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Atypical immune responses to bacterial pathogens in pigs

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Introduction

Bacterial pathogens may induce a wide range of immune responses depending on the production of specific virulence factors and interactions with the host (extracellular versus intracellular organisms).

Infection by extracellular bacteria usually induces the production of humoral antibodies, which are secreted by plasma cells in regional lymph nodes and submucosa of the respiratory and gastrointestinal tracts. The humoral immune response is the main protective response against extracellular bacteria. There are several antibody-mediated mechanisms for combating infection by extracellular bacteria:

- antibodies can neutralize toxins produced by bacteria;
- complement activation on bacterial surfaces leads to complement-mediated lysis of bacteria;
- antibody and the complement split product C3b bind to the bacteria, serving as opsonins to increase phagocytosis;
- C3a and C5a, generated by antibody-initiated complement activation, induce local mast cell degranulation, releasing substances that mediate vasodilatation and extravasation of lymphocytes and neutrophils;
- other complement split products are chemotactic for neutrophils and macrophages (Goldsby et al., 2000a).

Intracellular bacteria, on the other hand, induce primarily a cell-mediated immune response. Both activated T-helper cells (CD4⁺ T_H) and cytotoxic T-lymphocytes (CD8⁺ CTLs) serve as effector cells in cell-mediated immune reactions. Cytokines secreted by T_H cells can activate various phagocytic cells, enabling them to phagocytose and kill microorganisms more effectively. Effector T-cells may originate two distinguished responses (TH1 and TH2) depending on the panels of cytokines they secrete. The TH1 response is characterized by the secretion of IL-2, INF- γ , and TNF- α . These cytokines are associated with the promotion of excessive inflammation and tissue injury. IL-2 and INF- γ secretion by TH1-cells promote the differentiation of CD8 precursors to fully cytotoxic T-cells. This pattern of cytokine production makes the TH1 subset particularly suited to respond to viral infections and

intracellular pathogens. The other subset, called the T_H 2 subset, secretes IL-4, IL-5, IL-6, and IL-10. This subset functions more effectively as a helper for B-cell activation and is associated to allergic reactions. Natural Killer cells (NK) are also involved in the nonspecific cell-mediated immune response to intracellular bacteria (Goldsby et al., 2000).

Many extracellular and intracellular organisms have developed ways to evade the host immune system, originating atypical immune responses during infection. In these cases, a comprehensive understanding of host-pathogen interactions is required for rational design of control strategies. This article aims to present and discuss atypical immune responses to major bacterial pathogens affecting pigs in modern swine production.

Extracellular bacteria

Mycoplasmas

Mycoplasmas are distinguished phenotypically from other bacteria by their minute size and total lack of a cell wall. These organisms are extracellular surface parasites, although some species have been reported to occupy additional intracellular niches. Mycoplasmas have been known to lack metabolic capabilities not only for wall synthesis, but also for several other biosynthetic pathways. These characteristics reflect the association of mycoplasmas with a parasitic lifestyle (Razin et al., 1998; Citti et al., 1997).

Pathogenic swine mycoplasmas include *M. hyopneumoniae*, *M. hyohrinitis*, and *M. hyosynoviae* (Ross et al., 1999). The complex network of interactions between mycoplasmas and the host immune system involves both specific and nonspecific immune reactions. The specific reactions may play an important role in the development of lesions and exacerbation of mycoplasma-induced diseases. Nonspecific immune responses include suppression or stimulation of B- and T-lymphocytes, release of specific cytokines, and macrophage activation, among others (Razin et al., 1998).

Immunity to mycoplasmas

Mycoplasmas have a complex interaction with the host immune system. Although swine mycoplasmas are extra-

cellular organisms, they typically induce a strong cell-mediated response.

