

Detection of Enterobacterial Lipopolysaccharides and Experimental Endotoxemia by Means of an Immunolimus Assay Using Both Serotype-Specific and Cross-Reactive Antibodies

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The immunolimus (IML) assay system uses solid-phase endotoxin antibodies to capture lipopolysaccharide (LPS), which is then quantified by a modification of the chromogenic limulus amebocyte lysate (CLAL) method. Monoclonal antibodies (MAbs) reactive with selected O antigen serotypes of *Escherichia coli* (O18) and *Salmonella typhimurium* (O-9,12), when used in the IML, were shown to be highly specific in detecting their respective endotoxins in purified form and in plasma samples from experimentally infected animals. A murine MAb that was broadly cross-reactive with *E. coli*, *Salmonella*, and *Shigella* endotoxins also proved to be highly effective in the IML assay for capturing LPS molecules from both *E. coli* and *S. typhimurium* strains. These results indicate that IML assays can detect smooth-type enterobacterial endotoxins in plasma and suggest that such assays have potential for use in the rapid diagnosis of sepsis and endotoxemia caused by different enterobacterial species.

As many as 600,000 episodes of sepsis occur annually in the United States and about half of these cases are associated with positive blood cultures [1]. About half of all cases of sepsis are thought to be caused by gram-negative organisms [2] and the overall case-fatality rate associated with these infections is ~35% [3]. The incidence of severe infections caused by gram-negative bacteria has clearly increased during the past decade, most likely as the result of both the better survival of severely compromised patients and the greater use of invasive techniques in modern medicine. Accordingly, septic shock is one of the leading causes of mortality in hospitalized patients in the United States.

Despite advances in rapid diagnostic methods used in microbiology, the diagnosis of bacteremia and sepsis caused by gram-negative bacteria is still based on conventional and time-consuming blood culture methods. The need to develop a more rapid method for detection of gram-negative septicemia and endotoxemia is underscored by the imminent introduction of new and expensive anti-infective therapies, including endotoxin-binding proteins [4, 5] and cytokine-

directed antibodies or inhibitors [6]. Only certain subgroups of patients with sepsis or septic shock syndrome will realize benefits from these new therapies, and thus screening and identification of these particular persons is essential for both medical and economic reasons [7].

New diagnostic methods for detecting bacterial endotoxin have been investigated in our laboratory [8, 9], culminating in the development of a rapid method for the detection of the lipooligosaccharide (LOS) of *Haemophilus influenzae* type b (Hib). This immunolimus (IML) assay used Hib LOS-specific monoclonal antibodies (MAbs) in a solid-phase system to capture Hib LOS present in plasma samples from experimentally infected animals [10]. This capture step provided the necessary specificity for this method, while the requisite sensitivity was obtained by the use of the chromogenic limulus amebocyte lysate (CLAL) assay to detect the antibody-bound Hib LOS. We have tested the IML format for its suitability for detecting enterobacterial endotoxins, using both serotype-specific and broadly cross-reactive LPS antibodies as capture agents.

Materials and Methods

Bacterial strains and culture conditions. *Escherichia coli* O18:K1 IH3080 was isolated from a patient with neonatal meningitis and has been characterized extensively [11]. Similarly, *E. coli* O132:K1 C28 was isolated from a case of neonatal meningitis in Dallas. The *E. coli* strains were grown in brain-heart infusion broth (Difco, Detroit) at 37°C and harvested during the midlogarithmic phase of growth. *Salmonella typhimurium* SH4336 (serotype O-4,12) and SH4338 (serotype O-9,12) [12] were obtained from the collection at the National Public Health Institute, Helsinki.

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Endotoxins. Purified *E. coli* O111, O127, and J5 lipopolysaccharides (LPS) were purchased from List Biological Laboratories (Campbell, CA) as were *Pseudomonas aeruginosa* Fisher type 1 LPS and *Serratia marcescens* LPS. Purified LPS from *E. coli* IH3080 (serotype O18) and *S. typhimurium* SH2183 (serotype O-4,12) were provided by I. Helander (National Public Health Institute). *Salmonella paratyphi* A IS2 (serotype O-2,12) and *Salmonella thompson* (serotype O-6,7) were obtained from L. LeMinor (Institut Pasteur, Paris) and *Salmonella typhi* T2 (serotype O-9,12) was from B. A. D. Stocker (Stanford University School of Medicine, Stanford, CA). These three *Salmonella* strains were grown to late logarithmic phase at 37°C, and LPS was extracted by the hot phenol-water method [13], followed by further purification involving treatment with RNase and proteinase and ultracentrifugation. LOS was purified from Hib strain DL42 as described [14].

