basis. Thus, there is still no evidence to refute our earlier proposal that all such cultures are clonal (Lacey & Grinsted, 1973). Recent identification of a unique biochemical and genetic basis of the resistance gives further credibility to this notion.

If this view is correct, the trait of methicillin resistance provides an excellent background against which changes in genetic organization can be analysed.

One possibility that might be exploited arises from the observation that transposon Tn 551, coding for erythromycin resistance, inserts into the methicillin resistance gene and deletes its function (Berger-Bachi, 1983). It is possible that methicillin resistance could be controlled by the use of erythromycin. This is unlikely to be successful, however, as this transposon can also insert elsewhere in the chromosome.

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Anti-endotoxin immunotherapy in septic shock
Despite the relentless introduction of new antibiotics and the increasing sophistication of supportive care, Gram-negative septicemia continues to have a mortality of about 30%, increasing to 70% or more if shock supervenes (Kreger, Craven & McCabe, 1980). Much evidence is consistent with the view that bacterial endotoxin (lipopolysaccharide—LPS) is responsible, at least in part, for many of the pathophysiological features of septic shock (Rietschel et al., 1982), and lately there has been a resurgence of interest in the concept of anti-LPS immunotherapy as an alternative (or additional) strategy for the treatment of this condition. The experimental basis for this work has been reviewed elsewhere (Telzak & Wolff, 1985); it is the intention here to summarize the clinical experience that has been accumulated.

Two different approaches have been used to obtain an antiserum with anti-LPS activity. Braude et al. (1977) immunized healthy volunteers with a boiled whole cell vaccine of Escherichia coli J5, a rough mutant which seems to express LPS on its cell surface and which has been used extensively in animal studies of anti-LPS immunotherapy. Each volunteer donated a unit of serum before immunization to serve as a control, and a randomized double-blind study of passive immunotherapy in the treatment of Gram-negative septicemia was begun in 1977. The full results were published after 304 patients had been studied (Ziegler et al., 1982). Patients were entered into the trial if they were 'severely ill' with good clinical evidence of Gram-negative septicemia. They then received a single unit of J5 immune, or control, serum, as well as antibiotics and the best available supportive care. In 191 patients (63%) the diagnosis was confirmed by blood culture; a further 21 patients had Gram-negative organisms isolated from infected foci. The remaining 92 patients were excluded from further analysis. The control and immune groups were remarkably well matched, both in respect of the nature and severity of their infection, and the various risk factors known to affect outcome. The results were impressive: in the bacteraemic patients, the death rate was 38% of 100 recipients of control serum compared with 22 of 91 J5 recipients (P=0.04). The effect was particularly striking in the subset of patients with profound shock: the mortality was reduced from 76% to 46% (P=0.009). Only one important criticism can be levelled at this study, and that relates to the level of immunity to J5 prior to entry to the study. Naturally occurring antibody to J5 is present in most individuals to a variable degree (Mattsby-Baltzer & Alving, 1982). Patients with profound shock: the mortality was reduced from 76% to 46% (P=0.009). Only one important criticism can be levelled at this study, and that relates to the level of immunity to J5 prior to entry to the study. Naturally occurring antibody to J5 is present in most individuals to a variable degree (Mattsby-Baltzer & Alving, 1982). Patients with profound shock: the mortality was reduced from 76% to 46% (P=0.009). Only one important criticism can be levelled at this study, and that relates to the level of immunity to J5 prior to entry to the study. Naturally occurring antibody to J5 is present in most individuals to a variable degree (Mattsby-Baltzer & Alving, 1982). Patients with profound shock: the mortality was reduced from 76% to 46% (P=0.009). Only one important criticism can be levelled at this study, and that relates to the level of immunity to J5 prior to entry to the study. Naturally occurring antibody to J5 is present in most individuals to a variable degree (Mattsby-Baltzer & Alving, 1982). Patients with profound shock: the mortality was reduced from 76% to 46% (P=0.009). Only one important criticism can be levelled at this study, and that relates to the level of immunity to J5 prior to entry to the study. Naturally occurring antibody to J5 is present in most individuals to a variable degree (Mattsby-Baltzer & Alving, 1982). Patients with profound shock: the mortality was reduced from 76% to 46% (P=0.009).
coliform septicaemia is a life-threatening complication, and there is some evidence that the frequent episodes of 'culture negative fever' might be due to endotoxaemia (Harris et al., 1984). One hundred patients, most of whom had leukaemia, were randomized to receive a single unit of control or immune serum, given before they became neutropenic (McCutchan et al., 1983). Subsequent analysis showed that the J5 antiserum had no effect on the number of days with fever, or the total number of episodes (or fatal episodes) of Gram-negative bacteraemia. Two explanations were advanced for the failure of the study. Firstly, Gram-negative bacteraemia was infrequent: 16 episodes in 109 periods of neutropenia. Thus, a modest protective effect might have been overlooked (Type 2 error). This is true, but it does not explain the failure of the J5 serum to reduce the number of febrile days. The second suggestion was that the antiserum might have been no longer active by the time it was 'required', either because of natural catabolism or owing to neutralization by gut-derived endotoxin.

