

Disease Prevention Potential of Increased Immunity to Shared Core Lipopolysaccharide Antigens of Gram-Negative Bacteria by Immunization of Swine with *Escherichia coli* J5

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INTRODUCTION

The endotoxins of gram-negative bacteria are powerful initiators of a multitude of biological responses. The clinical consequences associated with infections with gram-negative bacteria 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. In fact, antibiotic mediated killing of bacteria can increase endotoxin release. Interactions between endotoxin and host cells initiate reactions which can cause irreversible shock and death. On the other hand, the release of 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.

Infections with gram-negative bacteria are common in veterinary medicine. The economic costs in terms of death losses is considerable, but only a fraction of the losses are due to reduced growth, decreased feed utilization, and medication costs. In addition, even subclinical infections have a dramatic metabolic effect causing a marked decrease in lean muscle growth with a proportional increase in fat production.

The growth proponent 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 proponents will almost certainly soon be prohibited. 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.

The purpose of this paper is to introduce the concept of reducing the biologic, and thus economic effects of clinical and subclinical infections with gram-negative bacteria in swine herds by increasing immunity against endotoxins.

COMMON STRUCTURAL AND BIOLOGICAL ACTIVITY OF ENDOTOXINS:

The wide variety of gram-negative bacterial species which can cause disease and the vast differences in type-specific oligosaccharide side chains (O antigens) between bacteria of the same species have caused researchers to focus their efforts to induce immunity against endotoxin on those portions of the molecule that are shared by all organisms (core sugar and lipid A). The concept was that by inducing immunity to portions of endotoxin that all gram-negative bacteria have in common, a degree of resistance to the biologic effects of infection without regard to the specific type of organism would be provided. To this end, cell wall-deficient mutants were identified which lacked various portions of the lipopolysaccharide complex.

One of the most common cell wall mutants to be tested was a strain of *Escherichia coli* 0111:B4 which lacked uridine 5'-diphosphogalactose 4-epimerase. This strain of *E. coli* was termed J5 and classified as an Rc lipopolysaccharide chemotype. *E. coli* J5 fails to completely produce the outer portion of the lipopolysaccharide and associated O polysaccharide side chains, thus leaving the core region fully exposed. Endotoxin core structures are highly conserved among pathogenic gram-negative bacteria.

Immunologic similarities of core antigens between various gram-negative bacteria have been identified using both *E. coli* J5 antisera (1,2) and monoclonal antibodies (3-5). Cross reactivity using isolated endotoxins was not initially identified (6), however it has been recognized that antibodies to outer lipopolysaccharides under some conditions may obscure the detection of cross-reactions between *E. coli* J5 antisera and purified gram-negative bacterial endotoxins (5,7). In addition, the physical state of the bacteria (growth phase, presence of capsule) can influence the immunologically measured cross reactivity (8-11). Finally, it has been suggested that sublethal exposure to antibiotics can increase antibody accessibility to core antigens because of alterations in O-polysaccharide side chains (12).

Monoclonal antibodies against *E. coli* J5 which cross-react with a broad spectrum of unrelated gram-negative bacteria block endotoxin mediated effect on polymorphonuclear leukocytes (13), as well as block tumor necrosis factor by macrophages (14).

PROTECTIVE EFFECTS OF INCREASED IMMUNITY TO SHARED LPS ANTIGENS

Initial studies concerning the protective potential of antibodies to common endotoxin antigens involved laboratory animals which were either immunized with *E. coli* J5 or passively protected by *E. coli* J5 immune serum. Using various models of endotoxemia and gram-negative infections, rabbits, mice, and guinea pigs were protected against various organisms including *E. coli*, *Pasteurella multocida*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* (9,15-22). The degree of protection appeared

to be greatest if the animals were immunologically compromised prior to being challenged with bacteria (18-21). Protection could however, be overcome if the animals were challenged with high enough numbers of bacteria (22). F(ab')₂ antibody fragments provided evidence that protection is via an anti-toxin effect rather than via increases in bacterial phagocytosis and clearance (17). Interestingly, increased immunity to *E. coli* J5 also delayed deaths due to hemorrhagic shock in rabbits (23) and the effects of graft versus host reactions in mice (24).

