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Lipopolysaccharide-Reactive Immunoglobulin E Is Associated with Lower Mortality and Organ Failure in Traumatically Injured Patients

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Antilipopolysaccharide (anti-LPS) immunoglobulin G (IgG) and IgM have been associated with protection from LPS effects in vivo. We investigated the presence of IgE and anti-LPS in 32 patients that had experienced severe traumatic injury and in 35 healthy volunteers; we also investigated whether IgE anti-LPS was associated with important clinical events. Plasma samples were collected daily from patients in the intensive care unit and on one occasion from volunteers; the samples were assayed for IgE anti-LPS. IgE anti-LPS was assayed by enzyme-linked immunosorbent assay with monoclonal anti-human IgE as the capture antibody. Detection was accomplished with biotin-labeled LPS (*Escherichia coli* J5 mutant) followed by streptavidin-peroxidase with 2,2'-azino(3-ethylbenzthiazoline)sulfonic acid as the substrate. The assay was demonstrated to be specific for IgE and LPS-biotin by nonreactivity of control sera with high-titer anti-LPS IgG and IgM and by inhibition with unlabeled LPS. IgE anti-LPS was detected in 1 of 35 healthy controls (2.9%) and 25 of 32 traumatically injured patients (78%) ($P < 0.001$). The presence of IgE anti-LPS was associated with a lower incidence of death ($P = 0.026$) and of renal failure ($P = 0.0012$). There was no apparent temporal relationship between detection of IgE anti-LPS and clinical events. IgG anti-LPS was detected more frequently in patients that were positive for IgE anti-LPS ($P = 0.06$) but was not associated with clinical events. The inability to detect IgE anti-LPS may be related to adverse clinical events through depletion of specific IgE due to LPS exposure after trauma or through saturation of the assay by IgE with other specificities. We have reported increased total IgE concentrations in these patients (J. T. DiPiro, R. G. Hamilton, T. R. Howdieshell, N. F. Adkinson, and A. R. Mansberger, *Ann. Surg.* 215:460–466, 1992).

Lipopolysaccharide (LPS) is a component of the cell membrane of gram-negative bacteria and is responsible for initiating the inflammatory events of gram-negative sepsis. LPS binds to LPS-binding protein, producing stimulation of macrophages and subsequent release of inflammatory mediators (such as tumor necrosis factor alpha, interleukin-1, and interleukin-6). This activation of inflammatory mediators initiates the physiologic changes characteristic of the sepsis syndrome, with continued activation leading to septic shock and multiple organ failure (10).

There are a number of mechanisms in immunocompetent hosts which modulate the response to LPS. The endogenous interleukin-1 receptor antagonist directly antagonizes the interleukin-1 proinflammatory effect. Potentially, binding of LPS to LPS-binding protein can be controlled through downregulation of LPS-binding-protein availability. Also, expression of the LPS receptor on macrophages (CD14) could be downregulated. The outcome following LPS exposure depends to a certain extent on the presence of endogenous antibody that is reactive with LPS. Endogenous anti-LPS immunoglobulin G (IgG) and IgM antibodies have been associated with protection from endotoxin effects in vivo (5, 7, 12). Other isotypes of anti-LPS antibody, including IgE, have not been studied.

We previously reported that total plasma IgE concentrations are elevated following major traumatic injury and sepsis (2).

Detection of IgE anti-LPS may indicate that allergic mechanisms are involved in the response to endotoxin. Classically, this would involve activation of basophils and mast cells. However, additional mechanisms may be involved, specifically, an LPS effect through the activation of cells having low-affinity IgE receptors (e.g., monocytes, eosinophils, platelets, and B lymphocytes). Alternatively, elevated IgE concentrations may be a marker for altered cytokine production after traumatic injury.

Therefore, we attempted to determine if IgE anti-LPS could be detected in the plasma of patients following major traumatic injury and if the presence of IgE anti-LPS was associated with important clinical events such as sepsis, adult respiratory distress syndrome, renal failure, and death.