In an attempt to overcome this problem, in the most recent trial of prophylactic anti-J5 (Baumgartner et al., 1985) a unit of antiserum was given at randomization and thereafter every 5 days while the patient was considered at risk of Gram-negative infection. Moreover, additional units were given if blood loss required replacement therapy and if septic shock occurred. A total of 265 high risk patients were studied. Administration of J5 immune plasma had no effect on the frequency or severity of focal Gram-negative infections. Coliform septicaemia occurred only six times and could not be evaluated. However, septic shock was less common in the anti-J5 recipients (6 of 16 with Gram-negative infection, compared with 15 of 23, P = 0.049), and this finding was particularly striking in patients who had had major abdominal surgery (2 of 8 compared with 13 of 15, P = 0.006). Inevitably, there is concern that a large, heterogeneous group of patients might lead to uneven randomization and once again the level of natural J5 immunity was not determined. Nevertheless, the results are consistent with the view that, as might be expected, anti-J5 serum protects against the acquisition of Gram-negative infection but against its consequences: endotoxin-induced shock.

The second approach to the production of anti-LPS immune sera has been developed by a group in Natal, South Africa. They screened blood donors for natural antibody to LPS by using an ELISA in which the 'antigen' was a pool of 12 purified endotoxins (Gaffin et al., 1982). Donors identified in this way provided the source of anti-LPS specific globulin or antibody-rich freeze dried plasma. This material was used in an open trial of septic shock complicating obstetric conditions in 33 African patients (Lachman, Pitsoe & Gaffin, 1984). Immune antiserum recipients were given two units rapidly, followed by a continuous infusion of one unit, 4-hourly. Additional units were given 'if complications were imminent'. Control patients received supportive care and normal freeze dried plasma for resuscitation. Inadequate data are provided to indicate whether the two groups were appropriately matched. The overall mortality was 47% in the control group and 7% in the treated group (P < 0.01). In addition, the authors reported beneficial effects on mean arterial blood pressure and duration of hospital stay. Although these results are encouraging, a formal double-blind study of well matched patients would be required to strengthen the evidence. Indeed, it is of interest that a small double-blind study of intramuscular anti-LPS globulin in septicaemic neonates found no important difference between control and treated groups (Adhikari et al., 1985).

One additional report describes seven patients with Gram-negative sepsis who were given a commercial human IgG preparation shown to have a high titre of antibody to Lipid A (Marget et al., 1985). Four patients survived, and a randomized, double blind study has begun.

Of the two strategies discussed, the results obtained with the J5 antiserum seem most promising. The experience with anti-LPS globulin is more difficult to assess: fewer patients have been treated and the study design was not ideal. Two further questions arise: how does anti-LPS immunotherapy work, and will it be practicable?

Anti-J5 serum contains both IgG and IgM antibody directed against the structurally-conserved core glycolipid ('endotoxin'). It is not a potent opsonin; its advocates believe that its effects depend primarily upon IgM antibody neutralizing the toxic properties of LPS, perhaps by steric hindrance, although enhanced bacterial clearance may also play a part (Ziegler et al., 1973; Young, Stevens & Ingram, 1975; Sakulramrung & Domingue, 1985; Trautmann & Hahn, 1985). But although core glycolipid may provide the immunodominant epitope it is by no means clear that it is the antigen to which the protective antibody is directed; recent studies have failed to demonstrate significant binding of anti-J5 serum to heterologous LPS (Siber, Kania & Warren, 1985), and a Swedish
group have reported that patients with bacteremia respond by making an antibody to a 34 kilodalton outer membrane protein of Salmonella, not to core glycolipid (Brauner et al., 1985). Furthermore, re-examination of the data from the original study by Ziegler et al. (1982), suggests that protection from shock was related to the generation of the highly conserved Re antibodies, rather than the Rc core associated with E. coli J5 (Glauser, M. P., personal communication, 1986).

The basis of the protection given by anti-LPS globulin is equally unclear. It is an IgG preparation, and Gaffin has argued that not only is it easier to prepare than IgM, but it will remain longer in the circulation. It is claimed that by possessing determinants against core and surface structures it may act both by an anti-LPS neutralizing effect and also by promoting opsonisation and complement—mediated bacterial killing. However, some doubt has been cast on this argument by the preliminary results of a recent study of septic shock, in which a single unit of a J5 hyperimmune immunoglobulin offered no advantage over a control preparation (Calandra et al., 1986).

The practicability of passive immunotherapy as a method of treatment depends upon widespread availability of a standardized preparation, and no approach is satisfactory at present. Ideally, monoclonal antibodies will provide a solution, and indeed a human monoclonal has already been produced and appears effective (Teng et al., 1985). The use of anti-LPS immunotherapy is an exciting development that may have application in such diverse areas as burns, shock lung, and graft-versus-host disease (Cuevas et al., 1974; Meyrick, 1986; Moore et al., 1986). The next stage must be to establish adequate supplies of a well-characterized and standardized material, and to evaluate it in carefully designed clinical trials.

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References


Leading articles

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