Sponsors

University of Minnesota
College of Veterinary Medicine

College of Agricultural, Food and Environmental Sciences

Extension Service

Swine Center

Editors
W. Christopher Scruton
Stephen Claas

Layout
David Brown

Logo Design
Ruth Cronje, and Jan Swanson;
based on the original design by Dr. Robert Dunlop

Cover Design
Shawn Welch

Immune responses to extracellular bacteria

Albert Rovira, DVM, MS
University of Minnesota

Bacterial diseases are one of the main concerns for pig health in modern production systems, and probably the cause of most of the mortality. Bacterial infections can be classified in those produced by intracellular and extracellular bacteria. These two types of infection elicit different immune responses. This paper reviews the immune responses against extracellular bacteria. Different mechanisms are presented and examples of bacteria-producing important diseases of swine are provided.

Innate immune response

The innate immune response is responsible for the control of many commensal bacteria and the early response against infection by pathogenic bacteria. The first line of innate defense against pathogens is provided by physical barriers, chemical factors, and the commensal flora.

Physical barriers

The skin and mucous membranes represent an impenetrable barrier to most bacteria. If the integrity of these surfaces is damaged some bacteria are able to invade the host. This is the case of *Clostridium tetani*, which sporadically infects young pigs via the umbilical cord or the castration wound. *Streptococcus suis* infects humans through skin cuts and this route may apply for infection by this bacterium in pigs as well.

Chemical factors

Mucosal secretions contain antimicrobial substances. Lysozyme is an enzyme present in most secretions that degrades bacterial peptidoglican. Antibacterial peptides in the intestine are toxic for important pathogens of swine such as *Escherichia coli* and *Salmonella*.

Commensal flora

The presence of commensal organisms on mucous membranes prevents the colonization by pathogenic bacteria through different mechanisms: competition for nutrients and receptors, production of toxic substances, generation of cross-immunity and non-specific stimulation of the immune system. Subclinical infection with a non-pathogenic strain of *Haemophilus parasuis* protected pigs from subsequent inoculation with a virulent strain. On the other hand, the efficacy of exposure of weaned pigs to non-toxigenic *E. coli* to reduce enteritis by pathogenic *E. coli* has been demonstrated in field trials. However, the mechanism responsible for protection in these two examples has not been investigated.

Main components of the innate immune response

The two main components of the innate immune response are the complement and the phagocytosis.

Complement

Bacteria can activate complement via the alternative pathway through capsular polysaccharides (Gram +) or the lipopolisaccharide (Gram -). Complement activation results in a cascade of biochemical reactions that leads to:

- Opsonization: Some products of the complement reactions (C3b) opsonize bacteria enhancing their recognition by phagocytes.
- Lysis: The end product of complement activation is the formation of the membrane attack complex, a pore-like structure that lyses the cell. This killing mechanism has been demonstrated against *E. coli* and *Neisseria* species and it is thought to play a role against gram-negative bacteria in general. However, there is no information on antibody-independent complement-mediated lysis of bacterial species isolated from pigs .
- Inflammation: Some complement by-products (C3a, C4a, C5a) are potent chemotactic factors that recruit and activate leukocytes.

Phagocytosis

Macrophages and polimorphonuclear neutrophils present in the mucosal surfaces are able to recognize and phagocyte bacteria. Neutrophils are more efficient phagocytes and can be massively recruited during inflammation. In contrast, the importance of macrophages is that they process the phagocyted bacteria and present protein antigens to lymphocytes to develop a humoral response. The ability to engulf and kill bacteria is greatly enhanced by opsonization with C3b or specific antibodies. This phenomenon has been demonstrated for important swine pathogens such as *H. parasuis*, *Actinobacillus*

pleuropneumoniae, and certain strains of *S. suis*. Activated phagocytes secrete cytokines, which induce leukocyte infiltration and synthesis of acute-phase proteins by hepatocytes.

Adaptive immune response

The adaptive immune response develops later but is more efficient and specific than the innate immune response. The humoral response is the most important response against extracellular bacteria. In humans, individuals affected by conditions that cause agammaglobulinemia suffer from recurrent extracellular bacterial infections. The most frequent agents causing these infections are *Streptococcus pneumoniae* and *Haemophilus influenzae*. In contrast, their response to viral and fungal infections is relatively unimpaired. Conversely, individuals affected by Di George's syndrome (absence of thymus), unable to mount a T-cell response, are frequently affected by viral and fungal infections and relatively resistant against bacterial infections.