MATERIALS AND METHODS

Clinical material. Prior to initiation of the study, banked sera from a panel of 270 subjects known to have high total serum IgE levels in the atopic range (>200 ng/ml as determined by the IMx [Abbott Diagnostics, North Chicago, Ill.]) were screened for the presence of IgE anti-LPS. This group was used for selection of positive reference sera. Histories and demographic data for these subjects were not available. All IgE anti-LPS-positive sera identified in the screening assay were confirmed by soluble-antibody inhibition in a second analysis as described below. Plasma or serum samples were then obtained from two groups of study subjects. Group 1 consisted of 32 subjects who sustained major traumatic injury and were

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admitted to the trauma intensive care unit. This group consisted of 29 males and 3 females with a mean age of 33 ± 8 years. All patients had an American College of Surgeons Hospital Trauma Index of 10 or greater (mean, 13.9 ± 2.9 ; range, 10 to 22), indicating a high degree of injury severity and risk of mortality (1). A substantial percentage of these patients developed sepsis (56%), respiratory distress syndrome (63%), or renal failure (34%), and eight patients died during their hospitalization (25%). These patients were selected from a group of 72 trauma patients previously studied (8a). They included all 18 patients of the 72-patient group who experienced a septic event and 14 patients who stayed for similar lengths of time in the trauma intensive care unit but who did not experience a septic event. Plasma samples were obtained daily from these patients while they were in the intensive care unit. Group 2 consisted of 35 healthy volunteers who were hospital employees. This group consisted of 16 males and 19 females with an average age of 31 ± 8.7 years. Allergy histories were not available for either group. All subjects provided written informed consent for blood collections.

Reagents. All chemicals, unless otherwise specified, were obtained from Sigma Chemical Company, St. Louis, Mo. All buffers were prepared with endotoxin-free deionized water. LPS-biotin conjugate was prepared as follows. One milligram of LPS (*E. coli* J5 rough mutant [catalog no. 301; List Biological, Campbell, Calif.]) was added to 1 ml of phosphate-buffered saline (PBS), pH 7.4, containing 4 μ l of triethylamine (T-0886; Sigma), and the mixture was gently vortexed. Sodium *m*-periodate (2.1 mg; S-1878, Sigma) was added, and the mixture was mixed gently for 20 min at room temperature. One microliter of anhydrous glycerol (2136-01; J. T. Baker, Phillipsburg, N.J.) was then added, and the mixture was vortexed. After completion of this reaction, 5.6 mg of crystalline, biotin-epsilon aminocaproyl hydrazide (Calbiochem Corporation, La Jolla, Calif.) was dissolved in 263 μ l of dimethyl sulfoxide (Fisher Scientific, Fair Lawn, N.J.) in a separate glass tube by vortexing. Then, 0.1 ml of the dimethyl sulfoxide-biotin-hydrazide mixture was added to the tube containing LPS-periodate. The mixture was rotated at room temperature for 2 h. Initially, LPS-biotin was dialyzed against PBS with a 2,000-molecular-weight membrane (200 ml for 2 h, repeated three times). This, however, did not improve the performance of the product, and dialysis was deleted from the procedure. LPS-biotin was stored at 4°C and prepared fresh each week.

Chromatographically purified human IgE-specific murine monoclonal antibody (HP 6061) obtained from Hybridoma Reagent Laboratory, Baltimore, Md., was used at 2 μ g/ml in PBS (13). Monoclonal anti-LPS core-lipid A (clone J18-8D2) and the LPS O chain of *E. coli* O111:B4 (clone 042E1) (11) were kindly provided by Martin Evans and Matthew Pollack (Uniformed Services University, Bethesda, Md.). Commercial intravenous IgG (Sandoglobulin; Sandoz Pharmaceuticals, East Hanover, N.J.) was used in the specificity analysis described below. Three high-titer serum samples containing IgG and IgM anti-LPSs were selected by using a previously reported enzyme immunoassay (4).

IgE, IgG, and IgM anti-LPS enzyme immunoassays. The assay for anti-LPS-specific IgE was performed as follows. Microtiter plates (Immunol 4 Removawell; Dynatech, Chantilly, Va.) were coated for 1 h at room temperature and overnight at 4°C with monoclonal anti-human IgE (HP 6061; 0.1 ml per well) that had been diluted with PBS (without protein) to a final concentration of 10 μ g/ml. The plates were then washed five times with PBS-0.05% Tween 20 (PBS-T) and blocked for 1 h at room temperature with PBS-0.5% bovine serum albumin (Cohn Fraction V; A-7906; Sigma)

