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Immunoprophylaxis and Immunotherapy of Gram-Negative Sepsis and Shock with Antibodies to Core Glycolipids and Lipid A of Bacterial Lipopolysaccharides

Introduction

Since the 1950s, the beginning of antibiotic era, the frequency of gram-negative sepsis and shock has increased to such an extent that it is now a common complication in seriously ill patients. Mortality rates are still very high despite intensive medical care and antibiotic therapy. Between 2 and 10% of all hospitalized patients acquire gram-negative infections, a large proportion developing bacteremia and shock [1]. The most frequent organisms causing bacteremia are *Escherichia coli*, *Klebsiella-Enterobacter-Serratia*, *Proteus-Providencia* and *Pseudomonas aeruginosa* [2]. The risk of gram-negative sepsis is particularly high among immunocompromised patients: the aged, the malnourished, those undergoing massive surgical interventions, those with malignant neoplasmas, etc. With few exceptions, such as colistin and polymyxin, antibiotics have almost no effect on pathologic lipopolysaccharide (LPS) activity [3]. In fact, antibiotic therapy may even increase the amount of endotoxin liberated as a result of bacteriolysis and may complicate the clinical course of the sepsis.

The widespread presence of gram-negative bacteria in both the gastrointestinal tract and the hospital environment together with the high incidence of antibiotic resistance and the increased number of immunocompromised patients explains why these organisms so frequently cause nosocomial infections and why preventive and antimicrobial treatment is relatively ineffective. As a result of these factors, researchers must continually seek additional approaches for specific prophylaxis and therapy of gram-negative sepsis. Because of the great serological diversity of gram-negative agents, their efforts are directed towards development of preparations containing antibodies to common (cross-reactive) LPS antigens [4–6]. The present review summarizes the possibilities for prophylaxis and therapy of gram-negative sepsis and shock in humans and experimental animals by means of hyperimmune sera and monoclonal antibodies against common LPS antigens.

Chemically, endotoxin is a lipopolysaccharide and has a similar structural organization in all gram-negative bacteria that comprises a hydrophylic polysaccharide region to which a lipoid component, named lipid A, is covalently bound. There are three parts in the LPS molecule, each possessing antigenic properties: 1) O-specific polysaccharide side chains (O-chains); 2) core polysaccharide (core); and 3) lipid A. Lipid A confers the toxicity while serological specificity is determined by the O-chains [7].

The great variety of sugars and the linkages between them comprising O-chains determine the existence of numerous O-antigenic specificities. This explains why O-antibodies are serotype-protective. Smooth (S) gram-negative bacteria may turn into rough (R) mutants. Their LPS loses the O-chains and is known as core glycolipid (CGL). Antibodies against R-mutants cross-react with a great number of gram-negative bacteria and extracted LPS due to the similarity in their core region. The core region is provisorily divided into outer and inner core, with the latter possessing a highly conserved structure among gram-negative bacteria [8].

Thus far nine core types have been identified in most clinically significant gram-negative pathogens. All *Salmonella* serovars have one core type [7,9,10]. Five different core types, similar to one another and to the Ra core of *Salmonella*, were identified chemically and serologically in *E. coli*, *Shigella* and *Citrobacter* [9]. The differences between most of them are found in the outer core, while the inner core is almost identical. In *Proteus*, three core types are known [11], and again the inner core has a common serological specificity. In *P. aeruginosa* there is one core type [12] common to all strains.

Depending on the level of genetic block, R-mutants of *Salmonella* can synthetize a complete (Ra) or incomplete (Rb to Re) core (Figure 1). Antigenic similarity between the LPS molecules is found in the Re to Rc serotypes. This could explain why efforts for the development of effective immunotherapy concentrate mostly on the Rc mutant of *E. coli* O111:B4 (J5-mutant) and *Salmonella minnesota* R595 (Re mutant).

At least eight different specificities in the core and lipid A were identified with monoclonal antibodies [13]. In J5 CGL there are at least three epitopes, and not all of them are cross-reactive [14].

Lipid A is the most conservative structure in the LPS molecule, and antibodies against it possess a marked cross-reactivity with a great variety of gram-negative bacteria [15,16]. The structure providing full antigenic activity of lipid A contains a glucosamine disaccharide and at least one amide-bound fatty acid; three antigenic specificities were found in this structure [16].

