INFECTION AND IMMUNITY, Aug. 1986, p. 298–304
0019-9567/86/080298-07$02.00/0
Copyright © 1986, American Society for Microbiology
Vol. 53, No. 2

# Mechanisms Involved in Protection Provided by Immunization against Core Lipopolysaccharides of *Escherichia coli* J5 from Lethal *Haemophilus pleuropneumoniae* Infections in Swine

BRADLEY W. FENWICK,†* JIM S. CULLOR, BENNIE I. OSBURN, AND HARVEY J. OLANDER
*Department of Pathology, School of Veterinary Medicine, University of California, Davis, California 95616*

Received 21 January 1986/Accepted 15 April 1986

**In an investigation of the potential protective effects of immunity against common lipopolysaccharide core antigens of gram-negative bacteria during a severe gram-negative infection in the natural host, we induced *Haemophilus pleuropneumoniae* infections in weanling pigs immunized with a vaccine of an Rc mutant of *Escherichia coli* (strain J5). To help define the mechanism involved in J5-mediated protection, we compared the clinical, hematologic, bacteriologic, and serologic responses following an *H. pleuropneumoniae* infection in J5-immunized pigs with those following an *H. pleuropneumoniae* infection in nonimmunized control animals. As a result of an intranasal inoculation, all of the control animals and the J5-immunized animals were infected with *H. pleuropneumoniae*. However, while 80% (4 of 5) of the nonimmunized pigs died within 24 h as a result of the infection, no deaths occurred in the J5-immunized animals. In the immunized group, J5 titers dropped during the acute stages of the infection and rebounded to well above the prechallenge levels during convalescence. The J5 titer also increased in the single surviving control animal. These findings suggest that antibodies against common subsurface components of gram-negative bacterial cell walls correlate with protection from an otherwise lethal challenge of *H. pleuropneumoniae* but do not prevent infection. Important growth-phase-dependent antigenic changes have been recognized to occur during the growth of *H. pleuropneumoniae* in cultures (R. Nielson, Nord. Veterinaermed. 28:337–348, 1976). In a study of these changes and during an inquiry into the mechanism of J5 antibody-mediated protection, measured quantities of *H. pleuropneumoniae* were removed from a broth culture at hourly intervals and used to adsorb hyperimmune equine J5 antiserum. Significantly greater amounts of J5-specific antibodies were adsorbed during the log phase of bacterial growth than during the early or late phase. The availability of epitopes recognized by J5 antibodies appears to be closely related to the rate of bacterial multiplication. The results of these experiments suggest a mechanism of protection provided by increased immunity to *E. coli* J5 during gram-negative infections.**

*Haemophilus pleuropneumoniae* is the cause of a highly contagious primary pneumonia in pigs. The disease is a major problem in the swine industry throughout the world and is increasingly diagnosed as a cause of mortality and lowered production. Presently used procedures of controlling the disease have failed to slow its spread or significantly reduce the losses associated with infections (30, 31). Little is known about the pathogenesis of the disease, but the clinical signs, pathology, and immunopathology suggests that bacterial toxins, possibly endotoxin, play a role in the pathogenesis (1, 29).

Previous studies involving a number of gram-negative infections suggested that antibodies to antigenically similar cell wall components of gram-negative bacteria may provide nonspecific resistance to infection (4, 18). More recently it was shown that antibodies produced against "core" glycolipids of *Enterobacteriaceae* strains can provide protection from experimental gram-negative infections in laboratory animals (17, 34, 35) and naturally occurring gram-negative septicemias in humans (36). In addition, many of the biological effects of purified endotoxin can be blocked (2, 3, 6). These studies and others demonstrated that immunity to lipopolysaccharide (LPS) core antigens confers a degree of protection from a wide variety of gram-negative bacterial diseases.

The purpose of this investigation was to determine the involvement and potential protective effect of immunity against shared LPS core antigens during a localized gram-negative infection in the natural host and to define the mechanisms involved. Severe pneumonias were therefore induced with *H. pleuropneumoniae* in weanling pigs which had been previously immunized with a vaccine of an Rc mutant of *Escherichia coli* O111:B4. This strain, J5, is rough, owing to a lack of UDP-galactose epimerase (10), and is genetically stable, owing to an as-yet-uncharacterized inability to incorporate exogenous galactose into the LPS (36). This results in the inability of J5 to complete the production of the outer polysaccharide portion of its cell wall and thus leaves the LPS core region exposed. At peak J5 antibody titers the pigs were intranasally challenged with virulent *H. pleuropneumoniae*.

The mechanism by which antibodies to LPS core antigens provide protection from the sequelae of gram-negative infections is unclear. To define the mechanism of protection, we performed adsorption studies with hyperimmune J5 antisera and viable *H. pleuropneumoniae* that was harvested at regular intervals for 20 h from broth cultures.