Comparison of response to experimental infection of pigs using *Actinobacillus pleuropneumoniae* and *M. hyopneumoniae* clearly shows the differences between the immune response to these pathogens. *Actinobacillus pleuropneumoniae* is known to be a good stimulator of the humoral immune system. The humoral immune response in the respiratory tract of pigs infected with *M. hyopneumoniae* seems to be quantitatively more limited than that observed in *A. pleuropneumoniae* infections. Only 0.4% of antigen-specific plasma cells were identified in mediastinal lymph nodes from pigs infected with *M. hyopneumoniae* compared with 2% recovered from pigs infected with *A. pleuropneumoniae*. These findings support observations that a stronger activation of the T-cell population, compared to the B-cell population, occurs in the mediastinal lymph nodes and lung tissue 4 weeks after infection with *M. hyopneumoniae* (Suter et al., 1985). Another interesting feature of *M. hyopneumoniae* infection is the fact that an intact immune system is necessary to produce lung lesions. One study showed that healthy pigs infected with *M. hyopneumoniae* developed lung lesions characterized by massive peribronchial, peribronchiolar, and perivascular lymphoid hyperplasia. These lesions were much less prominent in thymectomized and antithymocyte serum-treated pigs. These results suggest that cell-mediated immune mechanisms are important in the development of pneumonic lesions in enzootic pneumonia of pigs (Tajima et al., 1984).

Humoral and cell-mediated response

During infection with *M. hyopneumoniae*, the proportion of IgA to IgG-containing cells remained constant in the nasal mucosa and retropharyngeal lymph nodes (1:3), and changed to 1:15 in bronchial lymph nodes. In both lymph nodes, the maximum number of all isotypes of immunoglobulin containing cells occurred 3 weeks after infection, although IgG-containing cells in bronchial lymph nodes reached maximum number 1 week later. At 8 weeks after mycoplasma inoculation, the number of IgG-containing cells in bronchial lymph nodes was still elevated, while the number of IgA-containing cells was within normal limits. The local humoral response in tracheobronchial secretions was characterized by an early response by IgM and IgG, and a progressive increase in IgA levels. The antibody response in sera was somewhat different. The first detection of antibodies in an individual pig was achieved 2 weeks after infection, whereas 5 weeks following inoculation all infected pigs were positive (Suter et al., 1985).

Proinflammatory cytokines

M. hyopneumoniae and *M. hyorhinae* are known to stimulate macrophages and monocytes to secrete proinflammatory

cytokines such as IL-1, IL-6, and TNF- α . TNF- α and IL-1 are important cofactors in the activation of T- and B-lymphocytes and promote lymphocyte proliferation and differentiation into effector cells. Both cytokines upregulate the cytotoxic activity of macrophages and large granular NK cells and enhance the metabolic activity of polymorphonuclear cells. By inducing increased expression of MHC antigens they potentiate antigen presentation by antigen presenting cells (APCs). Immune system cells costimulated by TNF- α or IL-1 express enhanced levels of receptors for cytokines and produce cytokines, a- and b-chemokines, and prostaglandins. The ability of TNF- α and IL-1 to cause enhanced expression of adhesion molecules on endothelial cells, together with the chemotactic activities of chemokines and TNF- α -mediated expression of adhesion molecules on the surface of neutrophils, triggers the increased recruitment of leukocytes to local sites of inflammation. In addition, TNF- α and IL-1 exert local necrosis and tissue destruction. Although high doses of TNF- α inhibit in vitro replication of bone marrow progenitor cells, both TNF- α and IL-1 stimulate the production of GM-CSFs and enhance in vivo hematopoiesis, thereby increasing the pool of cells to be stimulated and chemoattracted to the site of inflammation. Such effects induced by TNF- α and IL-1, released in response to interactions with mycoplasmas, may provide an explanation for most of the inflammatory and pathologic manifestations observed in mycoplasmal infections. IL-6 is another pleiotropic, proinflammatory cytokine induced by many mycoplasmas. This cytokine is produced by monocytes and macrophages as well as by Th- and B-lymphocytes and other non-immune system cells. IL-6 exhibits some activities that overlap with those of TNF- α and IL-1. Nevertheless, its major activity is mediated by its ability to serve as a cofactor in B-cell differentiation and maturation into Ig-secreting cells. IL-6 also enhances the expression of IL-2 receptor (IL-2R) on activated cells and induces the production of IL-2 by T_H-cells. This cytokine promotes the proliferation of T-lymphocytes and amplifies hematopoiesis. IL-6, like TNF- α and IL-1, also triggers the production of acute-phase proteins in the liver (Razin et al., 1998).