Other antigens common for gram-negative bacteria are enterobacterial common antigen, outer membrane

Received: 4 April 1991/Revision accepted: 8 August 1991

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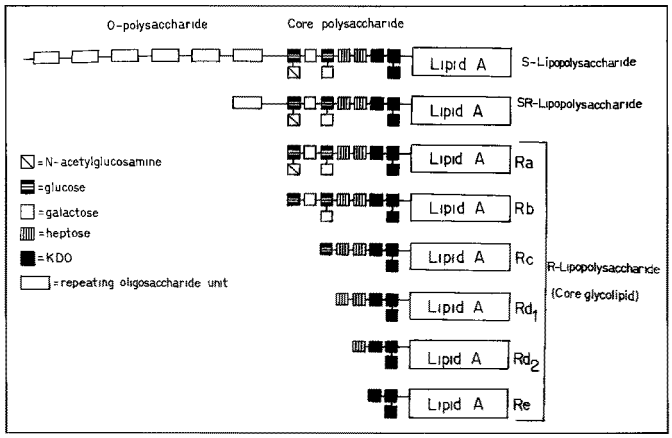

Figure 1: Structure of the R core and Ra - Re chemotypes of *Salmonella* LPS. The J5 mutant of *Escherichia coli* is similar to the Rc chemotype, and *Salmonella minnesota* strain R595 core glycolipid corresponds to the Re chemotype. Glc = glucose; Gal = galactose; Glc Nac = glucosamine (N-acetylglucosamine); Hep = heptose (L- glycero-D-mannoheptose); KDO = ketodeoxyoctonic acid (2-keto-3-deoxy- mannooctulosonic acid).

proteins, porins and the lipoprotein of *Braun* and *Bosch*. Data about the protective activity of these antigens are contradictory and are discussed elsewhere.

Cross-Protection with Antibodies to LPS Antigens

After immunization or infection with bacteria or LPS, most of the antibodies produced are against the O-chains. O-antibodies provide only type-specific protection against sepsis and endotoxemia [17,18], which limits their clinical application.

Immunization with R-mutants or CGL and lipid A induces antibodies to common epitopes in the CGL. Here we focus on the role of antibodies to CGL and lipid A as a protective factor during gram-negative sepsis and shock.

Cross-Reactivity with LPS Antigens

Accessibility of the Epitopes

Very often the shared epitopes in the core/lipid A region are inaccessible for antibodies against them because they are "hidden" by O-chains [19–21]. Varying or lack of binding activity to heterologous LPS has been reported for J5 polyclonal and monoclonal antibodies [21–24]. Many investigators found that the common epitopes in the LPS are localized in lipid A [14,25] or lipid A/KDO region [26], but not in the J5 core [27]. Other results point out that in intact LPS, lipid A is in a cryptic position and is expressed only after removal of the polysaccharide [16,28]. Despite this, many reports showed that lipid A could express its epitopes even in purified LPS and intact gram-negative bacteria [19,29–34]. According to our results (*I. Mitov, M. Freudenberg, U. Bamberger, C. Galanos*, in preparation) monoclonal antibodies to lipid A bind R, SR and some S LPS, depending on the oligosaccharide chain length (Table 1).

There are probably bacterial surface regions where the LPS molecule is incomplete and epitopes in the core and lipid A are exposed [35]. Thus differences in protective activity might be due to variations in epitope expression [36,37]. Cross-reactivity is limited by differences in antigenic determinants of lipid A and CGL [37], or they are weak immunogens compared to O-chains [31]. In some experiments heat treatment of bacteria or LPS either changes the conformation of or uncovers the core epitopes that might be inaccessible on live organisms [38–40].

Role of Bacterial Growth Phase

It is known that during sepsis most of the defence mechanisms subside and bacteria begin to multiply very rapidly. At this moment, cell wall synthesis is so intense that CGL may be incorporated before attachment of O-chains is completed [6,41] and antibodies to common LPS epitopes could bind intact smooth organisms [34,42,43]. The observed marked dependence of anti-J5 serum absorption by bacteria in their growth phase indicates that antibodies to CGL and lipid A would be most effective when the cell growth is unlimited, as in the early stage of infection or in immunocompromised hosts [44].