## MATERIALS AND METHODS

**Bacteria.** J5, a UDP-galactose 4-epimerase-deficient mutant of *E. coli* O111:B4, was supplied by E. J. Ziegler, University of California, San Diego. J5 was grown in Tryp-

* Corresponding author.
† Present address: Department of Comparative and Experimental Pathology, College of Veterinary Medicine, University of Florida, Gainesville, FL 32610.

ticase (BBL Microbiology Systems, Cockeysville, Md.) soy broth at 37°C. *H. pleuropneumoniae* J45 (serotype 5) had been isolated from a naturally occurring case of porcine pleuropneumonia by E. L. Biberstein, University of California, Davis. *H. pleuropneumoniae* was cultured on chocolate agar plates or in PPLO broth without crystal violet (Difco Laboratories, Detroit, Mich.), each supplemented with 7% neutralized equine serum, 1 μg of NAD (Eastman Kodak Co., Rochester, N.Y.) per ml, and 4.0% yeast extract (GIBCO Laboratories, Salt Lake City, Utah). Cultures were incubated in an atmosphere of 5% $CO_2$.

**Antigen.** A J5 vaccine was prepared as described by Ziegler et al. (36). Briefly, cells from an overnight culture of J5 in Trypticase soy broth were removed by centrifugation and washed three times with sterile saline. The bacterial concentration was adjusted with saline to approximately $10^7$ CFU/ml, and the suspension was boiled for 2.5 h. The bacterial concentration was spectrophotometrically readjusted to $5 \times 10^9$ CFU/ml, and phenol was added to a final concentration of 0.05 mg/ml. The vaccine was bottled, checked for sterility, and stored at −4°C.

**Serology.** Serum antibody titers against LPS core antigens were determined by a direct enzyme-linked immunosorbent assay (ELISA) by a modification of the procedures described by Ito et al. (14) and Dunn and Fergusen (8) in which intact *E. coli* J5 was used as the antigen. The same procedure used to produce the J5 vaccine was followed to prepare the antigen for the J5 ELISA, except that the final bacterial concentration was adjusted to $5 \times 10^8$ CFU/ml and no phenol was added. The antigen was stored at −4°C. *H. pleuropneumoniae* serum titers were determined by tube agglutination as described by Mittal et al. (21) and by an *H. pleuropneumoniae* LPS-specific ELISA (B. W. Fenwick, Ph.D. thesis, University of California, Davis, 1985). Peroxidase-conjugated, affinity-purified, heavy-chain-specific, rabbit anti-swine immunoglobulin G (IgG) (Cooper Biomedical, Inc., Malvern, Pa.) and IgM (Pel-Freez, Rogers, Ariz.) were used as conjugates in the ELISAs. The results of the ELISAs were read on a micro-ELISA plate reader (model MR600; Dynatech Laboratories, Inc., Alexandria, Va.), with titers defined as the reciprocal of the serum dilution in which the optical density was at least three times the average optical density of four control wells containing pooled *H. pleuropneumoniae*-negative and *E. coli* J5-adsorbed porcine sera.

**Experimental infection.** Ten-week-old pigs from the University of California, Davis, swine herd were confirmed to be free of *H. pleuropneumoniae* infections by repeated serologic and bacteriologic examinations. Twenty animals were intramuscularly immunized with 3 ml of the J5 vaccine and reimmunized in 2 weeks. Five pigs were used as nonimmunized controls. When J5 titers had apparently reached their maximum, the animals were challenged intranasally with $2 \times 10^7$ saline-washed viable organisms suspended in 5 ml of sterile saline.

Prior to the challenge and at 4-h intervals afterwards, physical examinations were performed, clinical scores were assigned, and rectal temperatures were recorded for all of the animals. Clinical scores were assigned on a scale of 1 to 4, with 1 being normal, 2 being depressed but standing, 3 being depressed and unable to stand, and 4 being comatose.

Complete blood counts, coagulation profiles, and blood cultures were performed for all of the control animals and 10 randomly selected vaccinates prior to and at 8 and 16 h after the *H. pleuropneumoniae* inoculation. Coagulation profiles included activated partial thromboplastin time (27), prothrombin time (28), fibrin degradation products (Thrombo-Wellcotest Kit; Burroughs Wellcome Co., Dartford, England), and circulating soluble fibrin monomer complexes (15). Blood cultures were performed with whole venous citrated blood and 10- and 100-fold dilutions of a 1-ml sample. A 1-ml quantity of blood and the dilutions were mixed with 9 ml of molten PPLO agar as a pour plate. Colony numbers were counted 24 and 48 h later, and the number of viable organisms per milliliter of venous blood was calculated.

J5 and *H. pleuropneumoniae* titers were determined at 4-day intervals by the ELISA and tube agglutination as previously described. Surviving animals were killed at regular intervals, and complete pathologic and microbiological examinations were performed.

**Immunoadsorption.** *H. pleuropneumoniae* was grown in 12 liters of modified PPLO broth in a 14-liter fermentor (Magnaferm bench fermenter model MA114; New Brunswick Scientific Co., Inc., Edison, N.J.) at 37°C. Agitation and aeration with 5% $CO_2$ were maintained at 400 rpm and 10 liters/min, respectively. The culture was started with a 24-h broth culture. At hourly intervals, samples of the culture were checked for contamination, and the number of viable bacteria was determined (20). At the same time, aliquots of the bacteria were removed by centrifugation. The cells were immediately washed with ice-cold sterile saline (three times) and spectrophotometrically adjusted to approximately $5 \times 10^8$ CFU/ml, as determined by a previous experiment.

