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# Immunotherapy of Gram-negative Infections in Oncological Patients

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**Abstract**—*While it is widely recognized that gram-negative bacteria (GNB) are a leading cause of morbidity and mortality among patients whose immune defenses are compromised both by their underlying malignancy as well as by its treatment, efforts to prevent or to treat such infections by immunological means have been hampered for several reasons. First, the natural anatomical barriers are often disrupted either by the tumor itself, by the chemotherapeutic agents, or, as recognized more recently, by viral (herpetic) or fungal infections, so that entrance of the gram-negative flora into the host tissue and blood stream is greatly facilitated. Efforts to diminish the bacterial load by means of oral decontamination, be it selective or not, to prevent such ingress have brought limited success. Second, active immunization is not likely to elicit a useful response in these patients, so that mainly passive immunotherapy has been considered and studied. However, since the most immunocompromised cancer patients are very often leucopenic, the opsono-phagocytic function of passively administered antibodies is not likely to help the patients to get rid of the invading gram-negative bacteria. This latter observation has been particularly well established in the case of* Pseudomonas aeruginosa *infections in leukemic patients (Young LS, Stevens P, Ingram J.* J Clin Invest 1975, **56**, 850–861).

## INTRODUCTION

*The concept of anti-core glycolipid antibodies*

SINCE many of the toxic manifestations induced by gram-negative bacterial infections are believed to be mediated by the lipopolysaccharide (LPS, endotoxin) component of the outer membrane of these bacteria, one approach has been to investigate whether antibodies directed against LPS could neutralize its detrimental effects. Studies in animals have shown that immunization with smooth GNB that possess a complete LPS molecule on their surface protects from many of the adverse effects of endotoxin and gram-negative bacterial infections. However, this approach has been hampered until recently by the wide variations of antigenicity of endotoxins isolated from GNB. Indeed, antibodies to complete LPS are directed primarily against the immunodominant, species-specific oligosaccharide side-chains and protect mainly against the immunizing bacterial strain. In contrast to oligosaccharide side-chains, the central part of the LPS molecule of GNB, the core glycolipid, which is responsible for LPS toxicity, shows little strain variation. Hence the working hypothesis that anti-core glycolipid antibody might be protective against a wide range of GNB.

Rough mutants of GNB are characterized by enzymatic deficiencies preventing the attachment of the lateral side-chains to the central core glycolipid. Depending on the type of the lacking enzyme, various rough mutants have been characterized and present different comparisons of their core sugars (Fig. 1). The simplest, roughest LPS is composed of lipid A and of a saccharide molecule called ketodeoxy-octulosonate (KDO), both of which have to be present on the cell wall of GNB, their absence being lethal. The rough mutants that harbor such LPS are called Re mutants.

## MATERIALS, METHODS AND RESULTS

*Experimental and clinical (retrospective) studies relating outcome from gram-negative infections to anti-endotoxin antibody levels*

The potential of antisera directed against rough mutants of GNB to protect against a wide variety of unrelated smooth GNB or endotoxins has been established in various experimental models. Passive immunization with *E. coli* J5, a rough mutant of *E. coli* O111, and with *S. minnesota* R595, the Re mutant of *S. minnesota* S128, prevented death in lethal challenge of mice and rabbits with various GNB or endotoxins, prevented localized and generalized Shwartzman reactions after injection of endotoxins in rabbits, and prevented hypotension following injection of endotoxins in dogs. Most of these studies demonstrated that immunization with rough

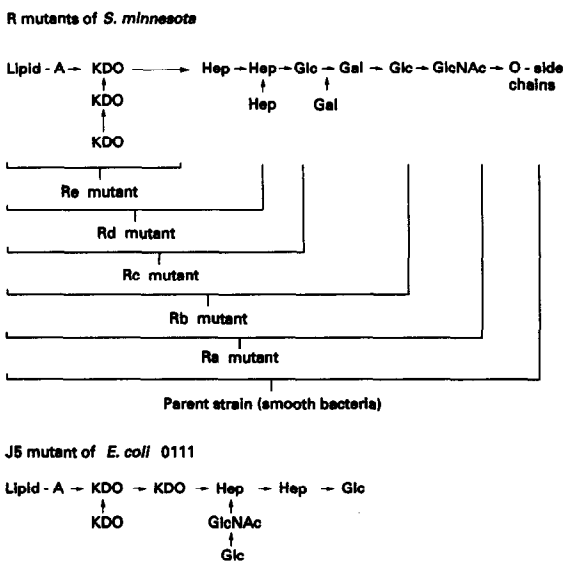


*Fig.* 1. *Schematized sugar composition of the core LPS of R mutants of* S. minnesota *and of the J5 mutant of* E. coli 0111.

