Haematology and Blood Transfusion Vol. 29
Modern Trends in Human Leukemia VI
Edited by Neth, Gallo, Greaves, Janka

Gram-Negative Bacteraemia: New Therapeutic Possibilities with Anti-Endotoxin Antibodies

S. L. Gaffin[1]

A. Introduction

Gram-negative bacteria are coated by highly toxic and chemically stable lipopolysaccharide (LPS, endotoxin) which is partially shed when the bacteria are destroyed [1], e.g. by antibiotics in the blood of the patient. Since antibiotics have no effect on the LPS [2], this toxic LPS persists in the circulation of affected patients and causes much of the mortality and morbidity in gram-negative bacteraemia. Moreover, LPS is also present in the gut in large amounts and it may enter the circulation and cause toxic reactions when the gut is damaged by almost any mechanism, including radiotherapy of cancer patients [3, 4]. Normally small amounts of LPS leaving the gut are cleared by the reticuloendothelial system (RES), but in the case of damage to the gut lining the massive amounts of LPS involved may overwhelm the capacity of the RES. In addition, the immunosuppressive effect of ionizing radiation may deplete the body of anti-LPS antibodies, which are involved in part, in LPS detoxification.

A number of studies have shown that passive administration of anti-LPS antibodies into an animal or patient can reduce mortality and morbidity of endotoxaemia [5–7]. For clinical exploitation however, the development of a source of adequate amounts of human anti-LPS is a fundamental problem. Active LPS is so toxic it cannot be used directly as an immunogen in humans. McCutchan, Ziegler, Braude and colleagues actively immunized volunteers with an Escherichia coli preparation containing an LPS mutant to produce an anti-"core" LPS hyperimmune serum in a most important study [8]. This serum significantly reduced the mortality of patients suffering from shock and bacteraemia. However, this serum is not at present available for general use, in part because of problems associated with the active immunization route. Moreover, there may be therapeutic benefit in using a different type of anti-LPS which is a mixture of antibodies, some directed to the core and others to the surface regions of LPS [9]. In order to overcome these problems we found that some plasma units donated to blood banks contained high concentrations of "natural" anti-LPS IgG [10]. We developed an ELISA for efficient screening of all blood units donated to a blood bank [11] in order to isolate them and have used such anti-LPS antibodies therapeutically.

In this paper I review our animal and human experiments which led to the present conclusion that anti-LPS produced by our simple blood bank screening procedure can significantly reduce morbidity and mortality in septic shock. Such anti-LPS might be expected to beneficially augment an immuno-suppressed patient's antibody stores.

1 Department of Physiology, University of Natal Medical School, Durban, South Africa

B. Human and Equine Anti-LPS Preparations

I. Human Anti-LPS

Plasma units donated to a blood bank (Natal Blood Transfusion Service, Pinetown, South Africa) were screened by an ELISA for the presence of high concentrations (> 40 μg/ml) of anti-LPS IgG [11]. Approximately 7% of donations had such antibodies in South Africa and Israel [12]. One full-time technician using semi-automated apparatus can process 200 samples per day with a yield of 14/day × 300 days/year = 4200 high titer plasma units (anti-LPS) per year. We found that adult patients in septic shock require 2–10 units (mean = 5–6) anti-LPS as a therapeutic dose [13]. Therefore 760 adult unit doses per year can be produced. We have pooled several hundred high titer units and fractionated them to produce an LPS-specific immunoglobulin with standardized properties. However, this was an intramuscular globulin preparation. We expect shortly to produce an intravenous form of this LPS-specific globulin. As an alternative anti-LPS source for i.v. administration, individual high titer plasma units have been freeze-dried (FDP) [13]. These have the added advantage of causing no licensing problems since this FDP is produced entirely by the routine licensed methods. Merely an additional test was done to determine whether the plasma contained LPS-specific IgG. There was no correlation between the presence of high concentrations of specific IgM and IgG in these plasma units. Since the IgG antibody is easier to prepare and has a longer half-life, we selected it rather than the IgM. While the anti-LPS FDP is more convenient and faster to prepare than the immunoglobulin, it has the disadvantage of not being standardized since each plasma unit contains a somewhat different distribution of anti-LPS antibodies from other units. In one unit they may be directed to LPS from *Shigella,* in another to *E. coli* and *Salmonella typhosa,* and still another mainly to *Klebsiella* and *Pseudomonas.* A pooled preparation containing mixtures of antibodies to a wide range of LPS sources is desirable. Practically, we found in our pooled LPS-specific globulin the following relative anti-LPS binding activities in decreasing order [12]: *Shigella flexneri, Salmonella abortus equi, Salmonella typhimurium, Klebsiella pneumoniae, Salmonella enteritidis, E. coli* 026 : B6, *Salmonella typhosa, E. coli* 055 : B5, *E. coli* 0111 : B4, *E. coli* 0128 : B12, *Salmonella minnesota, Salmonella marcescens, E. coli* 0127 : B8. Significantly almost the same *order* of anti-LPS binding activities was found in blood samples obtained from Australia, Papua New Guinea and England, but at much lower levels of activity[2].