Bacteria from 10 ml of the suspension were removed by centrifugation, and the bacterial pellet was mixed with 10 ml of hyperimmune equine J5 antiserum for 60 min at approximately 4°C while being gently rocked. A 1-ml sample of the suspension was collected immediately before the bacteria and serum were mixed and at the end of the incubation period to spectrophotometricly estimate bacterial cell numbers and to determine the number of CFU (20). A viability ratio was calculated by comparison of these measurements. The serum was separated from the remainder of the bacteria by centrifugation, followed by filtration through a 0.22-μm filter (Millex-GV; Millipore Corp., Bedford, Mass.). Samples of the adsorbed sera were frozen at −60°C, and antibody titers were simultaneously determined by the ELISA. ELISA titers were normalized for the actual number of CFU present at the start of the adsorbtion procedure. All assays were performed in triplicate.

**Statistical analysis.** The protective potential of the J5 vaccine was evaluated on the basis of differences in the clinical, bacteriologic, and serologic changes between vaccinated and control animals by analysis of variance with repeated measures (33). The significance of differences in antibody titers were determined by the Tukey *t* test (33). Protamine sulfate precipitation and clinical scores were examined by chi-square and two-tailed *t* tests (33). Mortality rates as related to treatment were evaluated by challenge experiment analysis techniques (16). Significance was determined at a probable error of <5%.

## RESULTS

**Titers.** Low J5 titers were present in most pigs prior to immunization. Immunization with the J5 vaccine produced high specific IgG antibody titers, which increased after the booster injection (Fig. 1). The specific IgM antibody titers did not appear to increase significantly as a result of vaccination with J5 (data not shown). However, the sensitivity of the ELISA was reduced by nonspecific binding of IgM to J5.

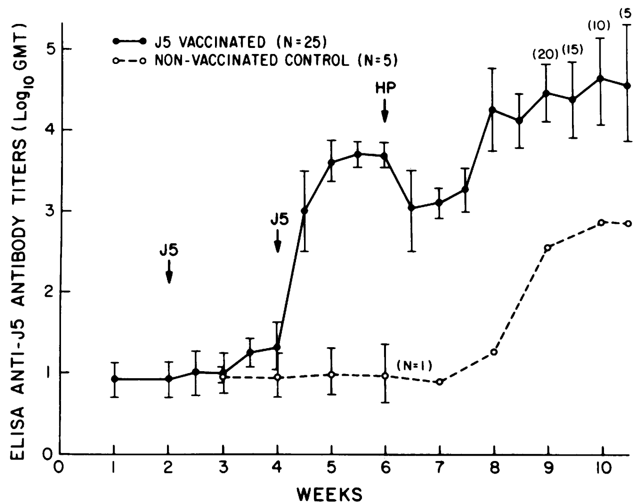


FIG. 1. Mean *E. coli* J5 IgG ELISA titers ± 1 standard deviation by treatment group during the experimental infection. Arrows indicate the time of immunization or challenge (J5, immunization with J5 vaccine; HP, bacterial challenge). Only one control animal survived the infection. The increasing titers suggest shared antigens between *H. pleuropneumoniae* and *E. coli* J5. The immunized animals were sequentially killed for pathologic examination; numbers in parentheses indicate the numbers of animals whose titers were measured at each time. GMT, Geometric mean titer.

There were no adverse reactions observed as a result of the immunization.

**Experimental infection.** Clinical, bacteriologic, serologic, and pathologic findings indicated that all pigs were infected with *H. pleuropneumoniae* as a result of the intranasal inoculation. No deaths occurred in J5-immunized pigs, while 4 of the 5 control pigs died within the first 24 h after the bacterial challenge ($P < 0.01$). In the J5-immunized group, J5 IgG titers dropped during the acute stages of the infection ($P < 0.05$) and rebounded to above the prechallenge levels (Fig. 1) during convalescence ($P < 0.01$ at 3 weeks after infection). The J5 IgG titer also increased in the single surviving control animal ($P < 0.01$). Prior to the experiment, serologic examination revealed no detectable antibodies to *H. pleuropneumoniae* in any animal. The *H. pleuropneumoniae* titers increased rapidly after the infection (Fig. 2).

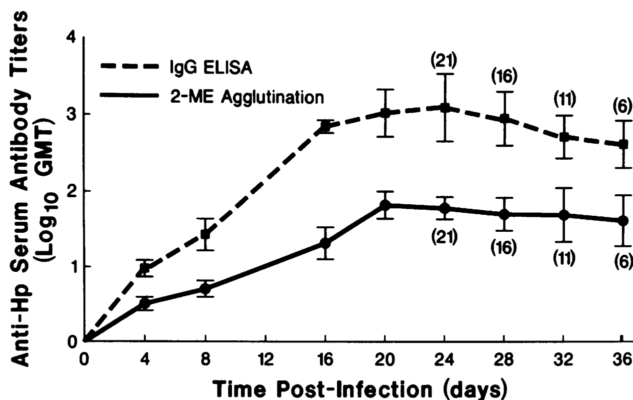


FIG. 2. *H. pleuropneumoniae* (HP) tube agglutination and ELISA titers ± 1 standard deviation by time as a result of experimental infection in 24 pigs. Animals were sequentially killed for pathologic examination. The numbers of remaining animals whose titers were measured at each time are shown in parentheses. GMT, Geometric mean titer. 2-ME, 2-Mercaptoethanol.

