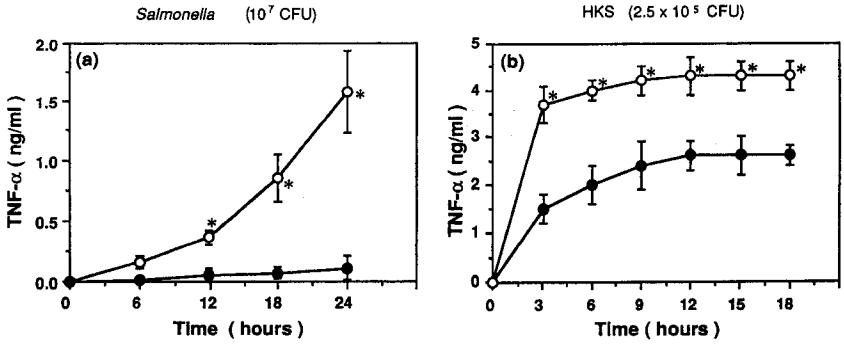

FIG. 3. Kinetics of salmonella-induced TNF-α production in vivo and in vitro of TCR $\delta^{+/-}$ mice (○) and TCR $\delta^{-/-}$ mice (●). TNF-α production in the sera of TCR $\delta^{-/-}$ and TCR $\delta^{+/-}$ mice after intraperitoneal injection with 10^7 CFU of *S. choleraesuis* (a) and by peritoneal macrophages of TCR $\delta^{-/-}$ and TCR $\delta^{+/-}$ mice after stimulation with HKS (b). Data were obtained from one of two experiments and are expressed as the means ± standard deviations of four mice. *, statistically significant difference in TNF-α levels between TCR $\delta^{-/-}$ mice and TCR $\delta^{+/-}$ mice ($P < 0.01$).

that γδ T cells produce gamma interferon in response to endogenous or exogenous heat shock proteins induced by various stress conditions in naive mice and prime macrophages at steady levels of activation for TNF-α production beforehand. However, because γδ T cells are present only in much smaller numbers in peripheral lymphoid tissues, it is unlikely that only γδ T cells play a priming role in TNF-α production by macrophages. Alternatively, it is possible that macrophages stimulated by γδ T cells preferentially produce interleukin-12, which in turn stimulates natural killer cells for gamma interferon production. Further studies of the level of macrophage activation in TCR $\delta^{-/-}$ mice are required to clarify this possibility.

In conclusion, we found that TCR $\delta^{-/-}$ mice were resistant to lethal infection with *S. choleraesuis*. Excessive TNF-α production, which is often detrimental in the pathogenesis of gram-negative bacteremia, was not evident in TCR $\delta^{-/-}$ mice after infection. The impaired capacity of macrophages in TCR $\delta^{-/-}$ mice for TNF-α production may be at least partly ascribed to the resistance in these mice against lethal infection with *S. choleraesuis*.

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