MAbs. One murine MAb (MATy-10) specific for the O-9 epitope of the *Salmonella* O-9,12 LPS molecule was used in this study for specific detection of the O-9,12 endotoxin [15]. This MAb was partly purified from ascites fluids by precipitation with ammonium sulfate. The human MAb SE111, reactive with *E. coli* O18 LPS, was used in purified form and will be described in detail elsewhere (unpublished data). The murine MAb WNI 222-5 is broadly cross-reactive with the LPS molecules of *E. coli*, *Salmonella*, and *Shigella* species [16] (unpublished data) and was used in purified form.

Animal models. The neonatal rat model for *E. coli* K1 infections [17] was used with some modifications. Briefly, *E. coli* strains O18:K1 and O132:K1 were grown to the late logarithmic phase in brain-heart infusion broth and harvested by centrifugation, washed once with pH 7.4 pyrogen-free (pf) PBS, and resuspended in this same buffer. This pf-PBS and other pf solutions were prepared by using nonpyrogenic sterile water for irrigation (Baxter Healthcare Products, Deerfield, IL). Five-day-old Sprague-Dawley rat pups (Simonsen Laboratories, Gilroy, CA) were injected intraperitoneally with 50–200 cfu of bacteria. Twenty-four hours later, the magnitude of bacteremia in each animal was determined by drawing 5 μ L of blood from the tail vein and spreading it onto a brain-heart infusion agar plate. Each animal was then immediately anesthetized, and cardiac puncture was used to draw 0.5 mL of blood into a syringe containing 0.05 mL of 3.8% (wt/vol) sodium citrate. This mixture was centrifuged for 10 min at 5000 rpm in an Eppendorf microcentrifuge (15-cm-diameter rotor; Brinkmann Instruments, Westbury, NY), and the resultant plasma was transferred to polypropylene tubes that were then frozen at –70°C. Forty-six infant rats were challenged with *E. coli* O18:K1; 16 animals were injected with *E. coli* O132:K1.

A mouse salmonellosis model [18] was used to obtain plasma samples containing *Salmonella* endotoxin. Female 8-week-old (CBA \times C57BL/6) F1 mice (Bomholtgard Breeding and Research Centre, Ry, Denmark) received an intraperitoneal injection of 10–50 times the LD₅₀ of *S. typhimurium* SH4338 and SH4336. Animals were euthenized by CO₂ narcosis at 72–144 h after infection. Immediately after death, blood was obtained by cardiac puncture for culture and for preparation of plasma as described above.

Dilution fluid for purified LPS. Blood samples from normal,

uninfected infant rats and adult mice were collected by cardiac puncture; plasma prepared from this blood was stored in multiple portions at –70°C and used for dilution of the purified LPS standards. These plasmas were diluted 1:10 in pf-PBS before their use as diluent for purified LPS standards in those CLAL and IML assays involving the detection of endotoxin in plasma samples. For detection of endotoxin in buffer, purified LPS preparations were diluted in pf-PBS before use in the CLAL and IML assays.

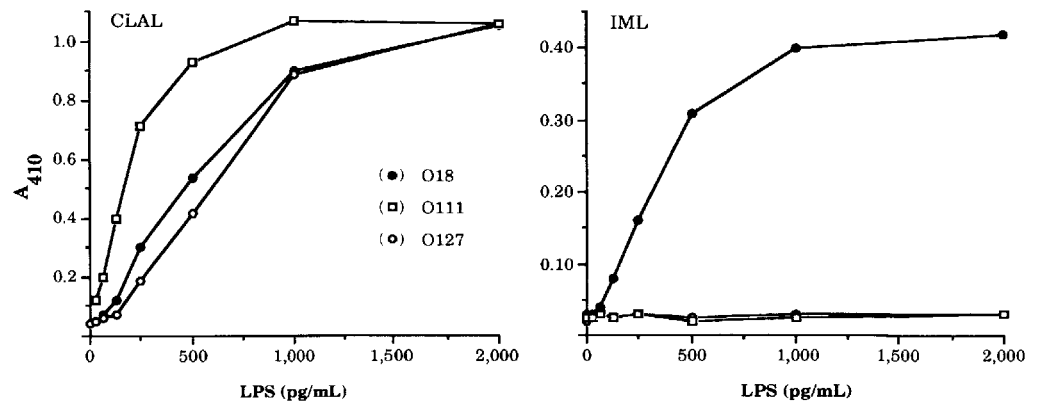
LAL extract and chromogenic substrate. Each vial of LAL extract (Pyrotell; Associates of Cape Cod, Woods Hole, MA), specified as suitable for use in the CLAL assay, was rehydrated in 10 mL of pf-water and stored in multiple portions at –20°C for <3 months before use in the CLAL assay. When used in the IML assay, LAL extract was rehydrated in 20 mL of pf-water before being stored at –20°C. The chromogenic substrate *N*-benzoyl-val-gly-arg *p*-nitroanilide hydrochloride (Sigma, St. Louis) was diluted to a concentration of 0.7 mg/mL in pf-water and stored at 4°C until used.