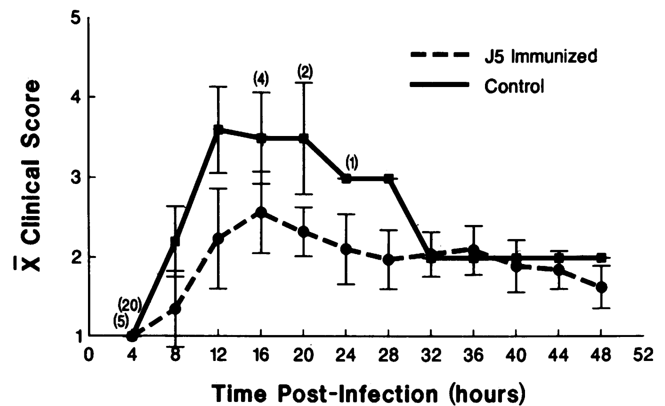


FIG. 3. Mean (X̄) clinical scores ± 1 standard deviation after infection with *H. pleuropneumoniae* by treatment group ($n = 20$ for the J5-immunized group and $n = 5$ for the control group). All but one of the control pigs died by 24 h postinfection. Numbers in parentheses indicate the remaining numbers of animals examined.

The clinical scores, temperatures, hemograms, and coagulation profiles were markedly different between the treatment groups (Fig. 3 and 4). The control animals rapidly developed severe clinical signs. There was a prompt and marked rise in temperature followed by a precipitous drop, a marked leukopenia without a left shift, thrombocytopenia, prolonged clotting times, hyperfibrinogenemia, elevated fibrin split products, and increased circulating soluble fibrin monomer complexes (Table 1). A statistical difference ($P < 0.05$) between the control and vaccinated pigs was found in the prothrombin time, partial thromboplastic time, and platelet number at 8 and 16 h after infection. The single surviving control pig had milder changes in most of the measured parameters than the control pigs that died as a result of infection with *H. pleuropneumoniae*.

The frequencies with which *H. pleuropneumoniae* was isolated from venous blood cultures of the J5-immunized and control animals were 80% (8 of 10) and 70% (14 of 20), respectively. The severity of the bacteremia was not significantly different between treatment groups at 8 h postinfection. At 16 h postinfection, the number of bacteria per milliliter of blood had increased dramatically in all control

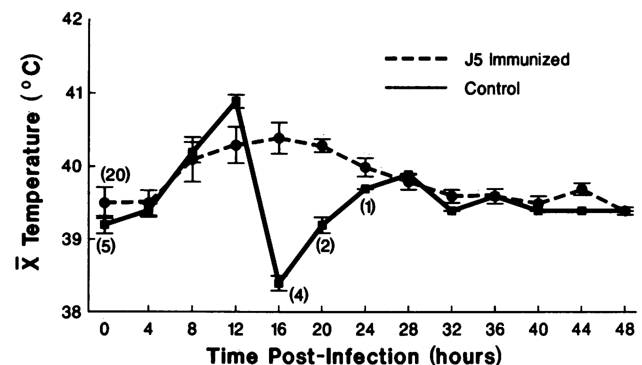


FIG. 4. Mean (X̄) body temperatures ± 1 standard deviation after infection with *H. pleuropneumoniae* by treatment group ($n = 20$ for the J5-immunized group and $n = 5$ for the control group). All but one of the control pigs died by 24 h postinfection. Numbers in parentheses indicate the remaining numbers of animals examined.

TABLE 1. Coagulation profiles of J5-immunized and control pigs by time after *H. pleuropneumoniae* infection

| Assay[a] | J5-immunized pigs ($n$ = 10) | | | Control pigs ($n$ = 5) | | |
|---|---|---|---|---|---|---|
| | Mean ± SD at 0 h | % change by: | | Mean ± SD at 0 h | % change by: | |
| | | 8 h | 16 h | | 8 h | 16 h |
| PT | 12.8 ± 1.1 | +5.2 | +7.2 | 13.0 ± 1.8 | +28.5 | +55.3 |
| PTT | 19.6 ± 2.4 | +7.5 | +7.8 | 20.2 ± 3.3 | +37.5 | +64.5 |
| Platelet numbers ($10^2$) | 828 ± 115 | −4.2 | −5.2 | 785 ± 145 | −45.3 | −50.5 |
| PP | 5.8 ± 0.3 | +2.4 | +12.6 | 6.1 ± 1.2 | +14.9 | +23.9 |
| Fib | 280 ± 78 | +30.3 | +40.2 | 300 ± 70 | +50.3 | +60.4 |
| $PSO_4$ | 0[b] | 1.2 | 2.1 | 0[b] | 1.6 | 2.4 |
| FDP | 0[b] | 0.6 | 1.5 | 0[b] | 1.4 | 2.1 |

[a] Abbreviations (units): PT, prothrombin time (min); PTT, partial thromboplastin time (min); PP, plasma protein; Fib, plasma fibrinogen; $PSO_4$, protamine sulfate precipitation of fibrin monomer complexes; FDP, fibrin degradation products.
[b] Standard deviations were not calculated.

animals except the one which survived. The severity of the bacteremia in the J5-immunized animals remained the same or had slightly decreased (Fig. 5). *H. pleuropneumoniae* was isolated from the lungs of all of the control pigs and many (12 of 20) of the J5-immunized pigs. Extrapulmonary isolations at the time of necropsy were taken only from the control pigs (Table 2).