In Vivo "Unmasking" of CGL and Lipid A Epitopes

The presence of antibodies to CGL and lipid A in normal human sera [45] suggests that although inaccessible for the antibodies *in vitro, in vivo* the epitopes become expressed. Immunization with LPS elicits synthesis of O-antibodies and of anti-CGL as well, perhaps due to synthesis of incomplete rough LPS *in vivo* or processing of LPS molecules by the host [46,47]. Another possibility is that S LPS associates with certain serum proteins *in vivo*, such as high-density lipoprotein, resulting in a structural change of the LPS molecule and expression of core and lipid A epitopes [23].

Cross-Protection with Antibodies to CGL and Lipid A Data From Experimental and Clinical Trials

The most conservative structure in LPS, lipid A, is considered to be a probable antigen for inducing cross-protection; this was proposed first by *Y. Kim* and *D. Watson* [48]. After development of a method for lipid A antiserum preparation, it was found that antibodies to lipid A opsonize *E. coli* for intraperitoneal (i.p.) phagocytosis [15]. It was demonstrated that antiserum to lipid A protects against skin necrosis and fever induced not only by lipid A but also by challenge with S- and R-LPS from *Salmonella*, *Shighella* and *E. coli* [49]. Antisera and monoclonal antibodies to lipid A inhibit some biological activities of LPS such as local Schwartzman reaction [30], abortive effect [50], LPS and lipid A mitogenicity [33] and lung injury [51]. Human monoclonal antibodies to *E. coli* J5 specific for lipid A enhanced survival of mice with sepsis caused by various gram-negative pathogens and endotoxin shock [30]. A single lipid A monoclonal antibody provided only

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Table 1: Binding activity of monoclonal antibodies against lipid A to different lipopolysaccharides,core glycolipids and lipid A in ELISA.

Antigens[b]	Reciprocal end titers with monoclonal antibodies[a]					
	1M	4M	9M	11M	18M	31G
Lipid A	256	1024	256	1024	64	2048
CGLs: Re	16	256	16	256	16	1024
Rd2	8	256	8	64	4	256
Rd1	4	64	8	64	4	16
Rc	8	16	4	16	16	32
Rb	4	16	4	4	4	16
Ra	0	4	16	2	2	2
Salmonella typhimurium:						
SR-LPS	16	64	16	64	16	32
S-LPS	16	64	16	32	64	64
Escherichia coli O75 LPS	16	16	4	64	16	64
Salmonella abortusequi						
LPS	0	4	0	4	0	16

[a] Supernatants of the cultured hybridoma clones.
[b] Lipid A and CGLs from *S. minnesota;* SR-form LPS from *S. typhimurium* strain 1511; S-form LPSs from *S. typhimurium* strain C5, *S. abortusequi* and *E. coli* serogroup O75.

moderate protection [52], while a combination of such monoclonal antibodies was fully protective [25]. Lipid A monoclonal antibodies produced by us increased the average survival time of mice infected with *K. pneumoniae* but were ineffective against *Salmonella typhimurium*. This could be explained by the inability of the monoclonal antibodies to activate complement (*I. Mitov, M. Freudenberg, U. Bamberger, C. Galanos,* in preparation).

It is not known yet to what extent antibodies to lipid A are protective during bacteremia. However, according to *W. Marget* [53], the lower incidence of septic shock in patients with elevated levels of lipid A antibodies indicates their relation to the favourable outcome. Human serum, which contains high titers of antibodies to lipid A, is effective in the treatment of gram-negative sepsis [5]. *L. Jaspers* et al. [54] found that patients treated with high-titered anti-lipid A human globulin had a significantly more favourable course of infection and a higher survival rate.

According to other investigators, passive transfer of lipid A antibodies or active immunization with lipid A did not protect patients from endotoxin shock or gram-negative sepsis [28,36]. It was suggested, however, that antibodies to lipid A might have protective, damaging or no effect [16].