mutants prevented the toxic manifestations of endotoxins from unrelated GNB. In humans, the importance of anti-endotoxin antibodies in the defense against gram-negative infections has been suggested clinically by retrospective studies relating anti-core glycolipid antibody levels with outcome in patients with bacteremia due to various gram-negative bacilli [2, 3] and to *P. aeruginosa* [4]. McCabe *et al.* [2] have shown that the survival of patients with bacteremia due to various gram-negative bacilli was related to their titers of anti-core glycolipid antibodies present at the onset of bacteremia, measured by indirect hemagglutination (Table 1). In a subsequent study of antibody titers measured by immunofluorescence [3], these authors found that IgG antibodies to O-specific antigens correlated also significantly with outcome, although less strikingly than anti-core glycolipid antibodies (Table 1). The correlation found between anti-core glycolipid antibodies and survival was independent of the levels of O-specific IgG antibodies. Studies by Pollack *et al.* [4] have concentrated on patients with *P. aeruginosa* septicemia (Table 2). In accordance with the observation by McCabe *et al.*, Pollack and Young observed a similar relationship between survival from *P. aeruginosa* septicemia and O-specific antibodies measured by indirect hemagglutination, using purified *P. aeruginosa* LPS. A similar correlation was subsequently found between anti-core glycolipid antibodies measured by ELISA and outcome from *P. aeruginosa* septicemia. Both IgG and IgM anti-core glycolipid antibodies were associated with a lower mortality. These studies suggested therefore that strain-specific as well as cross-reactive antibodies may protect patients from severe septic shock or death due to GNB, further suggesting that passive immunotherapy in critically ill patients may be of benefit.

*Therapeutic study with antiserum to* E. coli *J5 for patients with gram-negative bacteremia* (*Table* 3)

The observations reported above, both in animals and in humans, have prompted clinical studies with antiserum obtained by immunizing volunteers with the rough mutant *Escherichia coli* J5. The cross-protection afforded by this antiserum has been tested in the treatment of patients with gram-negative bacteremia, in a multicenter study coordinated by Ziegler from San Diego [5]. Serum was withdrawn from healthy volunteers before (control serum) and 2 weeks after immunization with J5 vaccine (J5 antiserum). In a randomized double-blind trial, the investigators administered test serum to 212 patients with microbiologically confirmed gram-negative bacteremic episodes, 109 having received control serum and 103 immune serum. The number of deaths in the bacteremic patients was 42 of 109 (39%) in controls and 23 of 103 (22%) in recipients of J5 antiserum ($P = 0.011$). In those patients with profound shock who needed treatment with vasopressors for more than 6 h, mortality was reduced from 30 of 39 (77%) in controls to 18 of 41 (44%) in recipients of J5 antiserum ($P = 0.003$) (Table 3). The protection afforded by anti-J5 plasma was the most striking in neutropenic patients and in patients with soft tissue infections. Overall, this study established that the administration of J5 serum from volunteers collected 2 weeks after vaccination with J5 *E. coli* decreased the mortality by half in patients with gram-negative bacteremia, even when profound septic shock was already present. However, when the clinical outcome was related to anti J5 antibody levels in the preparation administered to patients, not to whether the patient had received control or immune serum, no statistical correlation could be demonstrated (Table 3).