It was expected that prior exposure of the donor to subclinical gram-negative bacterial infections led to the distribution of anti-LPS antibodies in any particular plasma sample. However, no correlation was seen in our pooled anti-LPS between the antibody level to LPS from a particular bacterial strain and the frequency of this strain's being isolated by the hospitals serving the same area in which the donors live. As an example, *Shigella flexneri* was isolated only rarely in King Edward VIII Hospital, Durban, but pooled anti-LPS from donors in the Durban region had its highest activity against *Shigella flexneri* LPS.

II. Animal Anti-LPS

Equine anti-LPS hyperimmune plasma is currently produced on a large scale by the plasmapheresis of suitably immunized horses (ATOX Pharmaceutical Company, 14 Old Main Road, Gillitts, 3600 South Africa). The original minimum concentrations were 150 μg/ml [14], i.e. almost four times that acceptable for humans. Owing to improvements in methods, it is now produced at a concentration of 1500 μg/ml. Currently it is produced as a frozen solution and thawed just prior to administration. Specific IgG in it also bind to a wide range of gram-negative bacteria.

---

2 Courtesy of Dr. Brian Feery, Commonwealth Serum Laboratories, Parkville, Australia

C. Summary of Animal Studies Using Human and Equine Anti-LPS

I. SMAO Shock

Experimental rabbits received 2 ml/kg equine anti-LPS s.c.; 2 days later a laparotomy was performed on these rabbits and in untreated controls. The superior mesenteric arteries were occluded (SMAO) for 1 h and then released. The incisions were then closed. The ischaemia damaged the gut and led to endotoxaemia and shock. After 10 days survivors were observed. Survivors in the controls were 2/12 (17.7%), but in those receiving anti-LPS prophylaxis 7/8 (87.5%; $P < 0.001$) [15]. This has important implications in the field of abdominal surgery. Prophylactic anti-LPS appears useful whenever intestinal ischaemia may occur. This is becoming routine practice in South African equine veterinary medical practice.

II. Haemorrhagic Shock

Cats were bled via femoral arterial catheter into a reservoir and held at a MAP of 40 mmHg. After 4 h the shed blood was returned to the cat via femoral venous catheter. During the shock period the cats were infused with either high titer anti-LPS human plasma or normal human plasma. Survivors at 24 h were noted. Survivors in the controls were 1/8 (12.5%) compared with 7/8 (87.5%) in the anti-LPS group [10]. That is, haemorrhagic shock became "irreversible" unless anti-LPS was present to bind the LPS leaving the ischaemic bowel.