Microtiter plates. Sterile, polystyrene 96-well ELISA plates with flat-bottom wells (Corning Laboratory Sciences, Houston) were used throughout this study. These plates were shown to be pf by testing with the CLAL assay.

CLAL assay. A two-step assay system was used for detection of endotoxin by CLAL assay in microtiter plates [8, 19]. Commercially available, purified *E. coli* O111 LPS (List Biological Laboratories) was used as the standard in all experiments. Immediately before use in the CLAL assay, *E. coli* O111 LPS was diluted to concentrations of 0–800 pg of LPS/mL of dilution fluid, heated at 75°C for 12 min [19], cooled to room temperature, and loaded in duplicate 50- μ L portions into microtiter plates. Plasma samples from infected animals were diluted 1:10 in pf-PBS, heated as described above, and loaded in 50- μ L portions in duplicate wells. Serial 10-fold dilutions of the plasma samples in dilution fluid were used if the initial 1:10 dilution did not yield an absorbance reading within the range obtained with the LPS standards. After the addition of 50 μ L of LAL extract, the plates were incubated for 30 min at room temperature. Then, 50 μ L of the chromogenic substrate, diluted 1:2 with 0.054 *M* TRIS-HCl, pH 9.4, immediately before use, was added to each well and the plate was incubated for 20 min at room temperature. The absorbance at 410 nm of the contents of each well was determined by an automated ELISA reader (MR700; Dynatech Laboratories, Chantilly, VA). The endotoxin content of the test wells was determined by comparing the absorbance values of the test samples to those of the wells containing the LPS standards after the background absorbances were subtracted. The final endotoxin concentration was calculated by multiplying the derived LPS concentration by the respective dilution factor.

IML assay. Microtiter wells were coated overnight at room temperature with a 100- μ L volume of MAbs diluted in 0.1 *M* sodium carbonate buffer, pH 9.4. The optimal concentration of MAb for use in the IML was determined separately for each MAb; the final amount of antibody protein added per well was ~250 ng. Three wells were used for each endotoxin-containing sample; two were coated with the MAb and the third was left uncoated to serve as an internal control for specificity. The mi-

Figure 1. Detection of purified *Escherichia coli* lipopolysaccharide (LPS) using chromogenic limulus amoebocyte lysate (CLAL) and immunolimus (IML) systems. Monoclonal antibody used in IML system was O18-reactive SE111. *E. coli* LPS used were O18, O111, and O127. Results are means from duplicate wells.



cro-titer wells were washed three times with pf-PBS containing 0.05% (vol/vol) Tween-20 (pf-PBS-T) and then blocked with pf-PBS-T containing 1% (vol/vol) fetal bovine serum (Sterile Systems, Logan, UT). The plates were again washed with pf-PBS-T three times.

Plasma samples from infected animals were diluted 1:10 in pf-PBS, and the homologous LPS standard was prepared in 0–10,000 pg/mL concentrations as described above for the CLAL assay. After heating these endotoxin-containing preparations at 75°C for 12 min, 50- μ L portions of each test sample and the LPS standards were added to the microtiter wells and incubated for 1 h at 37°C. The wells were then washed six times with pf-PBS-T, and 50 μ L of LAL extract was added to each well. After a 20-min incubation at room temperature, 50 μ L of the chromogenic substrate was added and the absorbance in each well was measured by an ELISA reader after 30 and 60 min. The absorbance of the uncoated well was subtracted from the mean of the MAb-coated wells. In instances in which the absorbances in the uncoated wells exceeded the normal background limit (i.e., $A_{410} = 0.05$), the samples were diluted (before use in the IML assay) until the background absorbance was <0.05 at A_{410} .

The amount of LPS in the test samples was determined by comparison with standards on each plate. The LPS standards used in these assays varied with the MAb: Purified *E. coli* O18 LPS was used with the SE111 MAb, purified *E. coli* J5 LPS was used with cross-reactive MAb WN1 222-5, and purified *S. typhi* O-9,12 LPS was used with MATy-10 MAb.

Statistical analysis. Pearson's correlation coefficient was used to assess the strength of the relationship between results from the CLAL and IML assays and the magnitude of the bacteremia in the infected animals.

