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Interleukin-10 Protects Neonatal Mice from Lethal Group B Streptococcal Infection

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We investigated the role of interleukin-10 (IL-10) in a neonatal mouse model of lethal group B streptococci (GBS) sepsis. Plasma IL-10 levels significantly increased at 24 and 48 h after GBS inoculation. Neutralization of IL-10 with specific antibodies had no effect on lethality. Administration of recombinant IL-10 at 20 or 4 h before challenge, but not at later times, resulted in decreased tumor necrosis factor alpha levels and improved survival. IL-10 could be potentially useful for the treatment of GBS sepsis.

Interleukin 10 (IL-10), initially described as cytokine synthesis inhibitory factor, is an important regulator of the functions of lymphoid and myeloid cells (14). This cytokine, mainly produced by the Th2 subset of helper T cells (6, 21), macrophages (4), and B cells (16, 17), exhibits anti-inflammatory and immunosuppressive functions (14). Recombinant IL-10 (rIL-10) protects adult mice from lipopolysaccharide (LPS)- or staphylococcal enterotoxin B-induced lethal shock, presumably through its ability to reduce the release of pathophysiologic mediators, including tumor necrosis factor alpha (TNF-α) and gamma interferon (2, 7, 8, 11, 19).

Little is known about the role of IL-10 in neonatal septic shock, a major cause of morbidity and mortality. Therefore, we investigated the effects of IL-10 blockade and rIL-10 administration in mouse pups infected with group B streptococci (GBS), the most frequent cause of neonatal sepsis (1).

Neonatal (±24 h old) BALB/c mice were used. Parental mice were obtained from Harlan-Nossan (Milan, Italy). Pups from each litter were randomly assigned to control or experimental groups, marked, and kept with the mother. GBS strain COH1, a highly virulent strain originally isolated from a septic neonate, was kindly provided by Craig Rubens, University of Washington, Seattle (13). Bacteria were grown to the late logarithmic phase in Todd-Hewitt broth (Difco, Diagnostic International Distribution, Milan, Italy) diluted in phosphate-buffered saline (PBS; 0.01 M phosphate, 0.15 M NaCl [pH 7.2]) and were inoculated subcutaneously (25 μl) in neonatal mice. Inocula were adjusted photometrically to give 15 or 150 CFU in 25 μl of PBS, which produced 20 to 35 and 70 to 90% mortality, respectively, over a 96-h period. Deaths rarely occurred after this time (12).

To measure circulating levels of TNF-α and IL-10, animals were killed by decapitation under ether anesthesia at different times after challenge with 150 CFU. Mixed venous-arterial blood was collected in heparinized containers and centrifuged after saving 10 μl for colony counts. The latter were performed by standard pour plate methods. Pooled plasma from four animals was stored at −70°C until assayed for TNF-α and IL-10 levels. TNF activity was assessed by cytotoxicity in WEHI 164 clone 13 cells and expressed in units per milliliter, exactly as described previously (5).

Plasma IL-10 values were below the limit of detection of the assay in uninfected controls (not shown). In infected animals, IL-10 plasma levels significantly increased to 302 ± 183 and 1,330 ± 947 pg/ml at 24 and 48 h, respectively, after GBS challenge. In order to assess the role of endogenous IL-10 in GBS-induced lethality, pups were inoculated, at 6 h before challenge, with neutralizing rat anti-mouse IL-10 monoclonal antibodies (MAbs). These were purified from culture supernatants of hybridoma SXCl (15) (kindly provided by L. Romani, University of Perugia, Perugia, Italy) and inoculated subcutaneously in 25-μl volumes. Control animals received an equal amount of PBS vehicle. Pretreatment with anti-IL-10 MAb (125 or 250 μg per pup) at 6 h before challenge totally prevented the increase in circulating IL-10 levels but did not significantly affect survival or blood colony counts in pups infected with 15 or 150 CFU (not shown). These doses produced 21 and 79% lethality, respectively, in controls (not shown).

Since IL-10 protected mice from death by endotoxin (8, 11, 19) or staphylococcal enterotoxin B (7), we investigated whether administration of mouse rIL-10 (0.5 U/ng [Genzyme]) would be beneficial in our model. The rIL-10 was inoculated subcutaneously neat or diluted in PBS with 0.1% bovine serum albumin in 25- or 50-μl volumes at various times before challenge with 150 CFU. Pretreatment with 25 ng per pup at 20 h before challenge resulted in significant protection (P < 0.05), while no effect was observed with lower doses (Table 1). The time of rIL-10 pretreatment relative to challenge was critical (Table 2). No effects were noted when rIL-10 was given at the time of challenge or at later times. An increase in survival time, but not permanent protection, was observed when rIL-10 was given at 4 h before challenge (Table 2). Since IL-10 can modulate host defenses against infection (9, 10, 18), it was of interest to ascertain whether treatment with rIL-10 would affect the severity of infection. Colony counts performed on
blood samples obtained at 12, 24, and 48 h after challenge in animals treated with rIL-10 (25 ng) or anti-IL-10 (125 μg at 20 h before challenge) were not significantly different from those of control animals (not shown).