In 1966 *W. Tate* [18] and in 1968 *L. Chedid* [55] reported that antisera to R mutants protected against lethal effect of S-LPS. Results obtained thereafter indicated that antibodies to the minimal LPS structure, the CGL of *S. minnesota* R595 (Re mutant), possess protective properties during sepsis [56,57] and endotoxemia [58]. *W. McCabe* [36] found that Re and Rd2 chemotypes of *S. minnesota* and antibodies to them conferred significant protection against lethal *K. pneumoniae* and *E. coli* infection in mice. Pretreatment of animals with anti-Re sera inhibits some of the LPS activities causing disseminated intravascular coagulopathy (DIC) [59] and protects against shock and sepsis [60].

Most results about the protective activity of anti-R antibodies were obtained by investigations of sera and antibodies to Rc chemotype CGL. Antiserum to *E. coli* J5 prevents the lethal activities of S-LPS as well as the local and generalized Schwartzman reaction and is effective in the therapy of bacteremia caused by *E. coli, P. aeruginosa, K. pneumoniae* and *Haemophilus influenzae* [31,61]. Immunization with *E. coli* J5 elicits high levels of IgG antibodies in piglets and protects against otherwise lethal *Haemophilus pleuropneumoniae* infection [44]. *D. Dunn* et al. [42] showed that a single anti-J5 monoclonal antibody protects against a variety of gram-negative pathogens and isolated LPS. *H. Warren* et al. [62], using the Limulus assay inhibition test, found that anti-J5 and anti-Re sera neutralized a number of heterologous LPS.

Based on experimental results, several clinical trials were undertaken to assess the efficacy of human anti-J5 sera [45,63]. According to *E. Ziegler* et al. [6], therapy with J5 antiserum significantly reduces mortality due to gram-negative bacteremia and shock. Immune J5 plasma applied prophylactically to high-risk surgical patients prevented sepsis, shock and death [4]. Prophylactic administration of anti-J5 serum prevents graft-versus-host disease in patients following bone marrow transplantation [64], in which disease gram-negative bacteria are believed to play an important pathogenic role. In conclusion, prophylaxis and therapy with anti- Rc (J5) serum prevents the serious consequences of sepsis, shock and in many patients the lethal outcome; however, they do not reduce the incidence of infection.

There is still not enough data confirming that the obvious anti-endotoxic activity of anti-R sera is connected directly to antibodies to CGL and lipid A. This is also supported by the fact that some authors failed to demonstrate a correlation between antibody titers and a protective effect [62,65]. Antisera with high titers to Rc, Rd1 and Re were not more effective than preimmune sera [65,66]. *A. Fomsgaard* [67] found that human serum with antibodies to 11 different LPS, including Re CGL, neutralized LPS biological activity *in vitro* and exert anti-endotoxic and anti-infectious activity in mice; protection was serotype-specific [67]. Other researchers also failed to reveal effective protection against heterologous infection or LPS [68,69], probably because antibodies to CGL were unable to bind their epitopes [25,34].

Although on a volumetric basis O-antisera are more protective than anti-R, when activity is compared on the basis of the amount of antibodies, antisera to Re and J5 are more effective [70]. In favour of antibodies to CGL is also the fact that the protective factor can be concentrated by ammonium sulfate precipitation [71] and that the

Table 2: Protective effect of affinity-purified antibodies and hyperimmune sera absorbed by means of affinity chromatography on lethality due to *Klebsiella pneumoniae* and *Salmonella typhimurium* infection in C57B1/6 mice.

Pretreatment[a]		Challenged with: *Klebsiella pneumoniae* B	*Salmonella typhimurium* C5
Anti-Ra	– Antibody fraction[d]	4/24[b] (17)[c] NS	5/18[b] (28)[c] NS
	– Absorbed serum[e]	8/17 (47) **	3/ 7 (43)****
Anti-Rc	– Antibody fraction	7/22 (32)***	8/19 (42)**
	– Absorbed serum	14/17 (82)*	3/ 7 (43)****
Anti-J5	– Antibody fraction	13/19 (68)*	8/24 (33)***
	– Absorbed serum	ND	2/ 7 (29) NS
Anti-Rd1	– Antibody fraction	9/17 (53)*	8/19 (42)*
	– Absorbed serum	4/12 (33)****	2/ 7 (29) NS
Anti-lipid A	– Antibody fraction	19/23 (83)*	2/12 (17) NS
	– Absorbed serum	8/10 (80)*	ND
PBS		0/20	0/18