(PBS-BSA). Reference, positive control, and test sera diluted 1:2 to 1:20 in PBS-BSA were pipetted into duplicate wells (0.1 ml per well) and incubated for 2 h at room temperature. The plates were washed four times with PBS-T, and 0.1 ml of biotinylated LPS was added per well at 1 μ g/ml in PBS-BSA. Following a 2-h incubation at room temperature, the plates were again washed four times, and streptavidin-horseradish peroxidase (S-5512; Sigma) was added at 0.1 ml per well (1 μ g/ml in PBS-BSA) for 1 h at room temperature. After a final wash with PBS-T, 0.1 ml of 2,2'-azino(3-ethylbenzthiazoline)-sulfonic acid (ABTS) (55 mg/100 ml in citrate-phosphate buffer; A-1888; Sigma) was added. Color development was stopped after 16 h with 2 mM sodium azide (S-2202; Sigma), and the A_{410} was read with a microtiter plate reader (model 450; Bio-Rad, Rockville Center, N.Y.).

The immunoreactivity of the biotinylated LPS was tested with two murine monoclonal antiendotoxin antibodies. Microtiter plates were coated with 0.1 ml, diluted 1:1,000, of either J18-8D2, an antibody with specificity for the core and lipid A regions of LPS, or 042E1, an antibody with specificity for the O chain of *E. coli* O111:B4 (11). Each plate was incubated for 1 h at room temperature and overnight at 4°C. After being washed with PBS-T, each plate was blocked with 0.2 ml of 0.5% BSA for 1 h and then rewashed. Biotinylated LPS in 0.5% BSA was added in various dilutions. The detection system consisted of streptavidin-peroxidase (S-5512; Sigma), which was added at 1 μ g/ml (0.1 ml per well) for 1 h; this was followed by washing. ABTS as described above was then added, and the reaction was stopped with sodium azide at 15 min. The A_{410} was determined.

IgG and IgM anti-LPS were tested by using an enzyme immunoassay similar to that previously reported by Freudenberg et al. (4). Briefly, microtiter plates (Immunol 4 Removawell; Dynatech) were coated with LPS from *E. coli* (J5 mutant) at 12.5 μ g/ml. After the plates were blocked with PBS-BSA and washed, sera (diluted 1:16 to 1:128 in PBS) were added and incubated for 1 h at room temperature. After further washing, biotinylated mouse monoclonal anti-human IgG (HP 6017B) or anti-human IgM (HP 6081B; Hybridoma Reagent Laboratory) was added at 1 μ g/ml in PBS-BSA. The detection system consisted of streptavidin-peroxidase followed by ABTS as described above.

Controls and determinations of IgE assay specificity. The isotype specificity of the IgE anti-LPS assay was evaluated by using patient sera that we had previously determined, using the enzyme immunoassay described above, to contain IgG and IgM anti-LPS with end-point titers of at least 1:128. In addition, a commercial, purified IgG preparation (Sandoglobulin; Sandoz Pharmaceuticals) was tested. This IgG preparation was used at a 1:100 dilution in 0.5% BSA, while the sera were used at a 1:16 dilution.

A competitive inhibition test using nonbiotinylated LPS was performed to determine LPS specificity. For this test, microtiter plates were coated with murine monoclonal anti-human IgE (Fc) (HP 6061) as described above and then blocked with 0.5% BSA for 1 h. Serum determined to be highly reactive with IgE anti-LPS (from patient C-8097) by the assay described here was then added at a 1:8 dilution in 0.5% BSA to 12 wells. After incubation at room temperature for 2 h the plates were washed. Then, 0.1 ml of nonbiotinylated LPS (*E. coli* J5 mutant) was added to duplicate wells in the following concentrations: 1 mg/ml, 0.1 mg/ml, 10 μ g/ml, 1 μ g/ml, and 100 ng/ml. PBS alone (100 μ l) was added to the final two wells, and the plates were kept at room temperature for 1 h. After the plates were washed, biotinylated LPS was added; this was followed by

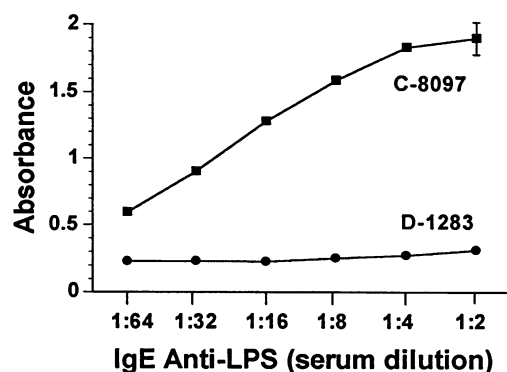


FIG. 1. Dilutions of proposed positive control serum which had IgE anti-LPS reactivity (from patient C-8097) and a negative control serum (from patient D-1283) by using the IgE anti-LPS assay (\pm standard deviation). Nonspecific binding is 0.210 absorbance units.