Results

Use of purified LPS to determine the sensitivity and specificity of the CLAL and IML assays. The sensitivity of the CLAL method in detecting *E. coli* LPS was determined using purified preparations of *E. coli* O18, O111, and O127 LPS. The limit of sensitivity of the CLAL, defined as the concentration of LPS yielding an absorbance (A_{410}) twofold higher than the mean of the background, was 50 pg/mL with O18 LPS, 150 pg/mL with O111 LPS, and 170 pg/mL with O127 LPS (figure 1). All three of these purified *E. coli* LPS prepara-

tions were also used to determine both the sensitivity and specificity of the IML method using the *E. coli* O18 LPS-reactive MAb SE111. Microtiter wells coated with this MAb readily bound the homologous O18 LPS, with a detection limit of ~ 120 pg/mL, whereas neither the O111 nor the O127 LPS molecules were reactive in this IML assay (figure 1).

Similar testing of the CLAL and IML assays was done using purified LPS from different *Salmonella* strains. With the CLAL, the limits of sensitivity for detection of four *Salmonella* LPS preparations (O-9,12, O-4,12, O-2,12, and O-6,7) ranged from 20 to 50 pg/mL (figure 2). Using the MAb MATy-10, which is specific for the O-9 epitope in O-9,12 LPS, the IML method could detect as little as 200 pg of the homologous LPS from *S. typhi* but did not detect any of the three other heterologous *Salmonella* LPS preparations (figure 2).

Characterization of the broadly cross-reactive MAb WN1 222-5. This MAb has been previously described as being reactive with the core oligosaccharide region of LPS molecules from *E. coli*, *Salmonella*, and *Shigella* species [16] (unpublished data). Testing of this MAb in the IML system with the various *E. coli* and *Salmonella* smooth LPS preparations as described indicated that this MAb bound all of the different smooth LPS molecules, with an average limit of sensitivity of ~ 500 pg/mL. In contrast, this MAb did not detect purified LPS (3000 pg/mL) from *P. aeruginosa* Fisher type 1 or *S. marcescens* in the IML assay. Similarly, purified LOS (3000 pg/mL) from Hib was unreactive in the IML with MAb WN1 222-5 (data not shown).

Detection of endotoxin in plasma samples from experimentally infected animals. The relative abilities of the IML and CLAL assays to detect LPS in plasma were compared by using animal models for invasive *E. coli* and *S. typhimurium* disease. Both of the O antigen-specific MAbs and the broadly cross-reactive MAb WN1 222-5 were used for endotoxin capture in the IML assays. Infant rats were infected with either *E. coli* O18:K1 or O132:K1, and blood was drawn for plasma preparation 24 h after infection. Of 46 animals challenged with *E. coli* O18:K1, 41 developed detect-

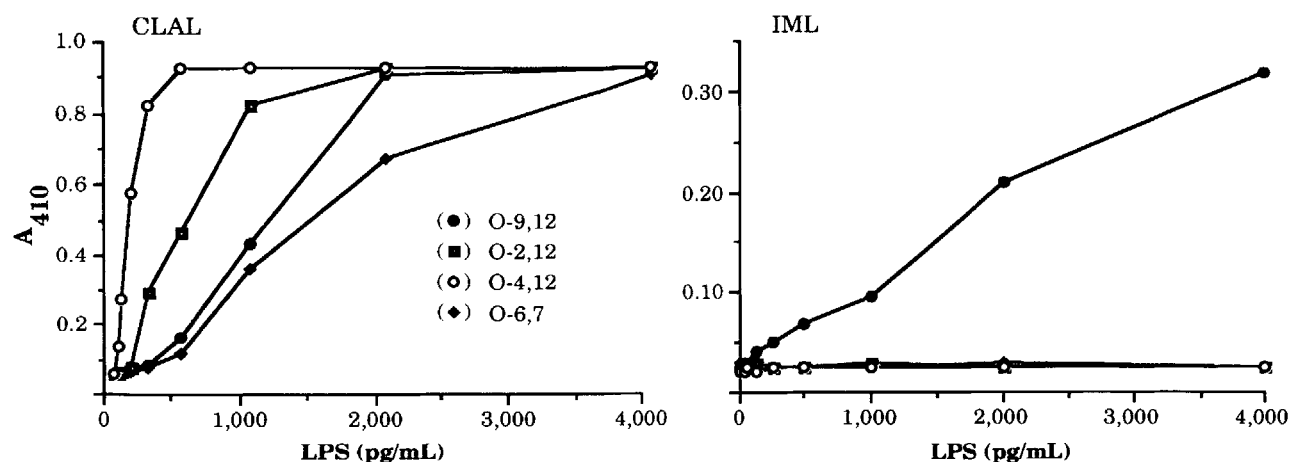


Figure 2. Detection of purified *Salmonella* lipopolysaccharide (LPS) using chromogenic limulus amoebocyte lysate (CLAL) and immunolimus (IML) systems. Monoclonal antibody used in IML system was O-9-reactive MATy-10, which binds O-9,12 LPS. Serotypes of *Salmonella* LPS used were O-9,12, O-2,12, O-4,12, and O-6,7. Results are means from duplicate wells.