III. Radiation Sickness

Mice (more than 200 animals) were irradiated with 630 rads in a hospital radiotherapy facility, 6 days later they received either normal equine plasma or anti-LPS equine plasma. Survival at 30 days post-radiation was observed. Survivors among the serum controls were 10%, but in the anti-LPS group survivors were 50% ($P < 0.025$) [16]. That is, radiation damage to the gut and plasma cells led to a high mortality. Anti-LPS inactivated LPS and "bought time" for the partially denuded gut to repair itself.

IV. Topical Infections

Pseudomonas keratitis of rabbit eyes led to inflammation, scarring and blindness. Such eyes were treated by equine anti-LPS as drops for 8 days. The infections were controlled, partial healing of the eye occurred and sight returned to some rabbits [17].

V. *Pseudomonas* Infection

Mice received 0.1 ml human anti-LPS and 1 h later received 0.1 ml *Pseudomonas aeruginosa* broth culture. Only 15% of controls survived compared with 85% of those receiving anti-LPS [12].

VI. Septic Abortion

Pregnant rats received anti-LPS or saline followed by low doses of *E. coli;* 2 weeks later fetuses were examined. Control fetuses were small and partially resorbed. Fetuses from anti-LPS-treated infected rats were of normal size. Placentas of control rats were inflamed and necrotic with viable *E. coli* present. Placentas in the anti-LPS-treated group were all normal [18]. Anti-LPS may have a potential value in preventing abortion of infected pregnant women.

VII. Veterinary Use

Equine anti-LPS is used in the veterinary medical industry of South Africa to treat a wide variety of diseases mediated in part by endotoxins. It is used parenterally, orally or topically. It has proved effective in treating the following cases: septic arthritis in Thoroughbred horses, septicaemia, peritonitis, diarrhoea, shock secondary to parvovirus infection, *Klebsiella* intrauterine infections, mastitis and *Pseudomonas* ear infections [14].

D. Summary of Human Studies Using Human Anti-LPS

I. Multicentre Septic Shock Study

In a multicentre study, consecutive cases of septic shock or imminent septic shock were treated with anti-LPS FDP or specific globulin; 20/22 improved [19].

II. Neonatal Septicaemia Study

Among severely septicaemic low birth weight infants no difference in survival was seen between those who received anti-LPS immunoglobulin and placebo, but anti-LPS-treated survivors had a much reduced period of hospitalization compared with the placebo-treated controls [20]. The slow rate of absorption of the intramuscular immunoglobulin may have caused the poor results in this trial. In addition this group of babies were of low birth weight and hence many had underdeveloped RES which might have been poorly responsive to any therapy. Because of the need for a rapid response in critically ill patients, an *intravenous* anti-LPS preparation is to be preferred. On the other hand there may be a place for the slowly absorbed immunoglobulin as a prophylaxis.

III. Single Centre Septic Shock Study

Women in a department of obstetrics and gynaecology who developed septic shock were admitted to the trials [13]. They received conventional antibiotic and supportive therapy and surgery where indicated. On a random basis some also received anti-LPS FDP. Mortality of controls was 9/19 (47.4%) and of the anti-LPS-treated group was 1/14 (7.1%). The anti-LPS group also developed fewer complications of shock and had a much reduced period of hospitalization.

IV. Current Studies

In essentially a continuation of the study on women in septic shock (sect C. III) the following results were obtained: mortality in controls = 11/27 (40.7%); mortality in anti-LPS-treated group = 1/23 (4.3%) [21].

E. Discussion

Human anti-LPS prepared by two different methods has been shown to protect patients therapeutically against septic shock. A wide variety of animal studies have previously shown similar results. The main problem had been to obtain reasonable amounts of human anti-LPS. In part, this has been solved by the use of our ELISA screening procedure which is appropriate to any medium to large-sized blood bank.

Some one-third of all cancer patients and two-thirds of leukaemia patients die of gram-negative bacteraemia. It is to be expected that radiotherapy or chemotherapy contribute to this bacteraemia by causing the partial denudation of the gut lining which permits an increased leakage of LPS into the circulation. At the same time this therapy reduces the rate of production of the natural protective anti-LPS antibodies and leucocytes. There appears to be a potential benefit in using anti-LPS in addition to conventional antibiotic therapy for treating septicaemia in immunosuppressed patients, particularly leukaemic patients. It must now be verified in clinical trials.

