Effects of anti-cytokine treatments in neonatal sepsis models

G. TETI - G. MANCUSO - V. CUSUMANO
G. BLANDINO* - M.T. FERA - M. CARBONE

INTRODUCTION

Infection with group B streptococcus (GBS) is associated with high morbidity and mortality rates in newborn infants. The clinical picture of "early-onset disease", occurring during the first week of life, has some common features with gram-negative and endotoxin shock. Septic shock has traditionally been recognized as a consequence of infection with gram-negative bacteria, but it may also be caused by gram-positive organisms. Many of the pathophysiologic changes of gram-negative shock have been related to endotoxin, the lipopolysaccharide (LPS) component of the bacterial cell wall. It is now believed that the toxic effects of LPS are mediated by endogenous cytokines such as tumor necrosis factor alpha (TNF-α), and interleukin-1 (IL-1). Indeed, while the activity of these mediators can be beneficial to the host in localized or intracellular infections, overproduction of circulating cytokines may lead to shock and death. The role of cytokines in gram-positive sepsis has only recently been addressed. Nathanson et al. have shown that the hemodynamic changes observed in human septic shock can be reproduced in dogs challenged with not only TNF-α or endotoxin, but also Staphylococcus aureus. Also, Wakabayashi et al. have reported elevations in circulating levels of TNF-α and IL-1β in rabbits injected with killed Staphylococcus epidermidis.

We have shown that TNF-α plays a pathophysiologic role in sepsis induced in rat pups by the intraperitoneal (i.p.) inoculation with a small number of virulent GBS. In this model significant elevations of plasma and/or spleen levels of TNF-α, IL-6, IFN-γ and IL-1α were detected. Cytokines were produced in a characteristic sequence (i.e. TNF-α followed, in the order, by IL-1 and IL-6, and, still later by IFN-γ). In addition TNF-α was at least in part responsible for the induction of IL-6 and IFN-γ.

In further studies, we have observed that heat-killed GBS, inoculated intracardially (i.c.) in neonatal rats, were effective in causing TNF-α production and TNF-α dependent lethality. In fact, in this model, lethality could be completely prevented by the administration of anti-TNF-α antibodies. This is in contrast with results of the live bacteria model, in which the same treatment was effective only in prolonging survival time. It has been shown that the site of inoculation and the compartmentalization of the inflammatory reaction can influence the levels of circulating cytokines and the response to anticytokine therapy. Since live GBS were inoculated i.p. and killed GBS were inoculated i.c. in our previous studies, we sought to ascertain if differences in the response to anti-TNF were related to the use of live versus killed bacteria or to the different routes of inoculation.

MATERIALS AND METHODS

Neonatal animals

Neonatal (24 to 48h old) Sprague-Dawley rats were used in this study. Parental animals were obtained from Charles River Italia (Calco, Italy). Pups from each litter were randomly assigned to control or experimental groups, marked and kept with the mother.
Bacteria

GBS-strains COH-1, originally isolated from a septic neonate, was kindly provided by C.E. Rubens, University of Washington, Seattle; bacteria were grown to the late logarithmic phase in Todd-Hewitt broth (Difco) and diluted to the appropriate concentration in phosphate-buffered saline (PBS) before inoculation into neonatal animals. Some bacteria were washed with H2O, killed by heating (80°C for 30 min.), and lyophilized.

Reagents

Rabbit anti-mouse TNF-α serum was purchased from Genzyme (Cinisello Balsamo, Italy).

Data expression and statistical analysis

Differences in lethality were assessed by the two-tailed Fisher exact test. With this test, differences were considered significant when P values were <0.05.

RESULTS

Table 1 shows that the administration of anti-TNF-α antibodies 6 h before challenge, extended survival time in pups infected i.c., i.p. or subcutaneously (s.c.). However, permanent protection was not afforded since differences in lethality were no longer significant at 96 h after challenge. On the contrary, complete protection by anti-TNF-α antibodies was observed in neonatal rats challenged with killed GBS.

DISCUSSION

Our data show that TNF-α has a detrimental role in both live and killed bacteria models of neonatal GBS disease. However the degree of protection afforded by anti-TNF-α antibodies was much higher in the killed GBS model. It is possible that these differences reflect different pathophysiologic mechanisms in these animal models. It is possible that the live bacteria model more appropriately reflects naturally occurring sepsis, while bolus injection with heat-killed bacteria may induce responses similar to those observed in endotoxin shock.