able bacteremia (limit of detection, 200 cfu/mL). Similarly, 14 of 16 rats infected with *E. coli* O132:K1 became bacteremic. Using the CLAL method, plasma from 21 (51%) of the 41 animals with detectable *E. coli* O18:K1 bacteremia yielded a positive reaction (table 1). Similarly, of the 14 animals with *E. coli* O132:K1 bacteremia, 13 (93%) had endotoxin detectable with the CLAL method, making a total of 34 bacteremic animals reactive in the CLAL test (table 1). The coefficient of correlation between the magnitude of bacteremia and detection of LPS in the CLAL assay was 0.585 for all animals in both groups; the limit of detectability of LPS in rat plasma was 200 pg/mL (figure 3).

Table 1. Detection of endotoxin in plasma from experimentally infected animals by means of chromogenic limulus amoebocyte lysate (CLAL) and immunolimus (IML) methods.

Challenge strain	CLAL-positive plasmas/bacteremic animals	IML (O antigen-specific)-positive plasmas/CLAL-positive plasmas	IML (cross-reactive)-positive plasmas/CLAL-positive plasmas*
<i>Escherichia coli</i>			
O18:K1	21/41 (51)	20/21 (93) [†]	18/21 (86)
O132:K1	13/14 (93)	0/13 [‡]	10/13 (77)
Total	34/55 (62)	—	28/34 (82)
<i>Salmonella typhimurium</i>			
O-9,12	12/17 (71)	9/12 (75) [‡]	10/12 (83)
O-4,12	8/17 (47)	0/8 (8) [‡]	6/8 (75)
Total	20/34 (59)	—	16/20 (80)

NOTE. Data are no./no. (%).

* Using monoclonal antibody (MAb) WN1 222-5.

[†] Using MAb SE111 reactive with *E. coli* O18 lipopolysaccharide (LPS).

[‡] Using MAb MATy-10 reactive with *S. typhimurium* O-9,12 LPS.

Use of the *E. coli* O18 LPS-reactive MAb SE111 in the IML assay allowed detection of endotoxin in 21 (51%) of 41 animals with *E. coli* O18:K1 bacteremia (figure 3). Of these 21 animals, 20 had yielded positive reactions in the CLAL (table 1). There was also 1 animal whose plasma yielded a negative result in the CLAL but was positive in this IML system. In contrast, none of the plasma samples from the 14 animals with *E. coli* O132:K1 bacteremia had a positive reaction in this IML assay (table 1, figure 3). The limit of detection of *E. coli* O18 LPS in rat plasma with this MAb was 400 pg/mL (figure 3).

When the broadly cross-reactive MAb WN1 222-5 was used as the capture agent in the IML system, LPS was detected in 21 (51%) of 41 animals with *E. coli* O18:K1 bacteremia and in 10 (71%) of 14 that were bacteremic with the other *E. coli* strain (figure 3). Of the 34 bacteremic animals with positive plasma by CLAL, 28 were reactive with the cross-reactive MAb in the IML (table 1). The limit of sensitivity in detecting LPS in plasma with this broadly cross-reactive MAb in the IML (100 pg/mL) was roughly similar to that achieved with the O antigen-reactive MAb (400 pg/mL), although the coefficient of correlation was better with the cross-reactive MAb (figure 3).

A mouse model of salmonellosis involving animals infected with *S. typhimurium* SH4338 or SH4336 (LPS serotypes O-9,12 or O-4,12, respectively) was also used in this study. All 17 animals challenged with SH4338 and the 17 challenged with SH4336 became bacteremic. The limit of sensitivity for detecting *Salmonella* LPS in mouse plasma with the CLAL was 200 pg/mL, and this method detected endotoxin in 20 of the 34 plasma samples (12 from animals infected with strain SH4338 [serotype O-9,12] and 8 from animals infected with SH4336 [serotype O-4,12]; table 1, figure 4). Use of the *Salmonella* LPS O-9 antigen-specific MAb MATy-10 in the IML allowed detection of LPS in 9 of