Acknowledgments. This work was supported by the SA MRC. Excellent technical work in these studies was carried out by Ms. Michelle Wells.

References

1. Goto H, Nakamura S (1980) Liberation of endotoxin from E. coli by addition of antibiotics. Jpn J Exp Med 50:35
2. Spink W, Braude AI, Castaneda MR, Goytia R (1948) Aureomycin therapy in human brucellosis due to Brucella militensis. JAMA 138:1145
3. Cuevas P, Ishiyama N, Koizumi S, Woodruff P, Kaufmann A, Fine J (1974) Role of endotoxemia of intestinal origin due to large burns. Surg Gynecol Obstet 138:725
4. Quastler H (1956) The nature of intestinal radiation death. Radiat Res 4:303–320

5. Davis D, Brown K, Douglas H, Take W, Braude AI (1969) Prevention of death from endotoxin with antiserum. Immunology 102:563
6. Milner KC (1973) Patterns of tolerance to endotoxin. J Infect Dis 128 [Suppl]: S237
7. Pollack M (1983) Antibody against Pseudomonas aeruginosa in immune globulins prepared for intravenous use. J Infect Dis 147:1090
8. Ziegler E, McCutchan J, Fierer J et al. (1982) Treatment of gram negative bacteremia and shock with human antiserum to a mutant E. coli. N Engl J Med 307:1226–1230
9. Gaffin SL (1983) Large scale production of antigram negative bacterial antibodies. Lancet 2:1420
10. Gaffin SL, Grinberg Z, Abraham C, Shechter Y, Birkhan J (1981) Protection against hemorrhagic shock in the cat by human plasma rich in endotoxin specific antibodies. J Surg Res 31:18–21
11. Gaffin SL, Badsha N, Brock-Utne J, Vorster B, Conradie J (1982) An ELISA procedure for detecting human anti-endotoxin antibodies in serum. Ann Clin Biochem 19:191–194
12. Badsha N, Vorster B, Gaffin SL (1985) Production and properties of human lipopolysaccharide specific globulin. Vox Sang (in press)
13. Lachman E, Pitsoe SB, Gaffin SL (1984) Anti-lipopolysaccharide immunotherapy in management of septic shock of obstetric and gynaecological origin. Lancet 1:981–983
14. Gaffin SL, Baker B, Du Preez J, Katzwinkel R, Fleming J (1982) Prophylaxis and therapy with antiendotoxin hyperimmune serum against gastroenteritis and endotoxemia in horses. Proc Am Assoc Eq Pract (Atlanta) 28:335–340
15. Zanotti A, Gaffin SL (1985) Protection against superior mesenteric artery occlusion shock by anti-lipopolysaccharide antibodies. J Surg Res (in press)
16. Gaffin SL, Zanotti A et al. (1984) Anti-LPS antibodies successfully treated shock, radiation sickness, surface infections and septic abortion in animals. S Afr J Sci 80:130
17. Welsh NH, Rauch A, Gaffin SL (1985) Topical immunotherapy for Pseudomonas keratitis in rabbits: Use of Anti-LPS plasma. Br J Ophthalmol (in press)
18. Lachman E, Gaffin SL, Sanker D, Pitsoe SB (1982) Prevention of septic abortion in rats by antiendotoxin antibodies. Abstract of the 3rd British Congress for Obstetrics and Gynaecology, July 1982, Birmingham, p 132
19. Gaffin SL, Lachman E (1984) The use of anti-lipopolysaccharide antibodies in the manegement of septic shock. S Afr Med J 65:158
20. Adhikari M, Coovadia H, Brock-Utne J, Pudifin D, Gaffin SL (to be published)
21. Lachman E, Pitsoe S, Gaffin SL (1984) Antilipopolysaccharide antibodies: Update. Lancet 2:875–876