Disease-reducing potential of increased immunity to shared lipopolysaccharide core antigens of Gram-negative bacteria by immunizing swine with *Escherichia coli* J5

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Summary: *This paper introduces the concept of reducing the biologic, and thus economic effects of clinical and subclinical infections with Gram-negative bacteria in swine herds by increased immunity to shared lipopolysaccharide (LPS) core antigens. While the outermost elements of the LPS of various Gram-negative bacteria are structurally and antigenically unique, their substructures (core region and Lipid-A) are structurally and antigenically closely related. Studies in humans and various animal species furnish evidence that increased immunity to these common antigens provides protection from the consequences of infections with a wide variety of Gram-negative bacteria. The most popular means of providing this immunity is by immunization with a cell wall-deficient mutant of* Escherichia coli *(termed J5). The practice of immunizing dairy cattle with J5 has increased considerably during the past year. It is important that veterinarians and producers understand the scientific basis for this protection in order to critically evaluate the likelihood that immunization with* E. coli *J5 will be justified in the profitable production of pork.*

The endotoxins of Gram-negative bacteria are powerful initiators of a multitude of biological responses, the most important of which include the production of interleukins, cachetins (e.g., tumor necrosis factor), and prostaglandins.[1] The clinical consequences associated with infections with Gram-negative bacteria, including fever, shock, hypoxia, and hypotension, are in large part due to release of endotoxins. Antibiotics often can control or eliminate the infection, but do not prevent the release of endotoxins.[2] In fact, antibiotic-mediated killing of bacteria can increase endotoxin release. Interactions between endotoxin and host cells initiate reactions that can cause irreversible shock and death. The release of even small amounts of endotoxin, associated with mild or even subclinical infections, can have significant biologic effects, including the production of acute-phase proteins, alterations in energy metabolism, and decreased appetite.[1,3]

Infections with Gram-negative bacteria are common in veterinary medicine. The economic cost in terms of death losses is considerable. Yet, death losses are only a fraction of the overall cost of Gram-negative bacteria to the producers. The greatest loss occurs because of reduced growth, decreased feed utilization, and medications. In addition, even subclinical infections have a dramatic metabolic effect, causing a marked decrease in lean muscle growth with a proportional increase in fat.

As consumer demand for residue-free pork intensifies, the swine industry is seriously exploring options to the use of antibiotics. The growth-promoting effect of antibiotics at subtherapeutic concentrations in the feed of food-producing animals is due to preventing or reducing the severity of subclinical infections. Because of increased bacterial resistance to antibiotics and the demand for antibiotic-free meat, use of antibiotics as growth promotants will almost certainly come under increased scrutiny and could one day be prohibited entirely. The dramatic increases in growth rate, feed efficiency, and carcass quality associated with early medicated weaning programs underscores the hidden costs associated with subclinical infections.

Common structure and biologic activity of endotoxin

The cell walls of Gram-negative bacteria all have the same fundamental architecture. The outermost membrane of Gram-negative bacteria is composed of a lipopolysaccharide (LPS) complex