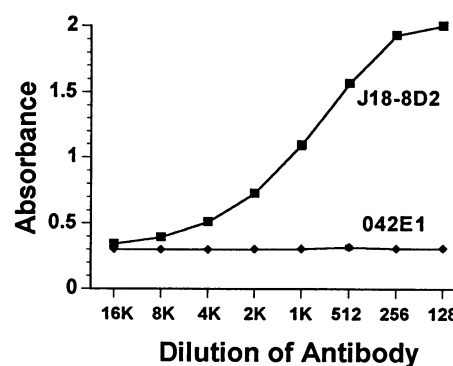


FIG. 2. Immunoreactivity of biotinylated LPS (from the J5 *E. coli* mutant, which does not possess an O side chain) with two murine monoclonal, anti-LPS antibodies. J18-8D2 is known to be reactive with the core and lipid A regions, while 042E1 is known to react only with the O side chain of *E. coli* O111:B4.

the addition of streptavidin-peroxidase and then ABTS as detailed above. The A_{410} was determined.

Total IgE determination. Total serum IgE was measured by an automated microparticle enzyme immunoassay (IMx; Abbott Diagnostics). The lower limit of detection of the assay is 0.1 ng/ml. Specificity was confirmed by using purified IgE, IgM, and IgG and albumin preparations. Further details for total IgE determinations and results were previously published (2).

Clinical data. A complete clinical data base was maintained for all trauma patients. This included the American College of Surgeons Hospital Trauma Index, physiologic parameters, a panel of chemistry and hematology laboratory tests, and notation of major hospital events and operations. Definitions used for sepsis syndrome, adult respiratory distress syndrome, and renal failure have been previously published (2).

Statistics. The *t* test was used to compare means between groups. The chi-square test was used for categorical variables. A *P* value of less than or equal to 0.05 was accepted as significant. Since the assay for IgE anti-LPS was qualitative but not quantitative, results were classified on the basis of antibody being detectable or nondetectable. Samples were considered positive if the absorbance value for the sample was at least twice that of negative serum control, with a *P* value (*t* test) of ≤ 0.001 .

RESULTS

Assay validation. A dilution curve for the most highly reactive patient serum (from patient C-8097) is shown in Fig. 1. This serum was used as a positive control for all further assays of IgE anti-LPS and was included on each assay plate. The coefficient of variation for replicates at a 1:16 dilution was between 2.5 and 10% (mean, $6.6\% \pm 3.0\%$; $n = 8$). Figure 2 illustrates the immunoreactivity of the biotinylated LPS with two murine anti-LPS antibodies. As shown, the conjugate was reactive with the antibody known to have specificity for the LPS core and lipid A regions (J18-8D2). There was minimal reactivity with the O-chain-specific antibody. This is as expected since the LPS used to prepare the biotin conjugate was from the J5 mutant, which does not have an O chain on the LPS.

The specificity of the assay for IgE and for LPS was demonstrated by three studies. In the first, sera that were determined to be reactive with IgG and IgM anti-LPSs (titers of at least 1:128) showed no reactivity in the assay system. Also, the commercially available, purified IgG preparation (Sando-globulin) was nonreactive. In the second study, the immunoreactivity of an IgE anti-LPS-positive test serum (C-8097)

was found to be completely inhibited by 100 ng of nonbiotinylated LPS per ml. Finally, sera from patients determined to have high total serum IgE levels (some greater than 10,000 ng/ml) were nonreactive in the assay system (data not shown).