Pathologic examination of the control animals that died revealed lesions typical of those previously described in acute outbreaks of *H. pleuropneumoniae* (27). In the surviving animals, lung lesions varied from an area of focal pulmonary thrombosis in the surviving control pig to patchy resolving interstitial pneumonia in the vaccinates.

**Immunoadsorption.** The results of the immunoadsorption experiment showed that antibody adsorption was growth phase dependent (Fig. 6). Significantly greater amounts of J5 antibodies (IgG) were removed from the equine J5 antisera during the period when bacterial numbers were increasing at the fastest rate. The percentage of dead bacteria increased with the age of the culture; however, the rate of J5 IgG adsorbed did not reflect this shift in viable cell proportions. Poor adsorption also occurred early in the growth phase, when the proportion of dead to viable bacteria was low. Agglutination of *H. pleuropneumoniae* by equine J5 antisera did not occur at any growth stage, and *H. pleuropneumoniae* serotype-specific antibodies were not identified by a complement fixation assay with *H. pleuropneumoniae* type strains. Exposure of *H. pleuropneumoniae* to hyperimmune equine J5 antisera did not significantly reduce the number of viable bacteria or their multiplication rate.

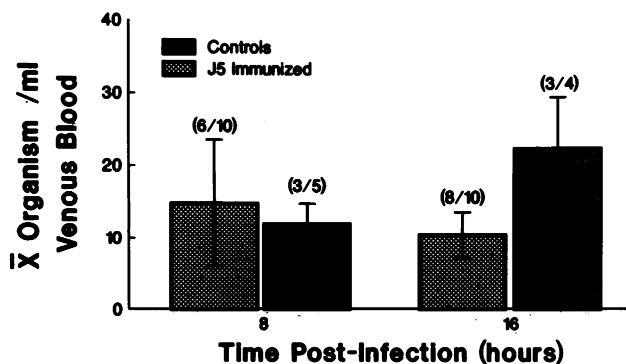


FIG. 5. Blood culture results (mean numbers [X̄] of organisms cultured per milliliter of blood) by treatment group and time. Numbers in parentheses indicate the numbers of animals examined.

## DISCUSSION

Immunization with *E. coli* J5 induces high specific IgG antibody levels in pigs and provides protection against an otherwise lethal infection with *H. pleuropneumoniae*. These findings extend previous studies which demonstrated that antibodies to J5 confer protection against gram-negative bacteremias (17, 34–36) as well as the effects of purified endotoxin (2, 3, 6). This is, however, the first time that protection from an infectious gram-negative disease in the natural host has been demonstrated. Previous studies of J5-mediated protection have involved experimental models of gram-negative sepsis in laboratory animals in which massive numbers of viable organisms were given or the animals were manipulated (drug-induced neutropenias) so as to be susceptible to the infection (17, 34, 35). If antibody levels were the protective principle, the results suggest that immunoglobulins of the IgG class play an important role, as was previously suggested (36). The potential involvement of IgM was not determined because of difficulties with the assay. The role of direct cell-mediated protection induced by J5 immunization during sepsis has not been investigated.

Although J5 immunization did not prevent *H. pleuropneumoniae* infection during a severe experimental challenge, there was a significant protection from mortality. There was also a notable reduction in the severity of clinical signs, hematologic alterations, and pulmonary lesions. The drop in the J5 titer during the acute phase of the infection and the increase in the J5 titer during convalescence support but do not prove the involvement of core antigen-specific antibodies in providing protection from lethal *H. pleuropneumoniae* infections. The increased J5 titers in pigs following *H. pleuropneumoniae* infection suggest the exposure of cross-reacting immunodeterminants. Additional evidence for the presence of common antigens is the increase in the J5 titer in the single surviving nonimmunized control pig.

TABLE 2. Positive *H. pleuropneumoniae* isolations at necropsy by location

| Treatment group | No. of animals with *H. pleuropneumoniae* isolated from: | | | | |
|---|---|---|---|---|---|
| | Lung | Spleen | Joints | Bronchial lymph node | Bone marrow |
| J5 ($n$ = 20) | 12 | 0 | 0 | 0 | 0 |
| Controls | | | | | |
| Died ($n$ = 4) | 4 | 4 | 2 | 2 | 4 |
| Surviving ($n$ = 1) | 1 | 0 | 0 | 0 | 0 |

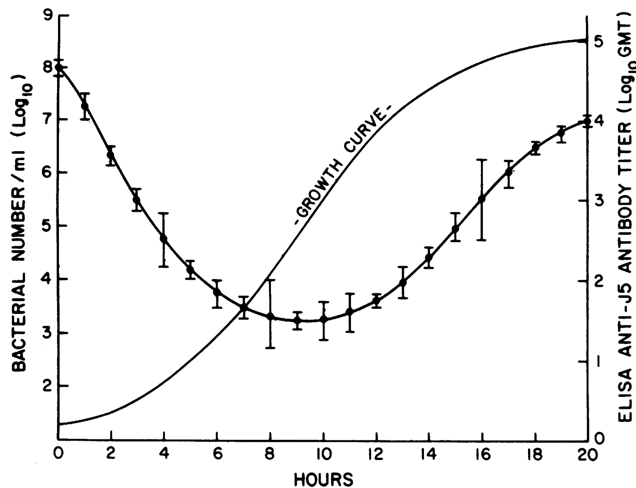


FIG. 6. Sequential adsorption of hyperimmune equine J5 antiserum with *H. pleuropneumoniae* during stages of growth in cultures. Bars represent 1 standard deviation. GMT, Geometric mean titer.