[a] Mice pretreated i.p. with 0.2 ml of each preparation or PBS and challenged i.p. 30–60 min thereafter with 10 LD_{50}.
[b] Number of survivors/number of challenged.
[c] Percentage of survivors.
[d] Fraction obtained after eluation of the antibodies bound to the Sepharose-4B-CGL (lipid A) immunosorbent.
[e] Serum, run 10 times through the homologous immunosorbent.
* $\chi^2>10.83$ – $p<0.001$; ** $\chi^2>6.63$ – $p<0.01$; *** $\chi^2>5.41$ – $p<0.02$; **** $\chi^2>3.84$ – $p<0.05$; NS = not significant ($\chi^2<3.84$ – $p>0.05$).
ND = not done.

absorption of sera with homologous antigen removes protective activity [23,61]. The drop in the J5 titer during the acute phase and its rise in convalescence supports the role of R-antibodies [44]. Even if elevated anti-J5 titers could not be detected in bacteremic patients, it does not mean that such antibodies are not protective but only suggests that CGL is not immunogenic enough or that the host immune response is suppressed.

Some of our results demonstrated that rabbit sera against Ra, Rc, J5, Rd1 CGL and lipid A but not to Rd2 and Re CGL were protective in mice infected with *K. pneumoniae* and *S. typhimurium*. Normal rabbit serum was also highly efficient, which complicated the evaluation of results. Affinity-purified antibodies from the same sera to Rc, J5, Rd1 and lipid A exhibited a significant protective activity (Table 2). The absorbed sera, even though containing no specific antibodies, also retained their activity (*I. Mitov, M. Freudenberg, C. Galanos,* in preparation). The reason for this apparent contradiction will be discussed later.

As a whole, the results presented support further investigations for the development of effective immunoprophylaxis and immunotherapy of high-risk patients with preparations containing antibodies against CGL and lipid A.

Serum Levels of Antibodies to CGL and Lipid A and the Risk of Gram-Negative Sepsis and Shock

As an essential part of the indigenous flora of the gastrointestinal tract, gram-negative bacteria permanently discharge endotoxin into the circulation. As a result, antibodies to LPS as well as significant titers to Rc and Re CGL [43] are often found in normal human serum. These antibodies are probably an important factor of host resistance [72]. Anti-lipid A titers have been found in 10–30% of healthy humans by means of passive hemolysis and ELISA [41,73]. During sepsis, anti-lipid A antibodies usually rise insignificantly [74], but chronic or recurrent gram-negative infections are associated with a rise in 70–80% of the patients [75]. High titers to CGL at the onset of bacteremia were associated with a significant lowering of the incidence of shock and mortality following sepsis [45,76]. A higher survival rate was observed in patients with titers to Re of 1:200 and more [48]. The inability for response to core epitopes might explain why some patients have sequential episodes of sepsis [77]. The higher incidence of septic shock in patients with decreased or lacking antibodies to core and lipid A [64,78,79] indicates a secondary immune deficiency [80]. It could be concluded that naturally occurring antibodies to CGL and lipid A possess protective properties during gram-negative sepsis and could be used as a marker of the immune status and the ability of the patients to overcome infection.

Influence of the Time of Antibody Administration

Experimental therapy with antibodies to CGL and lipid A is effective between the 2nd and the 8th hour after bacterial challenge [52], but not if delayed until the 24th hour [81]. Other data showed maximum protective activity in the first 15 min after challenge, with the effect vanishing after the 1st hour [70]. Passively administered anti-lipid A serum exhibited a significant hemolytical titer even after eight days, but protective activity was demonstrated immediately before or up to the 1st hour after challenge with lipid A [49]. This strongly suggests that such antibodies have no effect once pathological mechanisms are activated but rather block the binding of endotoxin to target cells and the triggering of endotoxic effects.

Judging from the experimental data, optimal protective activity could be expected when the antibody preparations are administered just before or at the very beginning of infection. The time before challenge should not exceed 1–2 hours because of the stimulating activity of LPS, which often contaminates the preparations.