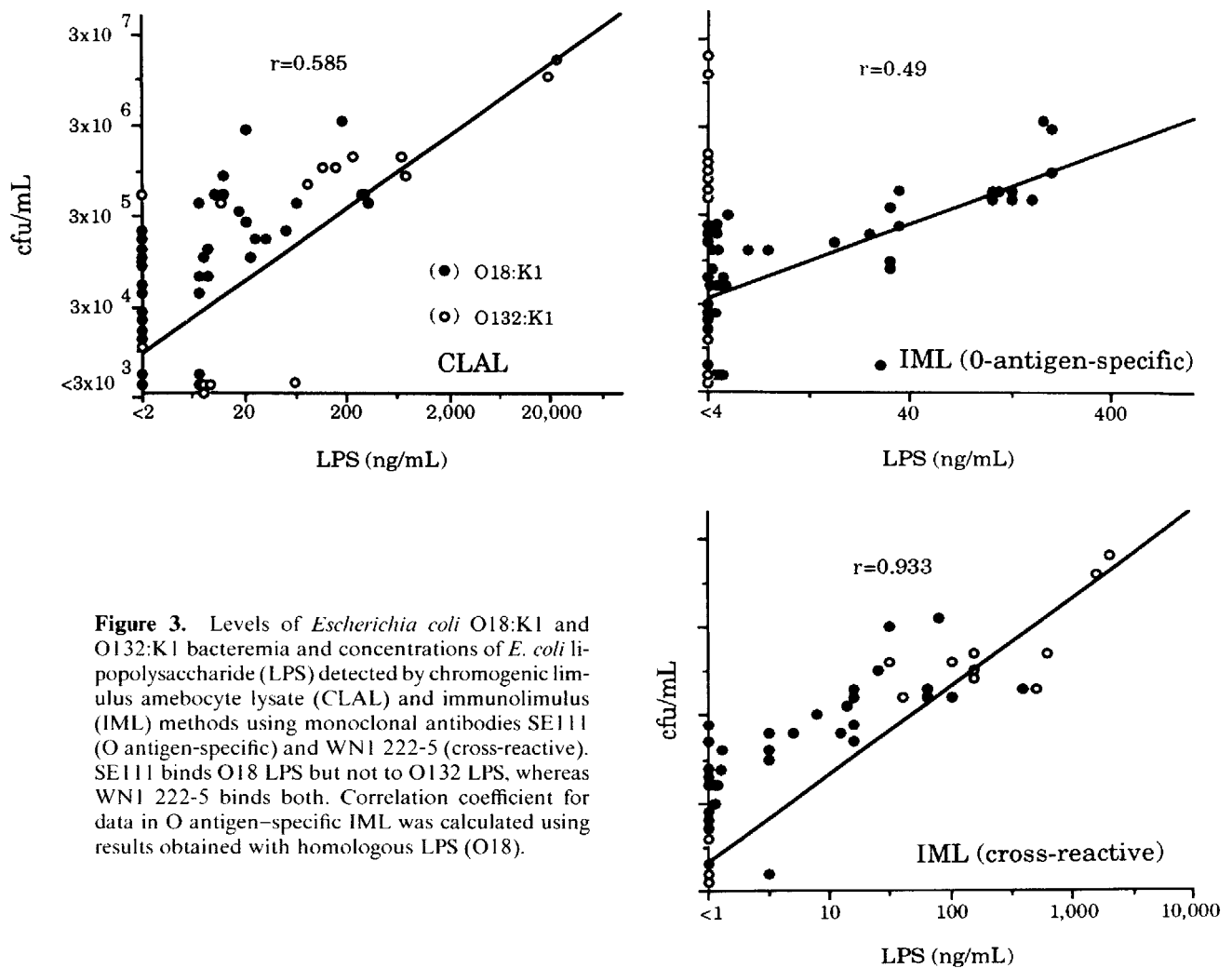


Figure 3. Levels of *Escherichia coli* O18:K1 and O132:K1 bacteremia and concentrations of *E. coli* lipopolysaccharide (LPS) detected by chromogenic limulus amoebocyte lysate (CLAL) and immunolimus (IML) methods using monoclonal antibodies SE111 (O antigen-specific) and WN1 222-5 (cross-reactive). SE111 binds O18 LPS but not to O132 LPS, whereas WN1 222-5 binds both. Correlation coefficient for data in O antigen-specific IML was calculated using results obtained with homologous LPS (O18).

17 plasma samples from animals infected with *S. typhimurium* SH4338 (serotype O-9,12; table 1, figure 4). Of the 12 bacteremic animals infected with strain SH4338 with plasma samples that yielded positive results in the CLAL assay, 9 were positive in the IML system using MAb MATy-10 (table 1, figure 4). None of the 17 plasma samples from animals infected with the heterologous *S. typhimurium* SH4336 (serotype O-4,12) reacted in this O antigen-specific assay (table 1, figure 4). About half (16) of the 34 plasma samples yielded a positive result with the broadly cross-reactive MAb WN1 222-5 in the IML system (table 1, figure 4). Among the total 20 bacteremic animals that yielded a positive CLAL reaction, 16 were positive in this IML using the cross-reactive MAb (table 1).

