Original Investigation

Monique Nys · Jacques Damas · Pierre Damas
Ruth Laub · Jean Michel Cloes · Maurice Lamy

Study of the protective effects of hyperimmune immunoglobulins G and M against endotoxin in mice and rats

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Abstract We prepared solutions of human IgM and IgG to various lipopolysaccharide (LPS) species. These were then tested, along with solutions of non-LPS specific human IgG or IgM, for their ability to confer passive immunity against experimental endotoxemia in two animal models. The immunoglobulins were first tested for an effect on the lethality induced by seven different LPSs in actinomycin-D sensitized mice, or by three different bacteria in normal mice. When the immunoglobulins were administered 1 h before challenge, a small protective effect was observed. This protection was dependent upon both the anti-LPS agent, the chemical composition of the LPS, or the strain of Gram-negative bacteria used for injection. The anti-LPS IgM and IgG preparations reduced the mortality induced by *Escherichia coli* but not by *Serratia marcescens* or *Klebsiella pneumoniae*, indicating protection by strain-specific antibodies. When the antibodies were preincubated with LPS or bacteria for 30 min before administration, almost complete protection was seen. The influence of these immunoglobulin preparations or of human albumin (as a control) on the hypotensive and vascular-permeabilizing effects of LPS in rats was then studied. A dose-dependent inhibitory effect was observed with IgG preparations and albumin. At 200 mg/kg, anti-LPS IgG reduced the effects of LPS, while at 400 mg/kg, both anti-LPS and normal IgG preparations showed protection, as did human albumin used at the same dose. The IgM-enriched preparation worsened the initial hypotensive phase after LPS, whereas the anti-LPS IgM significantly reduced the second phase of the hypotension, but only at the largest dose of 400 mg/kg. In this second model using the rat, a clear difference between the activity of IgG and IgM was thus observed. We conclude that pretreatment with human immunoglobulins from large plasma pools modestly, but significantly, attenuated the effects of murine and rat Gram-negative sepsis, but that protection was incomplete. Our results suggest that single regimen intervention strategies may not be sufficient to influence the course of the disease.

Key words Lipopolysaccharides · Endotoxemia · Septicemia · Immunotherapy · Anti-LPS antibodies

Introduction

A large percentage of deaths in hospitalized patients can be attributed to Gram-negative bacterial sepsis [8]. The number of episodes of bacterial sepsis in the United States was recently estimated to reach 400,000 annually, with a mortality rate of 10–40%, in spite of appropriate antimicrobial therapy and optimum supportive care [2, 10, 56]. Most of the toxic manifestations induced by Gram-negative bacteria are caused by endotoxin (lipopolysaccharide, LPS), which is a component of the outer membrane of these organisms. Antibiotic therapy does not prevent the toxic manifestations of LPS, and may even promote the release of LPS from bacteria [17, 28, 50]. The main alterations attributed to LPS are circulatory shock, disseminated intravascular coagulation, and
failure of numerous organs, including the central nervous system, heart, kidneys, gastrointestinal tract, lungs, and liver. The multitude of pathophysiological effects and targets of LPS explains the high mortality rate that accompanies exposure to this substance [27, 44].

The effects of antibodies directed against LPS have been studied in patients with presumed Gram-negative sepsis [6, 9, 23, 26, 35, 38, 49, 52, 55, 59, 60], but the results of these studies, while considered controversial, have not shown a significant therapeutic benefit. In addition, they have raised new questions about the role of endotoxin in bacterial infections [1, 12, 13, 20, 30, 54] and generated new experimental approaches [34, 43, 45]. Critical questions remain regarding the precise mechanisms by which exogenously administered anti-LPS antibodies may serve to attenuate the toxicity of LPS and afford protection during Gram-negative bacterial infection. On the other hand, the therapeutic advantage of the use of concentrated, specific IgM rather than of specific IgG preparations has been questioned.

We have developed a sensitive immunoassay for anti-LPS IgG and IgM antibodies (which can demonstrate anti-LPS cross-reactivity patterns in sera), employing a range of complete and incomplete (core glycolipid components) LPSs [42]. We used this assay to detect those plasma from healthy, volunteer Belgian blood donors that had high concentrations of antibodies to LPSs. To identify the antibody isotype(s) that provide optimal protection against sepsis, the plasma samples were pooled and fractionated to obtain anti-LPS intravenous immunoglobulin preparations from the Cohn fraction II (IgG preparation) and the Cohn fraction III (IgM-enriched preparation).

The aim of the present investigation was to study and compare the ability of hyperimmune anti-LPS IgM or IgG immunoglobulin preparations to confer passive immunity in vivo against LPS toxicity. This approach was conducted with two animal models: the mortality on LPS-induced mortality were estimated using actinomycin D-treated mice as previously described [41]. Briefly, serial dilutions of a 1 mg/ml stock suspension of LPS (S and R forms) were prepared in pyrogen-free physiological saline immediately prior to administration. The different LPS dilutions were injected with 25 µg of actinomycin D intraperitoneally (i.p., 0.5 ml) into the mice. Each dose of the LPS preparations was tested in groups of eight mice and deaths were recorded for 48 h after the treatment. The LD₅₀ of each preparation of LPS was calculated by the method of Reed and Muench [47]. In control experiments, no deaths occurred when mice were treated either with 1 µg of the different LPS preparations alone or with 25 µg of actinomycin D.