The protective antigens provided by the J5 vaccine certainly involve cross-reactive (possibly identical) epitopes present in the complex cell walls of gram-negative bacteria. Through the use of standard serologic techniques and monoclonal antibodies specific for the LPS of J5, extensive cross-reacting antigens have been identified for a wide variety of gram-negative bacteria (24, 25). Similar protective antibodies have also been found in antisera to other rough mutants of gram-negative bacteria (18). The use of whole (boiled) J5 bacteria likely induced immunity to immunodeterminants other than just the LPS core. It is also likely that IgG directed at these non-LPS core determinants was reflected in the whole-bacterial-cell ELISA titers. However, reexamination of the J5 titers with purified J5 LPS (List Biological Laboratories, Campbell, Calif.), revealed a direct correlation ($r = 0.95$) with the whole-bacterial-cell ELISA titers.

The mechanism of the protection provided by nonspecific immunity to gram-negative bacteria is likely multifactorial (19). However, there is evidence that J5 antiserum is not a strong nonspecific opsonin and does not increase complement-mediated serum bacteriolysis (34). The finding that *H. pleuropneumoniae* numbers were not significantly reduced after incubation with hyperimmune equine J5 antisera supports this notion. There is evidence that J5 antiserum can increase bacterial clearance by a mechanism yet to be defined (8).

The heterogeneity of LPS side chain length in gram-negative bacteria (12, 23) suggested to Ziegler et al. (36) that J5 antibodies act to block exposure of biologically active sites within the LPS. It was theorized that these components are exposed to a relatively greater degree during rapid bacterial growth when LPS side chain production is somewhat less complete. The growth-phase-dependent adsorption of J5-specific antibodies by *H. pleuropneumoniae* supports this concept.

The mechanism by which J5-specific immunity provides protection during *H. pleuropneumoniae* infections is unclear, largely because the pathogenesis of *H. pleuropneumoniae* is unknown. There is, however, good evidence that bacterium-mediated pulmonary venous thrombosis plays a major role in the pathogenesis. The lesions in the pigs that died during the acute stages of the infection were similar to endotoxin-induced disseminated intravascular coagulation and resulted in severe pulmonary edema. Morphologically, the lung lesions resembled those of adult acute respiratory distress syndrome in humans (13). It appears that some structural component or product of *H. pleuropneumoniae* is highly thrombogenic and is involved in the pathogenesis of the disease.

Swine whose plasma has been rendered hypocoagulable by treatment with Warfarin [3-(α-acetonylbenzyl)-4-hydroxycoumarin; Sigma Chemical Co., St. Louis, Mo.] prior to challenge with *H. pleuropneumoniae* are protected from acute lethal infections despite severe bacteremia and do not develop severe pneumonia or localized pulmonary infarction (B. W. Fenwick, unpublished data). Similarly, pigs immunized with J5 did not develop severe coagulopathy, had less severe pulmonary lesions, had milder clinical signs, and survived the infection despite being bacteremic. It appears that either an infection with *H. pleuropneumoniae* must be restricted to the lungs, possibly by pulmonary thrombosis and infarction, or, failing this, bacteremia occurs, causing disseminated intravascular coagulation and pulmonary edema, which frequently results in death. In other words, nonimmune animals who survive *H. pleuropneumoniae* infections rarely are bacteremic (7). The J5-immunized pigs, as well as those given Warfarin, became bacteremic but survived the infection. There may also be a relationship between the infecting dose of the bacteria and the severity of the resulting disease and thus of the potential of becoming bacteremic (32). This may explain why one control animal did not become bacteremic and survived the infection.

The similarities in the *H. pleuropneumoniae* infections between pigs with plasma rendered hypocoagulable by Warfarin and those immunized with J5 suggest that immunity to LPS core antigens reduces the thrombogenic potential of the bacteria. It is well established that portions of the cell walls of gram-negative bacteria as well as purified endotoxin is highly thrombogenic (22). Protection provided by immunity to J5 does not appear to be due to the prevention of bacteremia or to increases in bacterial clearance, as the frequency and number of *H. pleuropneumoniae* isolated from the blood of vaccinates and controls were approximately the same.

The therapeutic use of anticoagulants in experimental or naturally occurring septic shock improves coagulation abnormalities but usually fails to significantly improve long-term survival (5). Dunn et al. (9), using a guinea pig septicemia model in which viable *E. coli* was infused intravenously, found that pretreatment with J5 antisera in combination with heparin was necessary to enhance survival. However, the J5 antisera greatly increased bacterial clearance. In swine challenged with *H. pleuropneumoniae*, J5 immunization alone was sufficient to provide protection. Similar findings have been reported in connection with other models of gram-negative infections (17, 34, 35).

The pronounced growth-phase-dependent nature of J5 antibody adsorption to *H. pleuropneumoniae* suggests that antibodies to core antigens may be most effective when bacterial growth is unrestricted, as during the early stage of an infection or in a compromised host. Under these conditions, highly thrombogenic subsurface cell wall components may be exposed to a relatively greater extent. The immunologic masking of these components would effectively reduce the pathogenic potential of the bacteria. Additionally, there may be nonspecific opsonization and increased clearance. Such protection, as demonstrated by *H. pleuropneumoniae*

infections in pigs, would not be expected to prevent infection but rather to mediate the severity of the disease, especially in the acute stages.