Discussion

The LAL test for endotoxin, while exquisitely sensitive with in vitro samples, has several limitations when used to detect endotoxin in clinically relevant samples, especially

plasma. First, the high degree of sensitivity of the LAL system to endotoxin results in trace amounts of contaminating endotoxin, derived from sampling procedures or equipment, yielding false-positive results. Second, human plasma contains several inhibitors and activators of the enzymatic systems involved in the LAL cascade [20, 21]. Third, the color and turbidity of normal plasma lowers the sensitivity of the CLAL, the most recent modification of the standard LAL assay [22].

The IML method was developed specifically to circumvent these practical problems and possesses at least two significant advantages over the standard CLAL method. The use of antibodies as solid-phase endotoxin capture agents provides specificity in the detection of a given endotoxin, thereby alleviating the first problem described above. In addition, this endotoxin immobilization step permits thorough washing from the test system of those plasma constituents that adversely affect the CLAL system.

Previous experience with the IML had established that a MAb to Hib LOS would allow detection of this endotoxin in

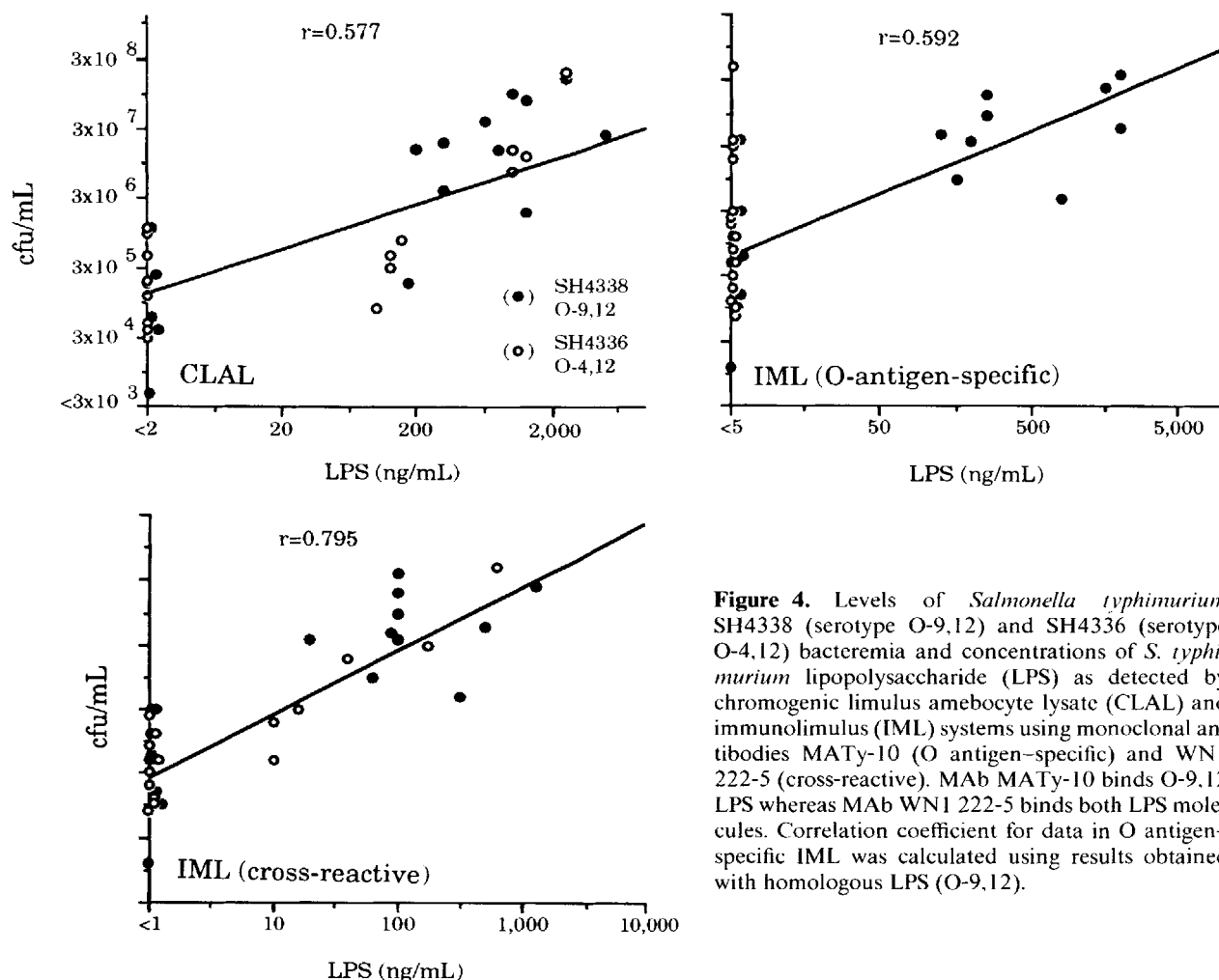


Figure 4. Levels of *Salmonella typhimurium* SH4338 (serotype O-9,12) and SH4336 (serotype O-4,12) bacteremia and concentrations of *S. typhimurium* lipopolysaccharide (LPS) as detected by chromogenic limulus amoebocyte lysate (CLAL) and immunolimus (IML) systems using monoclonal antibodies MATy-10 (O antigen-specific) and WN1 222-5 (cross-reactive). MAb MATy-10 binds O-9,12 LPS whereas MAb WN1 222-5 binds both LPS molecules. Correlation coefficient for data in O antigen-specific IML was calculated using results obtained with homologous LPS (O-9,12).

plasma samples from experimentally infected animals [10]. However, the MAb used in this earlier study bound to an epitope in the oligosaccharide of Hib LOS, which itself is similar to rough forms of enteric LPS molecules, being composed of only lipid A covalently coupled to a relatively small oligosaccharide [23]. One purpose of the present study was to determine whether a MAb directed to an O antigen epitope could efficiently capture smooth enteric LPS molecules present in plasma. Using MAbs to *E. coli* and *Salmonella* O antigens, it was found that the corresponding smooth LPS molecules could be detected in vitro at levels <200 pg/mL (figures 1, 2). Moreover, these same two MAbs readily detected their respective LPS molecules in plasma samples obtained from animals with *E. coli* or *S. typhimurium* infections, with the limit of sensitivity decreasing somewhat (figures 3, 4). A murine MAb (E5), described as being directed against *E. coli* J5 LPS, was recently reported to have a similar level of sensitivity (~ 250 pg/mL) in detecting purified enteric LPS molecules in buffer when used in a slightly modified version of the IML [24]; no results using in vivo-