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Figure 1

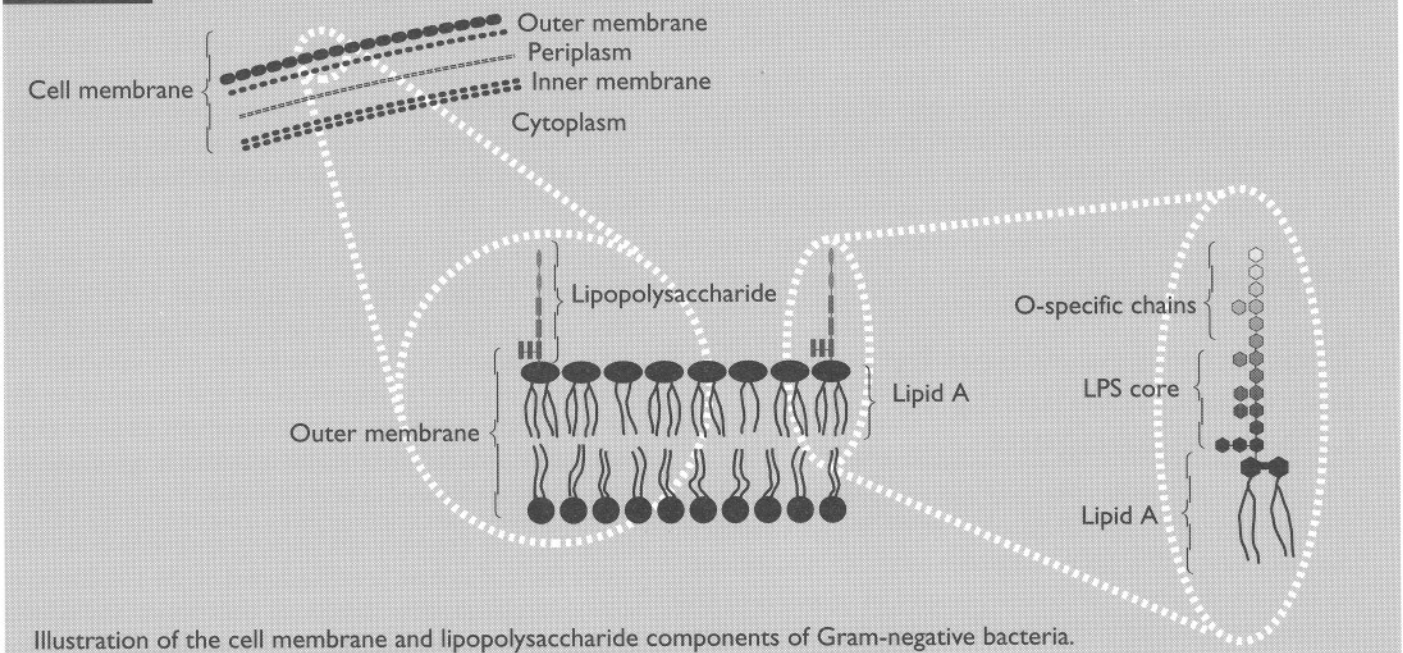

Illustration of the cell membrane and lipopolysaccharide components of Gram-negative bacteria.

which includes an inner lipid portion (termed lipid-A) and an outer polysaccharide component (Figure 1). The polysaccharide portion is further divided into O-specific chains of repeating oligosaccharide units which are on the surface and a "core" region which connects to lipid-A (Figure 1). The large number of different oligosaccharides as well as the many potential linkage combinations provides the basis for the vast array of different O-antigens among Gram-negative bacteria. In contrast, the structure and thus the antigenic characteristics of the LPS core region is much less variable. The implication of this is that antibodies directed at the LPS core region of one bacteria (e.g., *Escherichia coli* J5) will react with a large variety of different Gram-negative bacteria. The term 'endotoxin' is used to reflect the overall ability of the LPS complex without reference to specific subcomponents. While lipid-A accounts for most of this activity, the polysaccharide portion of the complex also contributes to the overall toxicity of the molecule.

When LPS is released from Gram-negative bacteria it binds to plasma proteins.[3] The complex then interacts with host cells via specific receptors that subsequently induce the production and release of inflammatory mediators.[1] Principle among these are interleukin-1 and tumor necrosis factor, which secondarily induce the systemic production of other inflammatory mediators (prostaglandins, leukotrienes, platelet-activating factor, etc.). The numerous metabolic, cardiovascular, and hematologic changes that follow are clinically recognized as septicemia or, in severe cases, as septic shock. In effect, the term 'endotoxin' is an obsolete vestige of our superficial understanding of the inflammatory process. In fact, endotoxin is not a toxin in the classic sense. Rather, it is an exceptionally potent initiator of the inflammatory process. It is the pathophysiologic potential of the mediators induced by endotoxin that are ultimately responsible for the 'toxic' consequences of many Gram-negative infections.

The wide variety of Gram-negative bacterial species that can cause disease and the vast differences in type-specific oligosaccharide chains (O antigens) between bacteria of the same species have caused researchers to focus their efforts toward inducing immunity against that portion of the LPS that is structurally and antigenically similar among a wide variety of Gram-negative bacteria species (core region and lipid A). The hope is that by inducing immunity to portions of the LPS complex that all Gram-negative bacteria have in common, it could provide a degree of resistance to the biologic effects of Gram-negative infections, regardless of the specific organism involved. To this end, cell wall-deficient bacterial mutants have been identified that lack various components of the outermost portions of the LPS complex.