*Prophylactic study with antiserum to* E. coli *J5 for the prevention of gram-negative infections in high risk surgical patients* (*Table* 4)

In view of the success of J5 antiserum in treating patients with established gram-negative bacteremia or shock, a randomized double-blind prophylactic trial in surgical patients at high risk of gram-negative infections was performed in Switzerland [6]. Immunization of the Swiss volunteers was performed as in the previous study, but plasma was used instead of serum because plasmapheresis allowed the collection of larger volumes. Two hun-

*Table* 1. *Relationship of shock and death to antibody titers in patients with gram-negative bacteremia*

| | Titers | Number of: Patients | Shock and death | *P* value |
|---|---|---|---|---|
| Type-specific antibodies | | | | |
| IgG to O antigens (IF)* | <1/80 | 96 | 63 (66%) | |
| | >1/80 | 92 | 32 (35%) | <0.001 |
| Indirect HA† | <1/640 | 29 | 19 (66%) | |
| | >1/640 | 139 | 54 (39%) | <0.01 |
| Cross-reactive antibodies | | | | |
| Indirect HA† | <1/80 | 112 | 67 (60%) | |
| | >1/80 | 39 | 6 (15%) | <0.01 |

from McCabe *et al.* [2] and Zinner *et al.* [3].

* Immunofluorescence.

† Indirect hemagglutination, measures mainly IgM. Type-specific antibodies are directed to the side-chains of gram-negative outer membrane lipopolysaccharides (LPS), called 'O' antigens, which are strain-specific. Cross-reactive antibodies are directed to the core region of LPS, which is highly conserved among gram-negative bacteria. LPS of the rough mutant Re of *Salmonella minnesota* has been used as antigen to detect anti-core antibodies.

*Table* 2. *Relationship of survival to antibody levels in* 43 *patients with* P. aeruginosa *septicemia*

| | | Percentage survival | *P* value |
|---|---|---|---|
| Type-specific antibodies | | | |
| Indirect HA* | ≤1:32 | 48% | |
| | >1:32 | 85% | 0.03 |
| | Levels (*g/ml) | | |
| Cross-reactive antibodies | | | |
| Anti-J5 core | <10 | 14% | |
| glycopipid IgG† | >10 | 79% | <0.001 |
| Anti-J5 core | <30 | 44% | |
| glycolipid IgM | >30 | 81% | 0.01 |

From Pollack *et al.* [4].

* Indirect hemagglutination, measures mainly IgM.

† Measured by ELISA.

For explanations on type-specific and cross-reactive antibodies, see footnotes to Table 1. In this experiment, the rough mutant J5 of *Escherichia coli* 0111 was used as antigen to measure anti-core glycolipid antibodies.

dred and sixty-two patients suitable for the study were randomized separately in each category of surgery, and received one unit of test plasma during the first 24 h after admission to surgical intensive care units, and booster units every 5 days as long as they remained severely ill in the intensive care units.

The results showed that anti-J5 plasma did not prevent the acquisition of new focal gram-negative infections, nor did it prevent shaking chills and febrile spikes consecutive to gram-negative infections. In contrast, anti-J5 plasma was very effective in preventing the severe consequences of gram-negative infections, i.e. shock and death. This protective effect was most striking in abdominal surgery patients, in whom the majority of the severe infections occurred during the study. Indeed, among this latter category of patients, gram-negative shock occurred in 13 of 83 control plasma recipients and in only two of 71 anti-J5 plasma recipients ($P = 0.006$), and consecutive death occurred in nine of 83 and one of 71 ($P = 0.017$) respectively (Table 4). The incidence of shock and death due to gram-negative bacteria or fungi were not different in the two study groups, demonstrating that the effect of anti-J5 plasma was specifically directed against GNB. These clinical results were therefore in accordance with previous studies in animals on the mode of action of anti-core glycolipid antiserum, which have suggested that it may primarily neutralize LPS (antitoxic effect), and not opsonize bacteria. Indeed, anti-J5 plasma in that study did not prevent the acquisition of new gram-negative infections, it only prevented their toxic consequences such as shock and death. As in the previous therapeutic study, an important note of caution against this interpretation was brought by the fact that no correlation could be demonstrated between anti-J5 antibody levels and outcome from GNB infection. Indeed, using a very sensitive ELISA assay to measure anti-J5 LPS as well as other anti-core glycolipid antibodies, we did not observe that those patients who survived gram-negative infections had either received, or had naturally occurring, antibody levels against J5 LPS higher than those who succumbed.