derived LPS-containing samples (e.g., plasma) were reported.

The O antigen-directed MAbs used in the present study also provided a high degree of specificity in endotoxin detection, even with plasma samples. This specificity occurred even when levels of heterologous LPS in plasma were >1000 ng/mL. The successful use of these O antigen-directed MAbs in the IML system prompted us to evaluate a recently described MAb that is cross-reactive with the endotoxin molecules of all *E. coli*, *Salmonella*, and *Shigella* strains tested to date [16] (unpublished data). This cross-reactive MAb worked readily in the IML with all samples of purified *E. coli* and *Salmonella* LPS tested in the present study and, when used with plasma samples from the experimentally infected animals, bound LPS from all 4 test strains (figures 3, 4). The fact that this MAb did not bind endotoxins from other pathogens (*P. aeruginosa* and Hib) indicates that appropriate antibody specificity was still operative in the IML.

Direct comparison of the relative levels of sensitivity of the CLAL and IML assays was not a specific aim of this study.

The crucial difference between these assays, however, is the use of extensive washing of the endotoxin immobilized in the microtiter wells in the IML system. This efficient elimination of interfering compounds and contaminating endotoxins means that the sensitivity of the IML method has the potential to exceed that of the CLAL. In addition, the fact that plasma samples used in the CLAL usually must be diluted at least 10-fold decreases the sensitivity of this method [25]. In contrast, samples for the IML do not have to be diluted before assay, and it is possible that multiple portions of a plasma sample could be sequentially incubated in the IML microtiter well, thereby increasing further the limit of sensitivity for endotoxin detection.

Taken together, our results indicate that the specificity of endotoxin detection in the IML is antibody-dependent. At the very least, the IML may prove to be an appropriate test vehicle for the identification of new endotoxin-directed MABs with therapeutic potential. In addition, the IML can be used to screen for new endotoxin-specific MABs that provide maximal sensitivity in detecting LPS; these MABs would allow improvement of the present IML technology. The current availability of MAb WN1 222-5 and similar cross-reactive MABs, together with the fact that the IML method can be completed in just 3 h, raise the possibility that these MABs might ultimately be incorporated into an IML-based system for rapid detection of *E. coli* and *Salmonella* bacteremia. Such a test, with a sensitivity comparable to that of the CLAL and a specificity that far exceeds it, might be useful for relatively quick identification of patients likely to benefit from new therapeutic modalities.

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