Activation of immune cells (mitogenic effect)

Many mycoplasmas possess the capacity to activate lymphocytes in a nonspecific, polyclonal manner. The mitogenicity of mycoplasmas is displayed by live organisms, nonviable cells, or crude extracts of lysed cells. Membranes of *M. hyopneumoniae* have a moderate nonspecific stimulatory effect on porcine lymphocytes, including blood lymphocytes and bronchial lymph node lymphocytes. *Mycoplasma hyorhinae* has also been shown to be mitogenic toward both B- and T-lymphocytes. The mitogenic components present in *M. hyorhinae* include a heat-stable 90-kDa soluble protein, and TX-100-extractable proteins weighing 53, 43, and 35 kDa. Proliferation

of B-lymphocytes induced by *M. hyorhinis* in vitro does not require the presence of accessory cells, such as macrophages (Messier and Ross, 1991; Razin et al., 1998).

Suppression of immune cells.

Suppression of immune cells by porcine mycoplasmas may be performed directly by contact with cell components or indirectly by means of cytokine regulation. *Mycoplasma hyorhinis* has been shown to release a protease-sensitive, 200-kDa factor that suppressed the induction of interleukin-2 (IL-2)-dependent cytotoxic T-cell responses to alloantigens. The suppression was not reversed by addition of excess recombinant IL-2 and was more profound when the mycoplasmal suppressive factor was added to cultures during the early stages of the cytotoxic reaction. The suppressive elements from *M. hyorhinis* were also inhibitory for mouse B-lymphocytes responding to *E. coli* lipopolysaccharide (LPS). Indirect suppression of polymorphonuclear phagocytes may also occur due to release of prostaglandins that down-regulate host cell functions, as demonstrated for *M. hyopneumoniae*. These data support the notion that perturbation of phagocyte functions exerted directly and/or indirectly by certain mycoplasmas may contribute to their capacity to evade the host natural defense mechanisms. (Razin et al., 1998).

Antigenic variation

The term “antigenic variation” (or “phenotypic switching”) refers to the ability of a microbial species to alter the antigenic character of its surface components that enhance the colonization of host tissues and evade phagocytosis. These surface components are the major targets of host antibody response; therefore, the ability of a microorganism to rapidly change the surface antigenic repertoire and consequently to vary the immunogenicity of these structures allows effective avoidance of immune recognition. One of the well-documented examples of a gene family providing an impressive surface variation system is the *VLP* gene family of *M. hyorhinis*. This system encodes a set of variable lipoproteins (VLP) that constitute the major coat protein of this mycoplasma. By combinatorial expression and high-frequency phase variation and size variation of the VLPs, an extensive array of antigenic variants can be generated. Different *M. hyorhinis* strains carry a variable number of *VLP* genes, which suggests that this organism has an expanded potential of structural diversity created by modulation of the *VLP* repertoire (Citti et al., 1997; Razin et al., 1998).

Antigens potentially associated with protective immunity

The swine immune system can recognize specific *M. hyopneumoniae* antigens. Convalescent sera from pigs infected with *M. hyopneumoniae* have been shown to have antibodies against heat shock proteins 60 (HSP60), which

indicates that these proteins are immunogenic in natural infection. These proteins are produced by the organism in response to sudden changes in the environment and have the function to maintain cellular functions (Scerm et al., 2002). Oral vaccination of mice with a *Salmonella typhimurium* strain expressing a 15 kDa protein, which is part of a 42 kDa *M. hyopneumoniae* NrdF protein (R2 subunit of the essential prokaryotic class I ribonucleotide reductase), induced a significant secretory immunoglobulin A immune response in the lung (Fagan et al., 1997). *Mycoplasma hyopneumoniae* L-lactate dehydrogenase (LDH) has been shown to be highly immunogenic after immunization of pigs with pure substance. Antibodies against LDH are usually detected after the acute phase of disease and may have significance for serological detection of infection (Frey et al., 1994). Although HSP60 and NrdF are immunogenic, their role in protection has not been established. Preliminary protection assays have shown that pigs immunized with recombinant LDH are not protected against experimental infection with *M. hyopneumoniae*.