Synergistic Effect of Immuno- and Chemotherapy

It was suggested that minimal inhibitory concentrations (MIC) of antibiotics could modulate S-strains in such a way that binding of antibodies to CGL and lipid A becomes possible. When *E. coli* O111 is cultured in the presence of sub-MIC amounts of β-lactam antibiotic carumonam, anti-J5 ELISA titers increase sixfold, probably because of O-chain damage [82]. *L. Young* [83] reported that anti-J5 monoclonal antibodies administered to mice together with one-fourth of the MIC of antibiotic enhanced survival after challenge with *P. aeruginosa*. Synergistic effect between hyperimmune globulin

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"Psomaglobin"N and tobramycin or ciprofloxacin was also found in the treatment of experimental pneumonia and burn wound sepsis [81,84]. The protective activity of antiserum to J5 observed by *B. Dunn* and *R. Ferguson* [31] was due in part to its application together with heparin, which decreases the pathologic consequences of diffuse intravascular coagulation and thrombocytopenia.

Combining immune preparations containing antibodies to CGL and lipid A with antibiotics or other drugs, thus preventing endotoxic effects, will prove to be a useful approach in the treatment of gram-negative infections.

Mechanisms of Action of Antibodies to Common LPS Antigens

Complement Activation and Enhancement of Phagocytosis

Data obtained by some investigators demonstrated that serum and monoclonal antibodies were not strong opsonins and did not enhance complement-mediated bacteriolysis but acted mainly as anti-endotoxins [21,44]. According to others, antibacterial activity of R antibodies was of greater importance than anti-endotoxic function [70]. Anti-R and lipid A antibodies are bactericidal and opsonizing in the presence of complement and enhance bacterial clearance [72,85]. These results are in accordance with the early findings that antibodies to lipid A opsonize *E. coli* for i.p. phagocytosis and, in the presence of complement, provide protection against *S. typhimurium* [15]. Antiserum to J5 probably acts by whole-cell opsonization [29], increasing bacterial clearance by phagocytosis [31].

Endotoxin Neutralization

Antibodies to the endotoxic principle of LPS, lipid A, cross-react with LPS of different bacterial origin and neutralize its toxicity [18,49,54]. It is possible that the antibodies bind the active portion of lipid A or an epitope near it [6,37], which provokes conformational changes in the lipid A molecule, the formation of a lower activity complex or the blockage of binding to target cells [41]. This becomes possible either by binding of antibodies to lipid A after the bacterial cell is subjected to defence mechanisms or by binding to liberated toxin [21,44]. Therefore, clinical efficacy of antibodies to CGL and lipid A depends to a great extent on molecular form and conformation of the LPS *in vivo* [34,39].

Which mechanism of antibody action plays a dominant role in protection is not strictly defined. Probably, at different stages of infection, antibodies act by combining some or all of them.

Role of the Antibody Isotype

The protective antibody isotype is important for clinical antibody application. IgM is usually infused intravenously, while IgG, though more stable and able to penetrate extravascular spaces, probably does not possess the molar potency of IgM. Many investigators found that their serotype-specific proctective murine monoclonal antibodies are IgG3 [35]. Since IgG3 is a minor component of murine serum, mechanisms probably exist for isotype restriction induced by O antigens.

It is still not clear which isotype mediates protection by anti-CGL and lipid A antibodies. Some data showed that IgM offered a higher protection [30,45,82] and that IgM titers correlate better with protection from septic shock [64]. The greater amount of IgM administered in comparison to IgG also supports this hypothesis [54]. The main role of IgG was found in other experiments [42,44]. It is not likely that cross-protective antibodies are of a class other than IgM and IgG [17], but the role of IgA could not be excluded [77].

Reasons for the Differences in the Results

Experimental Models, Test Systems and Antigen Preparations

The controversial and sometimes conflicting results might be due to differences in species and strains of experimental animals, in preparations and further processing of sera and monoclonal antibodies, in the use of substances for sensitization of animals, etc. [66].

The control animals are often treated with normal (NS) or preimmune sera used as a control. It is well known that NS protect against lethal gram-negative infections [36,65] due to humoral factors of natural resistance. Since differences between the lethality of animals pretreated with immune sera and those pretreated with preimmune sera or NS [65] are often negligible, it is difficult to evaluate precisely the role of antibodies to cross-reactive antigens.