Based on the promising results from 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 shock (7,25-27). While the prophylactic administration of *E. coli* J5 immune serum to surgical patients did not decrease the rate of post-operative infections, the medical consequences of these infections was not as serious (28). As was first demonstrated in mice, protection from graft-versus-host disease was related to anti-*E. coli* J5 titers (29). In humans, the antibody response induced by immunization by *E. coli* J5 is transient and not significantly enhanced by reimmunization (30).

Of all domestic animals, dairy cattle have received the most attention in terms of evaluating the potential benefits of being vaccinated with *E. coli* J5. Immunization with *E. coli* J5 significantly reduced the clinical signs associated experimentally induced coliform mastitis (31). Immunization was associated with increased serum mediated bacterial opsonization and phagocytosis (32).

Antibody titers against *E. coli* J5 that were half the population normal are associated with a 5.33 greater risk of clinical coliform mastitis (33). Immunization of dairy cows reduced the incidence of clinical gram-negative mastitis but did not appear to reduce the incidence of intramammary infections (34,35). Immunization with *E. coli* J5 increased profits by \$57 per dairy cow per year when more than 1 percent of the cows developed clinical coliform mastitis per year (36).

In calves, *E. coli* J5 titers decline at three times the rate as total IgG levels indicating a high rate of consumption, and by inference a role in providing protection from disease (37). Vaccination of calves with *E. coli* J5 is associated with over a two-fold reduction in the risk of death during the first 60 days of life (38). The use of oil emulsions increased vaccination induced titers significantly while age appeared to have little effect (39).

Perhaps more than any other domestic animal, pigs suffer more from infections with gram-negative bacteria. Infections, both clinical and subclinical, with *E. coli*, salmonella, pasteurilla, haemophilus, and actinobacillus are common. The economic losses associated with infections by these organism can only be roughly estimated as in many cases subclinical infections rather than overt disease is the rule. For example,

infections with *Actinobacillus pleuropneumoniae* can have a devastating effect on performance (40). It is important to note that current vaccines are for the most part 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 (41) and *E. coli* J5 titers were directly related to litter size, birth weight, and dam parity (42). In addition, conventionally reared pigs were found to have significantly higher *E. coli* J5 titers than gnotobiotic pigs (43). Finally, immunization with *E. coli* J5 provides significant protection against deaths due to experimentally induced porcine pleuropneumonia caused by *Actinobacillus pleuropneumoniae* (11).

It should be noted that not all attempts to demonstrate that *E. coli* J5 can provide protection against biological effects of endotoxin or mediate the severity of gram-negative infections have been successful (44-47). The basis for these apparently contradictory results is not fully understood however, various strains of *E. coli* J5 have been recognized. Variability is present in both the core region of the lipopolysaccharide and O-side chains (48). Failure of *E. coli* J5 to provide protection against gram-negative bacterial infections has been explained by immunization using an *E. coli* J5 strains which do not express cross-protective antigens (49). In addition, some strains of bacteria appear to be more sensitive to *E. coli* J5 antibodies than others (2,21).

CONCLUSIONS AND RECOMMENDATIONS:

There is conflicting data concerning the potential medical benefits of increased immunity to endotoxin and the fundamental mechanisms which might be involved. The basis for these conflicting results is unresolved. Nevertheless, the majority of the evidence indicates that in the long run, immunization with gram-negative lipopolysaccharide mutants such as *E. coli* J5 is beneficial.

It should be recognized that immunization with *E. coli* J5 or similar lipopolysaccharide mutants will not prevent gram-negative bacterial infections from occurring. When gram-negative infections do occur however, the clinical severity and thus economic consequences will be reduced. The use of *E. coli* J5 should not be thought of as a replacement for vaccinating against specific diseases.

The routine use of *E. coli* J5 vaccine in the cost effective production of pork should be carefully considered. Ultimately, as in the case of coliform mastitis in cattle, the economic justification for routinely immunizing pigs with *E. coli* J5 must be documented. A multi-herd study to establish the cost/benefit relationships of using *E. coli* J5 in Mexican swine herds will begin shortly.

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MECANISMOS PARA EL ESTABLECIMIENTO DE UN CENSO

PORCINO NACIONAL Y LA ELIACION DE ERRORES DEL LECHON

Y CERDO CRIBADO A NIVEL DE LONJAS O BOLSAS PECUARIAS

II ORGANIZACION ZOOTECNICA Y SANITARIA DE LAS

EXPLOTACIONES PORCINAS

1.1 Organización zootécnica

Jose Antonio del Barrio Martín

Asociación Nacional de Productores de Cerdo Porcino