*Table 3. Mortality of patients with gram-negative bacteremia treated with J5 antiserum*

| | Mortality from gram-negative bacteremia according to type of therapeutic serum | | |
|---|---|---|---|
| | Non-immune serum | Immune (J5) serum | *P* value |
| | (mean titer 1:6) | (mean titer 1:32) | <0.0001 |
| All patients | 38/100 (38%) | 22/91 (24%) | 0.04 |
| Patients in profound shock | 26/34 (76%) | 17/37 (46%) | 0.009 |
| | according to J5 titer in serum | | |
| | ≤1:8 | >1:8 | *P* value |
| Patients in profound shock | 20/27 (74%) | 28/53 (53%) | 0.07 |

Adapted from Ref. [5].

*Table 4. Prevention of gram-negative (GN) shock and death with anti-J5 plasma administered prophylactically to high-risk surgical patients*

| | Type of plasma | | |
|---|---|---|---|
| Category of surgery | Control | Anti-J5 | *P* value |
| Abdominal surgery | 83 | 71 | |
| Development of infection* | 21 | 15 | n.s. |
| Development of shock | 13 | 2 | 0.006 |
| Death from shock | 9 | 1 | 0.017 |
| All categories† | 136 | 126 | |
| Development of infection* | 33 | 29 | n.s. |
| Development of shock | 15 | 6 | 0.049 |
| Death from shock | 9 | 2 | 0.033 |
| Gram positive and fungal infections | | | |
| Development of infection | 10 | 14 | |
| Development of shock | 6 | 6 | n.s. |
| Death from shock | 3 | 3 | n.s. |

Adapted from ref. [6].
* Pneumonia, intra-abdominal and mediastinal infections.
† The categories of surgery other than abdominal surgery were multiple trauma and pulmonary surgery.

*Prophylactic study with antiserum to* E. coli *J5 for the prevention of gram-negative infections in patients with prolonged neutropenia*

One hundred patients, the majority of whom had acute non-lymphoblastic (63%) and lymphoblastic (29%) leukemia, presented 109 episodes of neutropenia at the city of Hope Medical Center in Duarte, California. Sixty of the 100 patients underwent bone marrow transplantation. All patients were given one unit of either pre-immune (control) or J5 antiserum serum from volunteers at the onset of neutropenia [7].

When compared to control serum, J5 antiserum given prophylactically did not reduce the number of febrile days, the number of gram-negative bacteremic episodes, or death from these infections. This inability to demonstrate a beneficial effect of prophylaxis with a single unit of J5 antiserum in prolonged neutropenia may have several explanations. First, antiserum to core glycolipid, which prevents the consequences of gram-negative bacteremia, shock and death, may not prevent the development of infection or bacteremia. If the major protective effect of J5 antiserum in treating established bacteremia depends on its neutralization of endotoxin, only those who developed bacteremia would test this effect of antiserum. The number of bacteremic gram-negative infections which placed patients at risk of shock and death was small (16 in 109 episodes of neutropenia). Mortality from infection in these 109 episodes was not frequent (6/109). Thus, the power of the study to demonstrate the same protective effect as seen in the treatment studies was necessarily low because relatively few patients developed the severe disease that was successfully treated in the previous study. Second, most patients developed gram-negative infections and bacteremia during the second week of neutropenia. This might be due to the fact that natural antibodies may have provided protection initially, but were progressively depleted, perhaps by invasion of the mucosal surfaces of the gut by gram-negative bacteria. Since many patients with GN bacteremia have natural antibody to core glycolipid, the amount of additional antibody to core glycolipid provided during the first week may not have substantially increased that present naturally. By the second week, both natural and passively transfused antibody may have been reduced below critical levels by a combination of absorption by endotoxins and physiological degradation of antibody.