Intracellular bacteria

Lawsonia intracellularis

Lawsonia intracellularis is the causative agent of proliferative enteropathy (PE), an intestinal infectious disease characterized by thickening of the aboral small and proximal large intestinal mucosa due to enterocyte proliferation associated with the presence of the intracellular bacterium. Little is known about the immunological response to *L. intracellularis*. Recent reports, however, have provided some data on humoral and cellular immune response during natural and experimental infection (Guedes et al., 2002; Guedes and Gebhart, 2003). Similar to most bacterial infections, exposure to *L. intracellularis* results in initial IgM antibody response, followed by a peak of IgG, with a subsequent decrease in antibody titers. Mucosal infection is associated with a high amount of IgA antibodies. Serum IgG is usually detected 2 weeks following experimental infection and may last for at least 13 weeks post-challenge. Considering that *L. intracellularis* is an obligatory intracellular bacterium, serum IgG is not likely to protect against infection. In fact, vaccinated pigs that did not seroconvert by the time they were challenged were still protected against infection, indicating that non-humoral factors may be involved in protective immunity. Cell-mediated immune response and secretory IgA, on the other hand, may play an important role in protection against *L. intracellularis* infection. Interferon-gamma (INF- γ) producing cells, which are an indicator of TH1 response, were detected in the peripheral blood of experimentally infected pigs 2–13 weeks after challenge. INF- γ -producing cells have been shown to play an important role on limiting *L. intracellularis* infection in mice. Al-

though the presence of INF- γ -producing cells was evident following experimental challenge of naïve pigs with *L. intracellularis*, further studies are necessary to determine the role of these cells in controlling infection by this agent (Guedes and Gebhart, 2003; Kroll et al., 2004).

Salmonella

Swine can be affected by a wide range of *Salmonella* serotypes, including *S. choleraesuis*, *S. typhimurium*, and *S. typhisuis*. Disease associated with host adapted *S. choleraesuis* is characterized by septicemia, enterocolitis, or bacteremic localization as pneumonia, hepatitis, and occasionally, meningitis and abortion. *S. typhimurium* usually causes colitis and *S. typhisuis* has been associated with caseous lymphadenitis. *S. choleraesuis* variety kuzendorf is the most frequent serovar causing disease in swine, followed by *S. typhimurium*. Other serovars that may be isolated from pigs include *S. heidelberg* (post-weaning diarrhea), *S. dublin*, and *S. enteritidis* (meningitis in suckling pigs) (Schwartz, 1999).

Salmonella infects the pig through the oral route and uses several mechanisms to cross the intestinal epithelium. Endocytosis by M-cells, which are located in the epithelium overlying Peyer's patches, is the major route of invasion. *Salmonella* can also invade enterocytes or be shuttled across the epithelium by cells that breach the epithelial layer and sample the gut lumen. Based on in vitro studies, the interaction between *Salmonella* and epithelial cells results in a proinflammatory response characterized by the release of several cytokines and chemokines, with interleukin IL-8 being one of the best studied factors. Bacterial flagella or flagellin monomers are the major bacterial determinant initiating this response. *Salmonella* must be flagellated and pathogenic in order to induce epithelial secretion of IL-8 (Wick, 2004).

Immune response to Salmonella

Immune cells

In vitro studies have demonstrated that neutrophils are rapidly recruited following bacterial administration. Neutrophils have the earliest detectable change in abundance, whereas changes in macrophage numbers are apparent a couple of days later. Following oral infection in mice, neutrophils are recruited to the spleen, mesenteric lymph nodes, and Peyer's Patches, which are sites normally depleted of these cells. Following exposure to *Salmonella*, macrophages increase the expression of several molecules, including major histocompatibility complex (MHC)-II and co-stimulatory molecules. Increased expression of these molecules suggests they might be competent to interact with T-cells. Dendritic cells also increase in the spleen of mice 5 days following *Salmonella* infection. Dendritic cells are responsible for initiating the adaptive immune response. Population changes are also observed among

lymphoid cells early during *Salmonella* infection. Five days following oral infection of mice, the B-cell population is stable in the spleen and elevated in the mesenteric lymph nodes. T-cells are reduced in the spleen by 25% and constant in mesenteric lymph nodes. These data suggest that there are organ-specific differences in cell population dynamics following *Salmonella* ingestion. Natural killer (NK) cells are also involved on the early response to *Salmonella*. These cells are activated early during infection. *Salmonella* has been shown to induce down-regulation of surface molecules in NK cells, such as NK1.1. The exact role of NK cells on *Salmonella* infection remains to be defined. (Wick, 2004). Some experiments have shown that the host immune response to virulent *Salmonella* appears to induce a form of immunosuppression. Mice vaccinated with an attenuated strain of *S. typhimurium* become immune to future challenge with virulent *S. typhimurium* but are unable to mount immune responses, 1–3 weeks post-immunization, against non-*Salmonella* antigens (Jones, 1996).