While a number of cell wall-deficient mutants have been developed and tested, the best studied is a strain of *E. coli* 0111:B4 that lacks uridine 5'-diphosphogalactose 4-epimerase. This strain of *E. coli* is termed J5 and classified as an Rc-LPS chemotype. *Escherichia coli* J5 fails to completely produce the outer portion of the LPS and associated O-polysaccharides, thus leaving the LPS core region fully exposed. The LPS core regions of Gram-negative bacteria are highly conserved.[3]

Immunologic similarities of core antigens between various Gram-negative bacteria have been identified using both *E. coli* J5 antisera and monoclonal antibodies.[5-9] Cross reactivity using isolated LPS was not initially identified; however, it has been recognized that antibodies to O-antigens may obscure the detection of cross-reactions between *E. coli* J5 antisera and purified Gram-negative

Antibodies induced by immunization with *E. coli* J5 recognize with the LPS core region of various other Gram-negative bacteria.

Escherichia coli J5 will not prevent bacterial infections but can reduce the severity of the associated disease.

bacterial LPS.[10–11] In addition, the physical state of the bacteria, growth phase, or presence of capsule can influence the immunologically measured cross-reactivity.[12–15] Finally, it has been suggested that sublethal exposure to antibiotics can increase antibody accessibility to core antigens because of alterations in the O-polysaccharides.[16] Monoclonal antibodies against *E. coli* J5 that cross-react with a broad spectrum of unrelated Gram-negative bacteria block LPS-mediated effects on polymorphonuclear leukocytes, and also block production of tumor necrosis factor by macrophages.[17,18]

Protection provided by increased immunity to LPS core antigens

A comprehensive review of the evidence concerning immunity to LPS core antigens is beyond the scope of this paper. A relatively comprehensive review has recently been published.[19] Initial studies concerned with the protective potential of immunity to LPS core antigens involved laboratory animals that were either immunized with *E. coli* J5 or passively protected by *E. coli* J5 immune serum. Various models of endotoxemia and Gram-negative infections have been used to demonstrate the ability of increased immunity to *E. coli* J5 to provide a degree of protection against *E. coli*, *Pasteurella multocida*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*.[20-27] The degree of protection appeared to be greatest if the animals were immunologically compromised prior to being challenged with bacteria.[23-25] Protection could, however, be overcome if the animals were challenged with high numbers of bacteria.[27] The protective ability of F(ab')2 antibody fragments to *E. coli* J5 provided evidence that immunity is via an antitoxin effect rather than an increase in bacterial phagocytosis and clearance.[22] Increased immunity to *E. coli* J5 also delayed deaths due to hemorrhagic shock in rabbits and the effects of graft-versus-host reactions in mice.[28,29]

Given the success of several laboratory animal experiments, human studies were soon undertaken. Increased immunity to *E. coli* J5 as provided by treatment with hyperimmune sera was found to provide a significant level of protection against deaths due to Gram-negative bacteremia and septic shock.[11,30-32] While the prophylactic administration of *E. coli* J5 immune serum to surgical patients did not decrease the rate of postoperative infections, the medical consequences of these infections were not as serious.[33] As was first demonstrated in mice, protection from graft-versus-host disease was related to anti-*E. coli* J5 titers.[34] In humans, the antibody response induced by immunization by *E. coli* J5 is transient and not significantly enhanced by re-immunization.[35]

Of all domestic animals, dairy cattle have received the most attention in evaluating the potential benefits of being vaccinated with *E. coli* J5. Immunization with *E. coli* J5 significantly reduced the clinical signs associated with experimentally induced coliform mastitis.[36] Immunization was associated with increased serum-mediated bacterial opsonization and phagocytosis.[37] When antibody titers against *E. coli* J5 are half the population mean, the risk of clinical coliform mastitis is five times greater.[38] Immunizing dairy cows reduced the incidence of clinical Gram-negative mastitis but did not appear to reduce the incidence of intramammary infections.[39,40] Immunization with *E. coli* J5 increased profits by $57 per dairy cow per year when more than 1% of the cows developed clinical coliform mastitis per year.[41] J5 vaccines labeled for this purpose are currently available from several sources.