A model of lethal infection induced by various clinically relevant Gram-negative bacteria was also studied. The microorganisms were grown overnight at 37 °C on tryptic soy agar plates. They were harvested by gentle washing with the help of cotton-tipped applicators, centrifuged and resuspended in sterile saline. A stock solution of the suspensions that corresponded to an optical density of Mac Farland number 1 was made. From these stock suspensions, serial tenfold dilutions were made in saline and then inoculated onto agar plates for quantitation of the organisms in suspension. Aliquots

Materials and methods

Antigens and chemicals

Human serum albumin (HSA, batch R&D 940119) was provided by the Belgian Red Cross (Brussels, Belgium). Smooth LPS from Escherichia coli 055:B5, E. coli 0127:B8, E. coli 0128:B12, Salmonella enteritidis, Serratia marcescens, Klebsiella pneumoniae (Westphal phenol extraction method) and rough LPS from E. coli J5, S. minnesota R595 (extracted by the Galanos method) were purchased from Sigma Chemicals Co. (St. Louis, Mo.), actinomycin D from Lyovac Cosmejen (MSD, Brussels, Belgium) and heparin from Leo (Therabel, Belgium). Blood culture isolates of E. coli, K. pneumoniae and S. marcescens were obtained from routine specimens in our hospital.

Preparation of antibodies

Sera from healthy volunteers (unpaid Belgian blood donors) were screened by ELISA [42] for IgM and IgG antibodies against 13 smooth (S)-LPS, 9 rough (R)-LPS and 2 monophosphoryl lipid A. Pooled plasma from 280 donors with high concentrations (> 40 µg/ml) of LPS-precipitable antibodies were used to prepare IgG- and IgM-enriched fractions for intravenous use by the Central Fractionation Department of the Belgian Red Cross, using β-propiolactone treatment of Cohn fractions II and III. A pool of non-selected plasma was similarly processed. Before use, the different lots of IgM-enriched standard (Penta, lot 230993) or anti-LPS (Penta-LPS, lot 300993) immunoglobulins and IgG-enriched standard (Ivegam, lot 931125) or anti-LPS (Ivegam-LPS, lot 931202) immunoglobulins underwent the standard quality controls applicable to all gamma globulin preparations. The anti-LPS preparations contained IgM, IgG and IgA antibodies against all of LPSs used for the selection of blood donors. The range of specificities to LPS was increased by the pooling of selected sera. In the Ivegam and Ivegam-LPS, no IgM anti-LPS antibodies was detected by ELISA (data not shown).

As described previously [42], differential absorption experiments showed that the four preparations contained a mixture of specific antibodies against different R- and S-forms of LPS and cross-reacting antibodies against a family of LPS (i.e., E. coli and Salmonella). We also detected predominantly anti-LPS activities due to the IgG₁ and IgG₂ subclasses. IgG₁ and IgG₂ were found more rarely in the preparations (data not shown).

In vitro, the four immunoglobulin preparations neutralized the biological activities of LPS, such as activation of the Limulus amoebocyte lysate (LAL) reaction and induction of interleukin-6 secretion from human whole blood, in a dose-dependent manner. For the seven LPS selected in mice, the four preparations have different neutralizing properties in the LAL assay; a maximal inhibition of more than 80% were obtained for E. coli 0128:B12 and K. pneumoniae, of 50% for S. enteritidis and E. coli 0127:B8 and of ≤20% for E. coli 35, S. minnesota R595 and S. marcescens, respectively (Nys et al., unpublished results).

Experiments in mice

Male BALB/cHeA mice, 4–6 weeks old, were used for the lethality studies. The animals were fed mouse chow ad libitum and were kept on a 12-h light, 12-h dark cycle. Their body weight was approximately 20 g. The experiments were approved by the University Hospital Subcommittee on Animal Care and Research.

Lethality studies

Effects on LPS-induced mortality were estimated using actinomycin D-treated mice as previously described [41]. Briefly, serial dilutions of a 1 mg/ml stock suspension of LPS (S and R forms) were prepared in pyrogen-free physiological saline immediately prior to administration. The different LPS dilutions were injected with 25 µg of actinomycin D intraperitoneally (i.p., 0.5 ml) into the mice. Each dose of the LPS preparations was tested in groups of eight mice and deaths were recorded for 48 h after the treatment. The LD₅₀ of each preparation of LPS was calculated by the method of Reed and Muench [47]. In control experiments, no deaths occurred when mice were treated either with 1 µg of the different LPS preparations alone or with 25 µg of actinomycin D.