*Clinical study with anti-J5 hyperimmune intravenous immunoglobulins for the treatment of patients with gram-negative shock*

Two of the three clinical studies summarized above have established the efficacy of J5 antiserum or plasma in the treatment of severe gram-negative bacteremia and in the prophylaxis of gram-negative shock in high risk surgical patients. Since serum or plasma are not suitable for mass production, purified intravenous immunoglobulin preparations (IVIG) were studied in a multicenter double-blind prospective study performed in Switzerland and the Netherlands [8]. All patients presenting with established septic shock due to gram-negative infections were treated either by a standard IVIG (Sando globulin), or by an hyperimmune anti-J5 IVIG prepared from plasma of volunteers immunized with J5 vaccine. One hundred patients were admitted into the study, 71 of them having microbiologically documented gram-negative shock. No significant difference were observed with respect to the number of deaths attributed to septic shock [15/30 (50%) in the J5 and 20/41 (49%) in the standard IVIG groups, respectively], and with respect to the interval between randomization and death. This study showed therefore that anti-J5 IVIG was not superior to standard IVIG in reducing mortality of gram-negative septic shock. This absence of a beneficial effect of anti-J5 IVIG compared to standard IVIG might be attributable to several possibilities:

1. an insufficient increase in antibodies directed to J5 LPS in the anti-J5 IVIG compared to that in the standard IVIG (only a 2.2-fold increase was measured),
2. a loss of protective antibodies during the manufacture of anti-J5 IVIG,
3. the absence in the anti-J5 IVIG of IgM antibodies or other serum factors that might be the protective factors which are present in J5 antiserum.

In addition, it has become clear that the procedure used for preparing hyperimmune IVIG in that study would not be applicable for a large scale preparation of anti-J5 IVIG, because the processes of immunization of volunteers and of plasmapheresis were too tedious. Therefore, to obtain a hyperimmune preparation, it will be necessary either to select donors with naturally occurring high titers of cross-protective antibodies as soon as a good *in vitro* test will be available for screening, or to improve the tolerability and immunogenicity of J5 vaccine before immunizing large numbers of people.

## CONCLUSION

The current view on the mechanism of protection afforded by J5 antiserum or plasma is that it neutralizes the harmful effects of endotoxins by means of cross-reactive anti-core glycolipid antibodies. In clinical trials, the demonstration of the mechanism responsible for the cross-protection afforded by antiserum to rough mutants has not been convincingly established. Two reasons may explain the difficulty in relating clinical outcome with levels of anti-core glycolipid antibodies in the therapeutic trial of J5 antiserum. The first reason is that many sera from non-immunized volunteers as well as sera from recipient-patients had naturally elevated anti-J5 LPS antibody levels. The second reason is that J5 vaccine is a weak immunogen, and induced in volunteers only a 3–5-fold mean increase of J5 LPS antibody titers. For these two reasons, there has been a great overlap in anti-J5 LPS antibody titers in immune and control serum or plasma administered to patients. Therefore, while the clinical data clearly showed a benefit from the administration of J5 antiserum or plasma, the relationship between anti-J5 LPS antibody levels administered to patients and improved outcome has not been established.

At the present time, in addition to the hypothesis attributing cross-protection to anti-core glycolipid antibodies, two alternative hypotheses have been proposed to explain the beneficial effects of immunization with *E. coli* J5 boiled cells. The first is that protection might be mediated by humoral factors other than antibody. Indeed, J5 vaccine may increase non-specifically some unrecognized acute phase reactants, capable of neutralizing LPS or altering its metabolism. The second hypothesis is that passive protection as seen after immunization with *E. coli* J5 might be due to a non-specific polyclonal antibody response of the immunized volunteers, a phenomenon known to occur after injections of both smooth and rough endotoxins. However, if either one or both of the two mechanisms mentioned above were operative in the protection afforded after anti-J5 plasma administration, it would not account for the experimental observation that only very limited cross-protection is observed after immunization with smooth bacteria.

In conclusion, two important questions remain to be answered: One is the determination of the epitope(s) of the LPS core molecule which is(are) the relevant antigen(s) that elicit(s) the best cross-reactive antibodies. Once cross-reactive antigen(s) and their corresponding antibody(ies) will have been characterized, it will be possible to investigate their cross-protective potential by further studies in humans. The answer to this problem is crucial at the present time, because it will permit the screening of blood donors for the preparation of immunoglobulins enriched in antibodies directed against this epitope(s), as well as to produce monoclonal antibodies.

The second question still not resolved is which type of antibodies, whether IgG or IgM, or both,

are most protective. This is important to determine if hyperimmune IVIG preparations are to be used, since these preparations contain at the present time almost exclusively IgG. Controlled trials of hyperimmune IVIG preparations and/or of monoclonal antibodies might help to solve these two questions.

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