Stimulated B-cells differentiate into plasma cells that produce specific antibodies against bacterial surface antigens and toxins. These antibodies confer protection against disease by different mechanisms:

- Neutralization of toxins: Attachment of the antibody to functional epitopes in the toxin molecule. Neutralizing antibodies to beta toxin are protective against *Clostridium perfringens* type C necrotizing enteritis. Similarly, neutralizing antibodies to the Apx toxins of *A. pleuropneumoniae* are essential for complete protection against porcine pleuropneumonia. The presence of toxoids in killed vaccines against infection of toxin-producing bacteria is highly recommended.
- Opsonization: Coating of bacteria with specific antibodies greatly increases phagocytosis and thus clearance of the bacterium.
- Complement activation: Antigen-antibody complexes can activate complement by the classical pathway. Activation of complement results in opsonization and lysis of bacteria and inflammatory stimulation, as mentioned above. Antibody-dependent complement-mediated lysis has been demonstrated for many genera such as *Bordetella*, *Haemophilus*, *Pasteurella*, and *Escherichia*.
- Mucosal protection: IgA and IgG on mucosal surfaces prevent bacterial adherence to receptors of the host cells. The immune response against *E. coli* infections is basically humoral and includes antibodies that neutralize the fimbrial antigens of the bacterium. Interestingly, a proportion of the B-cells stimulated in the gut return to the bloodstream and localize in other mucous membranes, where they differentiate into plasma cells and produce IgA. This mechanism explains the presence of IgA against *A. pleuropneumoniae* in bronchoalveolar secretions, after oral exposure. According to a similar mechanism, B-cells migrate from the digestive system to the mammary gland before farrowing. There, they produce high amounts of IgA against enteric pathogens that will be secreted in colostrum and milk, and will protect piglets the first weeks of age.

The other important component of the adaptive immune response is the CD4+ T-cell pool. Macrophages, B-cells and dendritic cells present protein antigens to CD4+ T-cells. In response, these cells produce cytokines that stimulate antibody production (IL-2, IL-4, IL-5), induce local inflammation (TNF), and activate macrophages (IFN-().

Bacterial pathogens have developed mechanisms for evading the host immune response. The presence of an external polysaccharide-rich capsule lends phagocytosis resistance to some bacteria; this is the case of *S. suis*. Other bacteria have surface molecules that inhibit complement activation, and still others secrete proteases that cleave IgA. Furthermore, some bacteria are able to modify their surface antigens to escape recognition by specific antibodies.

Immune response against intracellular bacteria includes a number of components: the antibodies, the phagocytosis, and the complement system. Information on the importance of these components on diseases affecting swine is still limited. However, available data should help understand the pathogenesis of these diseases and should be considered to plan treatment or preventive interventions.

References

Goldsby R, Kindt T, Osborne B, Kuby J. 2003. Immunology. Fifth edition. Freeman and company, New York.
Abbas A, Lichtman A. 2003. Cellular and molecular immunology. Fifth edition. Saunders, Philadelphia.
Roth J. 1999. The immune system. In Straw B, D'Allaire S, Mengeling W, Taylor D, Diseases of Swine, 8th edition. Iowa state university press. Amas, Iowa.
MacLaren D. 1998. Non-specific resistance to infection. In Collier L, Balows A, Sussman M, Microbiology and microbial infections, 9th edition (vol 3: Bacterial infections). Arnold, London.
Cushley W. 1998. Immunoglobulins. In Collier L, Balows A, Sussman M, Microbiology and microbial infections, 9th edition (vol 3: Bacterial infections). Arnold, London.
Sim R. 1998. In Collier L, Balows A, Sussman M, Microbiology and microbial infections, 9th edition (vol 3: Bacterial infections). Arnold, London.
Solano GI, Bautista E, Molitor TW, Segales J, Pijoan C. 1998. Effect of porcine reproductive and respiratory syndrome virus

infection on the clearance of *Haemophilus parasuis* by porcine alveolar macrophages. Can J Vet Res. 62:251-6.
Nielsen R. 1993. Pathogenicity and immunity studies of *Haemophilus* parasuis serotypes. Acta Vet Scand. 34:193-8.
Cruijsen TL, Van Leengoed LA, Dekker-Nooren TC, Schoevers EJ, Verheijden JH. 1992. Phagocytosis and killing of *Actinobacillus pleuropneumoniae* by alveolar macrophages and polymorphonuclear leukocytes isolated from pigs. Infect Immun. 60:4867-71.
Lun S, Willson PJ. 2004. Expression of green fluorescent protein and its application in pathogenesis studies of serotype 2 *Streptococcus suis*. J Microbiol Methods. 56:401-12.
Fuentes M, Pijoan C, Becton L, Morrison R, Pieters M. 2004. Inoculation of non-pathogenic *Escherichia coli* to control disease and reduce antibiotic usage. In Proceedings of the 18th IPVS congress, Hamburg, Germany.