Whether actively or passively acquired, immunity to LPS core antigens may provide the necessary time for activation of more specific host defenses by preventing acute mortality. Such a scheme may constitute an important part of the nonspecific early defense against gram-negative bacteria (4). The finding that most animals (B. W. Fenwick and J. S. Cullor, unpublished data) and humans (36) have naturally occurring titers to LPS core antigens and that the titers increase after gram-negative infections adds support to this concept.

Immunity to LPS core structures may also be beneficial during exposure of biologically active cell wall components occurring as a result of host- or antimicrobial agent-mediated bacteriolysis, as with Jarisch-Herxheimer-like reactions (11). Antisera to J5 appear to be able to reduce mortality during gram-negative infections and shock even with appropriate antibiotic and shock therapy (36).

It is apparent that immunity to *E. coli* J5 provides protection from mortality during the acute stages of *H. pleuropneumoniae* infections in swine. The mechanism involved in the protection from disease provided by immunization with *E. coli* J5 as well as immunization with purified LPS cores awaits complete definition. The data presented in this paper suggest that a reduction in the inherent thrombogenic potential of rapidly multiplying bacteria may be an important factor. Further studies with pigs passively immunized with porcine anti-J5, anti-lipid A, and anti-*H. pleuropneumoniae* sera will be helpful in determining the mechanism of protective immunity.

## ACKNOWLEDGMENTS

We gratefully acknowledge E. L. Biberstein for his aid in the preparation of this manuscript. We thank E. J. Ziegler and E. L. Biberstein for the contribution of bacterial strains. We thank D. Kelly and K. Parker for helping to perform certain parts of this project.

This study was supported in part by U.S. Department of Agriculture special grant 83-CRSR-2-2175.

## LITERATURE CITED

1. **Bendixen, P. H., P. E. Shewen, S. Rosendal, and B. W. Wilkie.** 1980. Toxicity of *Haemophilus pleuropnuemoniae* for porcine lung macrophages, peripheral blood monocytes, and testicular cells. Infect. Immun. **33**:673–676.
2. **Braude, A. I., and H. Douglas.** 1972. Passive immunization against the local Shwartzman reaction. J. Immunol. **108**: 505–512.
3. **Braude, A. I., H. Douglas, and C. E. Davis.** 1973. Treatment and prevention of intravascular coagulation with antiserum to endotoxin. J. Infect. Dis. **128**(Suppl.):157–164.
4. **Chedid, L., M. Parant, F. Parant, and F. Boyer.** 1968. A proposed mechanism for natural immunity to enterobacterial pathogens. J. Immunol. **100**:292–301.
5. **Corrigan, J. J., and C. M. Jorden.** 1970. Heparin therapy in septicemia with disseminated intravascular coagulation. N. Engl. J. Med. **282**:778–786.
6. **Davis, C. E., E. J. Ziegler, and K. Arnold.** 1978. Neutralization of meningococcal endotoxin by antibody to core glycolipid. J. Exp. Med. **147**:1007–1017.
7. **Didier, P. J., L. Perino, and J. Urbance.** 1984. Porcine *Haemophilus pleuropneumoniae*: microbiologic and pathologic findings. J. Am. Vet. Med. Assoc. **184**:716–719.
8. **Dunn, D. L., and R. M. Fergusen.** 1982. Immunotherapy of gram-negative bacterial sepsis: enhanced survival in a guinea pig model by use of rabbit antiserum to *Escherichia coli* J5. Surgery (Saint Louis) **99**:212–219.
9. **Dunn, D. L., P. A. Mach, F. B. Cerr, and R. M. Ferguson.** 1983. The role of heparin in guinea pig gram negative bacterial sepsis. J. Surg. Res. **34**:477–485.
10. **Elbein, A. D., and E. C. Heath.** 1965. The biosynthesis of cell wall lipopolysaccharide in *Escherichia coli*. I. The biochemical properties of a uridine diphosphate galactose 4-epimeraseless mutant. J. Biol. Chem. **240**:1919–1925.
11. **Fulford, K. W. M., N. Johnson, C. Loveday, J. Storey, and R. S. Tedder.** 1976. Changes in intra-vascular complement and anti-treponemal antibody titers preceding the Jarisch-Herxheimer reaction in secondary syphilis. Clin. Exp. Immunol. **24**:483–491.
12. **Goldman, R. C., and L. Leive.** 1980. Heterogeneity of antigenic-side-chain length in lipopolysaccharide from *Escherichia coli* O111 and *Salmonella typhimurium* LT2. Eur. J. Biochem. **107**:145–153.
13. **Hani, H., H. Konig, J. Nicolet, and E. Scholl.** 1973. *Haemophilus pleuropneumoniae* in swine. V. Pathomorphology. Schweiz. Arch. Tierheilkd. **115**:191–203.
14. **Ito, J. I., A. C. Wunderlich, and J. L. Lyons.** 1980. The role of magnesium in the enzyme-linked immunosorbent assay for lipopolysaccharide of rough *Escherichia coli* strain J5 and *Neisseria gonorrhoeae*. J. Infect. Dis. **142**:532–537.
15. **Kidder, W. R., J. L. Laurence, S. I. Rapaport, and M. J. Patch.** 1972. The plasma protamine paracoagulation test: clinical and laboratory evaluation. Am. J. Clin. Pathol. **58**:675–686.
16. **Liddle, F. D.** 1978. Evaluation of survival in challenge experiments. Microbiol. Rev. **42**:237–249.
17. **Marks, M. I., E. J. Ziegler, H. Douglas, L. B. Corbeil, and A. I. Braude.** 1982. Induction of immunity against *Hemophilus influenzae* type b infection by *Escherichia coli* core lipopolysaccharide. J. Clin. Invest. **69**:742–749.
18. **McCabe, W. R.** 1972. Immunization with R mutants of *S. minnesota*. I. Protection against challenge with heterologous gram-negative bacilli. J. Immunol. **108**:601–610.
19. **McCabe, W. R., S. C. Bruins, D. E. Craven, and M. Johns.** 1977. Cross-reactive antigens: their potential for immunization-induced immunity to gram-negative bacteria. J. Infect. Dis. **136**(Suppl.):161–168.
20. **Miles, A. A., and S. S. Misra.** 1938. The estimation of the bactericidal power of blood. J. Hyg. **38**:732–748.
21. **Mittal, K. R., R. Higgins, S. Lariviere, and D. Lablanc.** 1984. A 2-mercaptoethanol tube agglutination test for diagnosis of *Haemophilus pleuropneumoniae* infection in pigs. Am. J. Vet. Res. **45**:715–719.
22. **Morrison, D. C., and C. G. Cochran.** 1973. Direct evidence for Hageman factor (factor XII) activation by bacterial lipopolysaccharide. J. Exp. Med. **140**:787–811.
23. **Munford, R. S., C. L. Hall, and P. D. Rick.** 1980. Size heterogeneity of *Salmonella typhimurium* lipopolysaccharides in outer membranes and culture supernatant membrane fragments. J. Bacteriol. **144**:630–640.
24. **Mutharia, L. M., G. Crockford, W. C. Bogard, Jr., and R. E. W. Hancock.** 1984. Monoclonal antibodies specific for *Escherichia coli* J5 lipopolysaccharide: cross-reaction with other gram-negative bacterial species. Infect. Immun. **45**:631–636.
25. **Nells, M. J., and C. A. Niswander.** 1984. Mouse monoclonal antibodies reactive with J5 lipopolysaccharide exhibit extensive serological cross-reactivity with a variety of gram-negative bacteria. Infect. Immun. **46**:677–681.
26. **Nielson, R.** 1976. Pleuropneumonia in swine caused by *Haemophilus parahaemolyticus*. Studies on the protection obtained by vaccination. Nord. Veterinaermed. **28**:337–348.
27. **Proctor, R. B., and S. I. Rapaport.** 1961. The partial thromboplastic time with kaolin: a simple screening test for first stage plasma clotting factor deficiencies. Am. J. Clin. Pathol. **36**:213–219.
28. **Quick, A. J., M. S. Brown, and F. W. Bancroft.** 1935. A study of the coagulation defect in hemophilia and in jaundice. Am. J. Med. Sci. **190**:501–511.