Cytokines

INF- γ , TNF- α , IL-12, and IL-8 are some of the cytokines that participate in the early response to *Salmonella* infection. The lymphoid cells within intestinal Peyer's patches produce large amounts of INF- γ in response to stimulation with *S. typhimurium*, indicating that this cytokine plays an important role in the host response to infection. INF- γ and TNF- α usually increase resistance of nonphagocytic tissue culture cells to *Salmonella* invasion. INF- γ may also inhibit bacterial growth and increase the fusion of phagosomes containing bacteria with lysosomes. IL-12 seems to have a role in maintenance rather than induction of INF- γ production by T-cells during *Salmonella* infection. *Salmonella*-induced IL-8 attracts neutrophils that subsequently migrate through the epithelial layer into the intestinal lumen. Early sources of INF- γ include NK and T-cells of undefined specificity. TNF- α production during the early stage of *Salmonella* infection (5 days post-infection) is numerically dominated by neutrophils and macrophages. An increase in TNF- α + dendritic cells is also evident in splenocytes from mice. IL-8 is mainly produced by epithelial cells in the intestine (Jones, 1996; Wick, 2004).

Evasion of host innate immunity

To move into deeper tissue, these bacteria must be able to avoid and/or survive the oxygen-dependent and oxygen-independent killing mechanisms of professional phagocytes following internalization. Some oxygen-dependent mechanisms involve the production of superoxide anions, hydrogen peroxide, hypochlorite, and hydroxyl radicals that are pumped into the phagosomes. Oxygen-independent killing mechanisms include acidification of the phagolysosome and the introduction of degradative enzymes and small bacteriocidal peptides known as

defensins. Some microbial pathogens prevent fusion of phagosomes and lysosomes to avoid exposure to the toxic contents of the lysosome, while others have evolved strategies to protect themselves from such an environment. *S. typhimurium* significantly inhibits phagolysosomal fusion and that dividing organisms are primarily found within unfused vesicles. Another group observed that acidification of phagosomes containing live *S. typhimurium* was delayed 4–5 hours, while acidification of vacuoles with dead organisms occurred within 1 hour. *Salmonella* may synthesize over 30 bacterial proteins when exposed to macrophages, including the heat shock proteins. It seems probable that many of these gene products are employed in protecting the bacteria against the diverse killing mechanisms of macrophages and in circumventing host defense mechanisms (Jones, 1996).

Phagocytosis of *S. typhimurium* by macrophages is unconventional, both in mechanism of entry and in morphology of the phagosome formed. The *Salmonella*-containing vacuoles are large (2–5µm) and are termed “spacious phagosomes.” Spacious phagosome formation likely promotes *Salmonella* survival by dilution of toxic lysosomal compounds or attenuation of antimicrobial factors, including decreased phagosomal acidification. Neutrophils rapidly kill *Salmonellae*, with <10% of an initial inoculum surviving after phagocytosis in vitro. Therefore, the ability of the *Salmonella* organism to induce its own uptake into epithelial cells and macrophages may be a way to avoid neutrophil-mediated killing. *Salmonella* may also promote structural changes in the lipid A section of the LPS molecule to avoid the host immune system (Ernst et al., 1999).

Summary

Some extracellular and intracellular bacteria that affect swine may promote unique immune responses during infection. They may regulate the function of immune cells by having a mitogenic or suppressive effect. They may also develop complex evasion mechanisms based on expression of different surface antigens, secretion of substances that neutralize host defense factors, or even by “disguising” themselves from the immune system by invading host cells. Most commonly, the host immune response developed in the presence of these agents is the main cause of lesions observed during infection. Regulation of the immune response to these organisms may be the key to reducing the detrimental effects promoted by these agents.

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