In calves, *E. coli* J5 titers decline at three times the rate that total IgG levels decline.[42] This data indicates a high rate of consumption, and, by inference, a role in providing protection from disease. Vaccination of calves with *E. coli* J5 is associated with a more than two-fold reduction in the risk of death during the first 60 days of life.[43] The use of an oil emulsion adjuvant increased vaccination-induced titers significantly, while age at the time of immunization appeared to have little effect.[44]

Infections with Gram-negative bacteria are common in swine, yet only a few studies have been conducted on the potential use of *E. coli* J5 vaccine in swine. Infections, both clinical and subclinical, with *E. coli*, *Salmonella*, *Pasteurella*, *Haemophilus*, and *Actinobacillus* are common in swine. The economic losses associated with infections by these organisms can only be roughly estimated because the infections are often subclinical. For example, infections with *Actinobacillus pleuropneumoniae* can have a devastating effect on performance.[45] It is important to note that for the most part current vaccines are only partially effective against these organisms.

In piglets, the decline in anti-*E. coli* J5 titers was found to be more than twice as fast as the decline in total IgG concentrations and *E. coli* J5 titers were directly related to litter size, birth weight, and dam parity.[46,47] In addition, conventionally reared pigs were found to have significantly higher *E. coli* J5 titers than gnotobiotic pigs.[48] Finally, immunization with *E. coli* J5 provides significant protection against deaths due to experimentally induced porcine pleuropneumonia caused by *A. pleuropneumoniae*.[15] In field trials, immunization of pigs with *E. coli* J5 provided protection against *A. pleuropneumoniae* that was similar to that provided by a commercial pleuropneumonia vaccine.[49]

It should be noted that not all attempts to demonstrate that *E. coli* J5 provides protection against the biological effects of endotoxin or to mediate the severity of Gram-negative infections have been successful.[50–53] The basis for these apparently contradictory results is not fully understood. Appelmelk, et al., hypothesized that when *E. coli* J5 fails to provide protection against Gram-negative bacterial infections it is because the vaccine used *E. coli* J5 strains that do not express cross-protective antigens.[54] Variability has been noted in the core region of the LPS of various strains of *E. coli* J5.[55] In addition, some strains of Gram-negative bacteria appear to be more sensitive to effects of *E. coli* J5 antibodies than others.[6,26]

There is conflicting data concerning the potential medical benefits of increased immunity to endotoxin and the fundamental mechanisms that might be involved. The basis for these conflict-

ing results is unresolved. Nevertheless, the majority of the evidence indicates that in the long run, immunization with Gram-negative LPS mutants such as *E. coli* J5 is beneficial. It should be recognized that immunizing with *E. coli* J5 or similar LPS mutants will not prevent Gram-negative bacterial infections from occurring. When Gram-negative infections do occur, however, the clinical severity, and thus the economic consequences, will be reduced. The use of *E. coli* J5 should not be thought of as a replacement for vaccinating against specific diseases.

Discussion

The use of *E. coli* J5 vaccine in the cost-effective production of pork should be considered carefully. Of special concern are reports that producers and veterinarians are experimenting with immunizing swine with commercial *E. coli* J5 vaccines labeled for use in cattle. Positive results have been claimed, but have not as yet been documented. Recent studies suggest immunization of pigs with *E. coli* J5 will be most cost effective in those herds that suffer from high rates of Gram-negative infections that cannot be controlled by disease-specific vaccines or antibiotics.[56] It is less likely that *E. coli* J5 vaccines will be of value in minimal-disease swine herds. Ultimately, as in the case of coliform mastitis in dairy cattle, the economic justification for immunizing pigs with *E. coli* J5 must be documented, and vaccines labeled for specific purposes in swine approved.

Implications

- Increased immunity to LPS core antigens as provided by immunization with *E. coli* J5 can reduce the clinical consequences of Gram-negative infections.
- Immunization with *E. coli* J5 will not prevent infection, and thus is best used for supplemental protection rather than as a replacement for organism-specific vaccines.
- Immunization with *E. coli* J5 will be more effective against systemic infections, pneumonia, and mastitis than against mucosally oriented Gram-negative diseases, (e.g., enterotoxigenic *E. coli*, atrophic rhinitis).
- Additional research and disease-specific cost:benefit analyses are necessary before the routine immunization of swine with *E. coli* J5 can be justified.

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