29. **Rosendal, S., W. R. Mitchell, and M. Weber.** 1981. Haemophilus pleuropneumoniae lung lesions induced by sonicated bacteria and sterile culture supernatant, p. 221. Proceedings of the International Pig Veterinary Society Congress.
30. **Schultz, R. A., T. F. Young, R. F. Riss, and D. R. Jeske.** 1982. Prevalence of antibody to *Haemophilus pleuropneumoniae* in Iowa swine. Am. J. Vet. Res. **43:**1848–1851.
31. **Sebunya, T. N. K., and J. R. Saunders.** 1983. *Haemophilus pleuropneumoniae* infection in swine: a review. J. Am. Vet. Med. Assoc. **182:**1331–1337.
32. **Sebunya, T. N. K., J. R. Saunders, and A. D. Osburn.** 1983. Dose response relationship of *Haemophilus pleuropneumoniae* aerosols in pigs. Can. J. Comp. Med. **47:**54–56.
33. **Snedecor, G. W., and W. G. Cochran.** 1976. Statistical methods, p. 66–88, 260–275, and 329–334. Iowa State University Press, Ames.
34. **Ziegler, E. J., H. Douglas, J. E. Sherman, C. E. Davis, and A. I. Braude.** 1973. Treatment of *E. coli* and *Klebsiella* bacteremia in agranulocytic animals with antiserum to a UPD-Gal epimerase-deficient mutant. J. Immunol. **111:**433–438.
35. **Ziegler, E. J., J. A. McCutchan, H. Douglas, and A. I. Braude.** 1975. Prevention of lethal pseudomonas bacteremia with epimerase-deficient *E. coli* antiserum. Trans. Assoc. Am. Physicians **88:**101–108.
36. **Ziegler, E. J., J. A. McCutchan, J. Fierer, M. P. Glauser, J. C. Sadoff, H. Douglas, and A. I. Braude.** 1982. Treatment of gram-negative bacteremia and shock with human antiserum to a mutant *Escherichia coli*. N. Engl. J. Med. **307:**1225–1230.