The findings reported here bear some resemblance to those of a recent study investigating the role of IL-6 in a neonatal mouse model. Prophylaxis with the recombinant IL-6 significantly extended survival, but did not afford permanent protection in animals infected with live bacteria. However the same pretreatment totally abrogated lethality in killed GBS or endotoxin models (G. Teti and G. Mancuso, unpublished).

Little is known of bacterial components

<table>
<thead>
<tr>
<th>Challenge</th>
<th>Pretreatment</th>
<th>Route of inoculation</th>
<th>24h</th>
<th>No. dead/total no. (%) 48h</th>
<th>72h</th>
<th>96h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live GBSb</td>
<td>Anti-TNF-α</td>
<td>i.v.</td>
<td>0/15(0)</td>
<td>2/15(13)c</td>
<td>6/15(40)c</td>
<td>14/15(93)</td>
</tr>
<tr>
<td>Normal serum</td>
<td>3/15(20)</td>
<td>11/15(73)</td>
<td>15/15(100)</td>
<td>15/15(100)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Live GBS</td>
<td>Anti-TNF-α</td>
<td>i.p.</td>
<td>0/15(0)</td>
<td>1/15(6)c</td>
<td>5/15(33)c</td>
<td>12/15(80)</td>
</tr>
<tr>
<td>Normal serum</td>
<td>2/15(13)</td>
<td>8/15(53)</td>
<td>14/15(93)</td>
<td>15/15(100)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Live GBS</td>
<td>Anti-TNF-α</td>
<td>s.c.</td>
<td>0/15(0)</td>
<td>1/15(6)c</td>
<td>4/15(26)c</td>
<td>13/15(86)</td>
</tr>
<tr>
<td>Normal serum</td>
<td>1/15(6)</td>
<td>7/15(46)</td>
<td>14/15(93)</td>
<td>15/15(100)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heat-killed GBS</td>
<td>Anti-TNF-α</td>
<td>i.v.</td>
<td>0/15(0)c</td>
<td>0/15(0)c</td>
<td>0/15(0)c</td>
<td></td>
</tr>
<tr>
<td>Normal serum</td>
<td>8/15(53)</td>
<td>13/15(86)</td>
<td>15/15(100)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Rat pups were injected intraperitoneally with 50 μl of anti-TNF-α or normal rabbit serum 6 hours before challenge.
- One lethal dose 90 of COH-1 strain grown to the late log phase was used. The lethal dose 90 was 0.5, 1, and 1x10^6 CFU/ml for bacteria inoculated intravenously (i.v.), intraperitoneally (i.p.) and subcutaneously (s.c.), respectively.
- P < 0.05 by Fisher's exact test as compared with the respective control.
- Seventy mg/kg were inoculated in 50 μl of PBS.
other than LPS responsible for TNF-α release, including gram-positive components. Streptococcal lipoteichoic acid (LTA) can induce TNF-α production by human monocytes, although it is much less potent in this activity than LPS. However, the addition of anti-LTA antibodies or F(ab')₂ fragments markedly enhanced the aggregation of LTA receptors, as evidenced by indirect immunofluorescence and the release of tumor necrosis factor alpha and interleukin-1β. These findings suggest that aggregation of LTA receptors of monocytes is required for triggering marked cytokine responses. Both the type and group-specific polysaccharides of GBS can induce significant TNF-α responses and TNF-α dependent lethality in vivo. Collectively, our results are compatible with the hypothesis that, by increasing TNF-α levels, the type and group-polysaccharides and possibly LTA presented as a multivalent ligand on the bacterial surface, contribute to accelerating the development of shock and mortality during GBS sepsis. This notion would strengthen the argument for the therapeutic use of type-specific antibodies in the form of intravenous immunoglobulins or group-specific human monoclonal antibodies. Further studies, however, are needed to confirm our hypothesis.

The recognition of the paramount role of inflammatory mediators in the pathogenesis of sepsis and the increase in survival time produced by anti-TNF-α and IL-6 prophylaxis in septic neonatal animals suggest that these agents deserve some attention as possible adjunctive measures in the treatment of neonatal sepsis.

REFERENCES