Clinical samples. IgE anti-LPS was detected in 3 of the 270 nontrauma subjects with elevated total IgE levels (1.1%), 1 of the 35 healthy control subjects (2.9%), and 25 of the 32 traumatically injured patients (78%) ($P < 0.001$). Titers ranged from 1:2 to 1:128. In approximately 50% of the traumatically injured patients with detectable IgE anti-LPS, antibody was detected within 48 h of admission to the hospital (12 of 25 patients) and was detectable in samples collected from two patients in the emergency room. The relationships of IgE anti-LPS detection to clinical events and the presence of IgG and IgM anti-LPS are shown in Table 1. The presence of IgE anti-LPS was associated with lower incidences of death and renal failure. The incidence of sepsis was lower in those patients that were IgE anti-LPS positive, but this was not significant ($P = 0.075$). IgE anti-LPS detection was not associated with the development of adult respiratory distress syndrome or related to splenectomy. There was no apparent temporal relationship between the detection of IgE anti-LPS and the occurrence of clinical events such as sepsis, adult respiratory distress syndrome, renal failure, and death.

IgG and IgM anti-LPSs were detected in 59 and 53% of the trauma patients, respectively. IgG anti-LPS was not associated with any of the selected clinical features, with the exception of a higher frequency of detectable IgG anti-LPS in patients

TABLE 1. Association of IgE anti-LPS with clinical events

Clinical event or feature	No. (%) of patients experiencing event		<i>P</i> value
	IgE anti-LPS positive ($n = 25$)	IgE anti-LPS negative ($n = 7$)	
Sepsis	12 (48)	6 (86)	0.075
ARDS ^a	15 (60)	5 (71)	0.580
Renal failure	5 (20)	6 (86)	0.0012
Splenectomy	7 (28)	2 (29)	0.980
Death	4 (16)	4 (57)	0.026
Positive anti-LPS result			
IgG	17 (68)	2 (29)	0.060
IgM	14 (56)	3 (43)	0.540

^a Adult respiratory distress syndrome.

following splenectomy (89 versus 48%; $P = 0.033$). IgM anti-LPS was not associated with selected clinical events with the exception of mortality. Seven of eight patients that died did not have detectable IgM anti-LPS during their intensive care unit stay ($P = 0.0078$). We have previously reported that these patients had relatively low total IgM concentrations (2). Also, patients that were positive for IgE anti-LPS were more likely to be positive for IgG anti-LPS (68 versus 29%), but this was not significant ($P = 0.06$).

Total IgE was measured in plasma samples collected on admission to the emergency room and then collected weekly while the patient remained in the intensive care unit. Although total IgE concentrations increased in most patients during recovery, there was no apparent association between the concentration of total IgE in plasma and the detection of IgE anti-LPS ($P = 0.56$). Finally, there was no association between the detection of IgE anti-LPS and the American College of Surgeons Hospital Trauma Index.

DISCUSSION

This is the first known report describing the existence of LPS-specific IgE. The detection of this antibody suggests one additional mechanism for immune system activation by LPS. IgE specific for LPS may be involved in the activation of basophils, mast cells, and Langerhans' cells through binding of high-affinity receptors and in the activation of macrophages, platelets, and B lymphocytes through binding of low-affinity receptors.

LPS-reactive IgE was detected in the sera of a substantial majority of patients following traumatic injury (78%) and in the serum of only 1 of 35 healthy subjects (2.9%). In the injured patients, the absence of detectable IgE anti-LPS was associated with higher frequencies of renal failure and death. The 32 trauma patients selected for this study were among the most severely injured of approximately 3,600 patients admitted to the trauma service during the study period. The severity of injury is substantiated by the high percentage of patients experiencing organ dysfunction and death.

We have reported (2) that the same trauma patient group had elevated total plasma IgE concentrations compared with healthy volunteer controls and with patients following elective abdominal surgery. Within the injured group, total IgE concentrations were significantly higher in patients that developed sepsis syndrome than in those that did not. Given the relatively low titers of IgE anti-LPS and the high concentrations of total IgE in plasma (greater than 200 ng/ml), most of the IgE produced in this condition is not LPS specific. The current finding that IgE anti-LPS is associated with lower frequencies of organ failure, sepsis, and death appears inconsistent with the findings for total IgE. One explanation for the findings for total IgE and IgE anti-LPS is that the traumatic injury and sepsis syndrome cause altered cytokine regulation which results in increased IgE production. A number of findings for traumatized and septic patients suggest that increased IgE production may be favored. Substances that enhance IgE production, e.g., interleukin-6 and prostaglandin E_2 , are reported to be increased after trauma and sepsis (3, 6, 9). Also, an inhibitor of interleukin-4-induced IgE production, gamma interferon, is decreased (8). Production of IgE anti-LPS, which may be present in undetectable amounts in most healthy individuals, may be increased with nonspecific IgE upregulation after trauma. However, a portion of injured patients may be exposed to LPS in amounts great enough to deplete IgE anti-LPS. Therefore, since LPS exposure may result in organ failure and death, depleted IgE anti-LPS may be associated with these

adverse events. Certainly, further studies must be conducted to confirm this hypothesis. An alternative explanation is that upregulation of nonspecific IgE production after injury may result in saturation of the assay, inhibiting detection of IgE anti-LPS.