Because IL-10 can modulate TNF-α production, an important pathophysiologic mediator in this model (20), the effects of rIL-10 or anti-IL-10 on circulating TNF-α levels in septic pups were investigated. Figure 1 shows that TNF-α elevations were measured at 12, 24, and 48 h after GBS challenge and in control animals, confirming our previous results (12). Pretreatment with IL-10 significantly (P, 0.05) decreased TNF-α levels at 24 h. Pretreatment with anti-IL-10 (125 μg per pup), however, did not affect plasma TNF-α (Fig. 1). In further experiments, increasing the dose of anti-IL-10 to 250 μg per pup also had no significant effect on TNF-α levels.

The present data demonstrate for the first time induction of IL-10 by GBS, as evidenced by increased plasma levels in neonatal mice at 24 and 48 h after infection. Whether these increased endogenous IL-10 levels are essential in determining neonatal responsiveness to GBS remains to be clarified. Anti-IL-10 treatment with neutralizing anti-IL-10 MAb did not modulate survival or modify CFU. Thus, the IL-10 elevations in GBS-infected pups had apparently no major influence on septic outcome. High levels of IL-10 have been observed during the initial phase of fulminant meningococcemia septic shock (3). However, the high levels of IL-10 were found both in survivors and in those who died. In addition, the IL-10 levels correlated weakly with the severity of the disease and the prognostic score.

Our previous studies have demonstrated that the GBS neonatal model of infection is TNF-α dependent in that administration of anti-TNF-α is greatly protective in this model (20). Thus, we were particularly interested in determining the effects of IL-10 blockade or rIL-10 treatment on TNF-α levels. Indeed, previous studies have shown that IL-10 is a potent inhibitor of TNF-α production (4, 8).

In this study, neutralizing anti-IL-10 antibodies did not modify TNF-α levels in GBS-infected pups. Conversely, pretreatment with rIL-10 was quite effective in decreasing plasma TNF-α. This suggests that (i) unlike IL-6 (12), endogenous IL-10 is not involved in regulation of TNF-α production in GBS infection and (ii) delayed appearance of endogenous IL-10 relative to TNF may at least partially account for this lack of regulatory effects. Unlike TNF-α levels, which peaked at 24 h and were declining by 48 h, the IL-10 levels were increased at 24 h and were continuing to rise at 48 h.

Prophylactically, rIL-10 when given up to 20 or 4 h before challenge was protective against GBS infections, and there was a correlation between improved survival and TNF suppression. However, we cannot ascertain from the present data whether

### TABLE 1. Effects of rIL-10 on lethality induced by GBS in neonatal mice

<table>
<thead>
<tr>
<th>Expt no.</th>
<th>IL-10 dose (ng/mouse)</th>
<th>No. dead/total (%) at h:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>24</td>
</tr>
<tr>
<td>1</td>
<td>0 (vehicle)</td>
<td>3/14 (21)</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>1/14 (7)</td>
</tr>
<tr>
<td>2</td>
<td>0 (vehicle)</td>
<td>2/14 (14)</td>
</tr>
</tbody>
</table>

a Mouse pups were challenged with 150 CFU of strain COH1.

b P < 0.05 by Fisher exact test, compared with the respective vehicle controls.

### TABLE 2. Effects of time of administration of rIL-10 on lethality induced by GBS in neonatal mice

<table>
<thead>
<tr>
<th>Expt no.</th>
<th>Time of administration (h)</th>
<th>No. dead/total (%) at h:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>24</td>
</tr>
<tr>
<td>1</td>
<td>– (vehicle)</td>
<td>2/14 (14)</td>
</tr>
<tr>
<td></td>
<td>–4</td>
<td>0/14 (0)</td>
</tr>
<tr>
<td></td>
<td>–24</td>
<td>2/14 (14)</td>
</tr>
<tr>
<td>2</td>
<td>– (vehicle)</td>
<td>3/14 (21)</td>
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<tr>
<td></td>
<td>0</td>
<td>2/14 (14)</td>
</tr>
<tr>
<td></td>
<td>+24</td>
<td>2/14 (14)</td>
</tr>
</tbody>
</table>

a Mouse pups were challenged with 150 CFU of strain COH1.

b Relative to time of challenge.

c P < 0.05 by Fisher exact test, compared with the respective vehicle controls.
this was the only or even the most essential anti-inflammatory action of IL-10. The sustained protection for up to 20 h before challenge suggests a profound change in the host inflammatory response similar to that initiated by endotoxin tolerance (22). This requirement for a longer period of prophylaxis is quite different from endotoxin shock in which IL-10 could be protective when given simultaneously or immediately post-LPS (11). This difference may be accounted for by differences in the inflammatory response and kinetics of cytokine production after bolus injection of LPS versus infection with a small number of live virulent bacteria.

Regardless of the mechanisms of action, the present data clearly demonstrate a protective role of IL-10 in a severe gram-positive sepsis model. Since IL-10 has been shown at pharmacological doses to be highly effective in protection against staphylococcal enterotoxin B as well as endotoxemia, this suggests therapeutic application. It will be interesting to compare the efficacy of IL-10 with that of other potential candidates for treatment of sepsis.

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