The protective capacity of normal rabbit serum (NRS) was also confirmed by our results. As shown in Table 2, besides protection conferred by NRS, hyperimmune rabbit sera against CGL and lipid A absorbed by affinity chromatography remained protective to an extent comparable to non-absorbed sera. These results could explain why immune plasma might be preferable to the globulin preparations. When sera are obtained after immunization with R- mutants, one cannot exclude that the protection is due to antibodies against other cross-reactive antigens. Even when immunogens are pure CGL and lipid A, the sera often contain antibodies to unrelated gram-negative bacteria, which is attributable to the polyclonal immunostimulating properties of LPS [16]. Besides this, many absorption experiments are difficult to interpret [70], and small amounts of LPS may remain in the absorbed sera and influence the results [61,86].

Selecting the test system may also affect the results. According to some authors, inadequate methods for detecting cross-reactive antibodies are used [23,43]; furthermore, the assay may have profound effects on whether cross- reactivity occurs or the extent to which it occurs [39,87].

Differences may be due to the unique features of antigen preparations [37] and R-mutants [29]. *E. coli* J5 CGL is represented by several molecular forms [88], to which J5 induces synthesis of antibodies with different combining

sites [14]. Some cross-react with gram-negative clinical isolates, while others do not [19,21]. Antibodies to *E. coli* J5 are predominantly type-specific and could obscure results due to cross-reactive ones [88].

Challenge Dose, Antibody Amount and Specificity

Often the precise specificity of administered antibody preparations is not strictly defined. Although clinical trials relate survival to antibodies to CGL/lipid A [76], the effect is also connected to the whole IgG content and O-antibodies [45]. It is possible that in some cases an inadequate amount of antibodies was administered [66]. Large challenge inocula of a highly virulent strain are a serious insult upon the host, so that the antibody therapy has little effect [42,66]. On the other hand, when a less virulent strain is used, large amounts of LPS are introduced, which may stimulate host resistance and affect the results.

Cell Mitogenicity and Stimulation of Host Resistance

It has been known for years that LPS is a B-cell mitogen and that immunization with R-mutants or CGL and lipid A stimulates antibody response to various O [16] and other bacterial antigens [22,23]. Possibly, part of the protective activity of anti-R sera is due to this effect [29]. Some S-strains stimulate synthesis of cross-reactive antibodies to CGL [35], and the number of cells secreting antibodies to CGL four days after immunization is greater than those secreting O-antibodies [89]. The O-antibodies frequently discovered in anti-R sera [90] could also be explained by the fact that some rfa mutants synthesize O-chains not attached to CGL [10]. These antibodies could contribute to the protection.

Endotoxin in low concentration stimulates different mechanisms of host natural resistance. Procedures involved in production of antisera and antibody preparations are usually accompanied by contamination with trace amount of endotoxin. When administered 2–48 hours before challenge, they may protect regardless of antibody specificity [86]. Endotoxin strongly stimulatesmacrophages and lymphocytes and, in cases in which the specific antibodies are not detected in the preimmune sera, the effects of liberated cell mediators should not be excluded.

There is ample information indicating that many antisera against CGL and lipid A also contain other factors that block LPS toxic activity. Certain serum acute phase proteins, such as alpha-2 macroglobulin, protect animals from endotoxin shock. Passive transfer of heterologous tolerance without antibodies to LPS as well as data showing anti-endotoxin activity of NS or plasma have shown that serum factors other than antibodies also exert protective activity during gram-negative infection [3].

Conclusions

The results obtained by numerous investigators reveal the ability of antibodies to cross-reactive LPS antigens to protect against infections caused by various gram-negative pathogens and their endotoxins. Based upon experimental and clinical experience, one could postulate that these antibodies are a promising approach for the prophylaxis and therapy of sepsis. The recognition of high-risk patients would allow early start of combined therapy with appropriate immune preparations and antibiotics, which along with life-supporting measures (corticosteroids, electrolyte and nutrition infusions, etc.), would be a useful tool in the treatment of gram-negative sepsis and endotoxin shock.

Further studies using monoclonal and affinity-purified antibodies are needed in order to explain the existing controversies and define precisely the role of cross-reactive LPS antibodies in the host defence against gram-negative infections.

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