From this study and our previously published data, it appears that IgE production is upregulated after traumatic injury and sepsis. IgE may serve either as a mechanism for cell activation or as a marker of cytokine dysregulation following traumatic injury. It is possible, but we believe unlikely, that IgE anti-LPS is protective. Detection of IgE anti-LPS in selected trauma patients is an important finding in that depletion of IgE anti-LPS from plasma may indicate substantial LPS exposure with subsequent mediator release; this exposure increases the risk of organ failure and death. Whether the absence of IgE anti-LPS in injured patients is a better predictor of morbidity and mortality than direct measurement of LPS or of cytokines (such as interleukin-1, interleukin-6, or tumor necrosis factor alpha) remains to be determined.

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REFERENCES

1. American College of Surgeons Committee on Trauma. 1980. Field categorization of trauma patients and hospital trauma index. *Bull. Am. Coll. Surgeons* 65:28-33.
2. DiPiro, J. T., R. G. Hamilton, T. R. Howdieshell, N. F. Adkinson, and A. R. Mansberger. 1992. Total IgE in plasma is elevated after traumatic injury and is associated with sepsis syndrome. *Ann. Surg.* 215:460-466.
3. Faist, E., A. Mewes, C. C. Baker, et al. 1987. Prostaglandin E_2 (PGE_2)-dependent suppression of interleukin 2 (IL-2) production in patients with major trauma. *J. Trauma* 27:837-848.
4. Freudenberg, M. A., A. Fomsgaard, I. Mitov, and C. Galanos. 1989. ELISA for antibodies to lipid A, lipopolysaccharide and other hydrophilic antigens. *Infection* 17:322-328.
5. Gould, F. K., J. A. Harvey, and J. K. Dytrych. 1989. Antibody to endotoxin is associated with decreased frequency of postoperative infection. *Am. J. Obstet. Gynecol.* 160:317-319.
6. Hack, C. E., E. R. DeGroot, J. F. Richelle, et al. 1989. Increased plasma levels of interleukin-6 in sepsis. *Blood* 74:1704-1710.
7. Klumbeck, N., G. Delespesse, and J. P. Butzler. 1980. Humoral and circulating immune complexes in gram-negative bacteremia and septic shock. *In* M. K. Agarwal (ed.), *Bacterial endotoxins and host response*. Elsevier/North Holland Biomedical Press, Amsterdam.
8. Livingston, D. H., S. H. Appel, S. R. Wellhausen, et al. 1988. Depressed interferon gamma production and monocyte HLA-DR expression after severe injury. *Arch. Surg.* 123:1309-1312.
- 8a. Mansberger, A. R., J. E. Doran, R. C. Treat, et al. 1989. The influence of fibronectin administration on the incidence of sepsis and septic mortality in severely injured patients. *Ann. Surg.* 210:297-307.
9. Miller-Graziano, C. L., G. Szabo, K. Kodys, and K. Griffey. 1990. Aberrations in posttrauma monocyte subpopulation: role in septic shock syndrome. *J. Trauma* 30:S86-S97.
10. Morrison, D. C., and J. L. Ryan. 1987. Endotoxins and disease mechanisms. *Annu. Rev. Med.* 38:417-432.
11. Pollack, M., J. K. S. Chia, L. Koles, et al. 1989. Specificity and cross-reactivity of monoclonal antibodies reactive with the core and lipid A regions of bacterial lipopolysaccharide. *J. Infect. Dis.* 159:168-188.
12. Pollack, M., A. I. Huang, R. K. Prescott, et al. 1983. Enhanced survival in *Pseudomonas aeruginosa* septicemia associated with high levels of circulating antibody to *Escherichia coli*. *J. Clin. Invest.* 72:1874-1881.
13. Reimer, C. B. 1986. Five hybridomas secreting monoclonal antibodies against human IgE. *Monoclonal Antibody News* 4:1-4.