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Swine immune responses to viral infection

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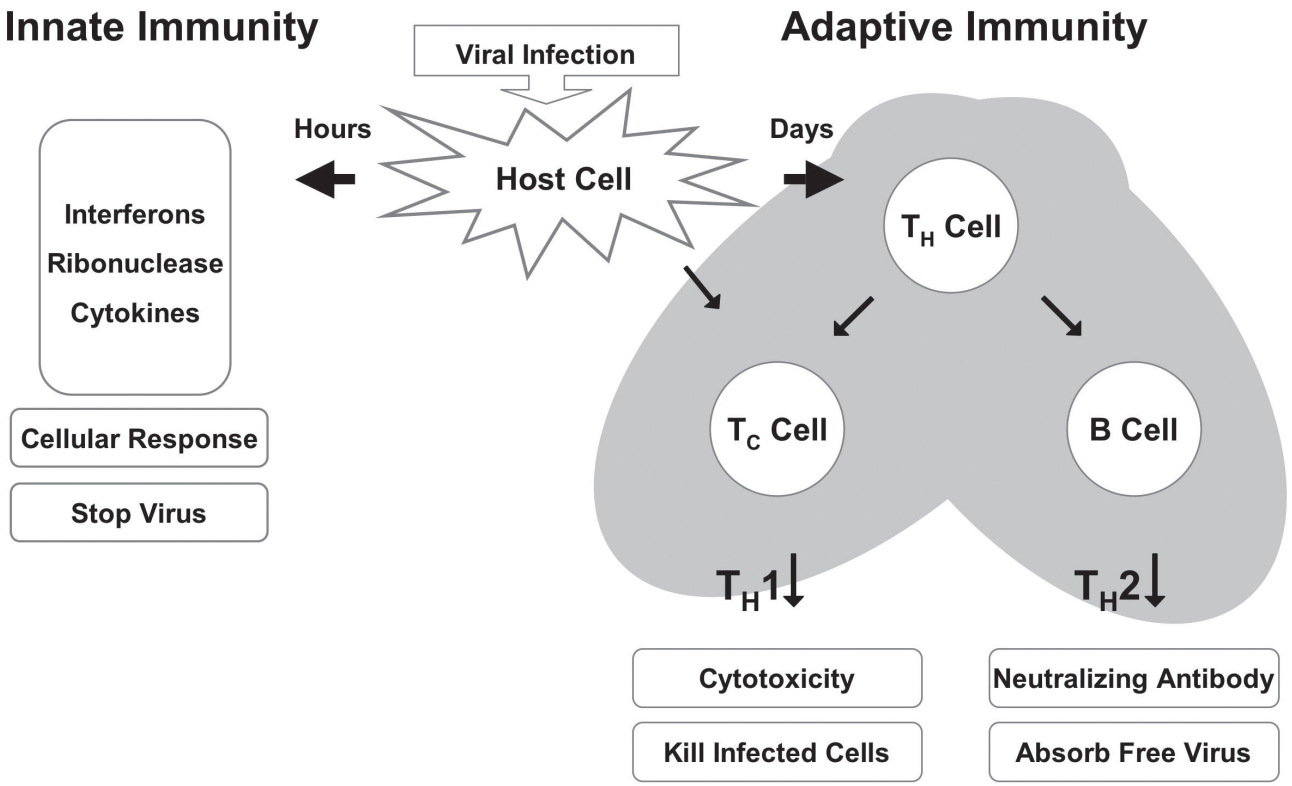
The immune system of animals evolved to protect against invading microorganisms, to repair injury, and to distinguish self from non-self. Host defense against infection, including viral infection, is generally divided into innate immunity and adaptive immunity. Innate immunity is the totality of cells, molecules, and physical barriers that provide constant surveillance and monitoring for foreign antigens, infectious agents, and noninfectious substances. Natural killer (NK) cells are a specialized lymphocyte that recognizes and destroys cells infected with viruses. NK cells are a primary effector cell of innate immunity. Macrophages play a central, controlling role in initiating immune responses and in directing the transition from innate to adaptive response by secreting cytokines that stimulate inflammatory responses and mobilize endothelium, liver, and other organ systems in a so-called acute phase response.

Innate immunity is triggered within minutes to hours of an infectious challenge and is estimated to completely resolve more than 95% of infections without further involvement of the immune system. Innate immune cells have receptors that sense the presence of substances unique to viruses. Viruses, especially RNA viruses like TGEV and PRRSV, tend to contain double-stranded RNA structures in their genome or produce them intracellularly during the viral life cycle. Binding of these biochemical structures to receptors activates innate anti-viral responses that efficiently stop infection and remove damaged cells and tissues. The antiviral responses include activation or enzymes to degrade the viral genetic material and to limit synthesis of viral proteins. A family of receptors known as Toll-like receptors play a central role in recognition of these viral features and are evolutionarily ancient, with a widespread distribution in plants, invertebrates, and vertebrates. Viruses that cause serious disease in pigs have acquired features and mechanisms of immune evasion and subversion that provide a window of opportunity for proliferation such that a race ensues between pathogen multiplication and innate immune defense. Infection with TGEV or rotavirus, if not quickly controlled, elicits a rapid, overwhelming enteric pathology that kills pigs long before adaptive, antigen-specific immune responses can be mobilized.

In the course of an innate immune response, specialized dendritic cells that are resident in tissues or that differentiate from inflammatory macrophages capture proteins from dead cells and phagocytosed viruses, migrate to lymph nodes, and present the antigens to B- and T-lymphocytes. Dendritic cells and macrophages are the interface between innate and adaptive immunity. Dendritic cells present polypeptide fragments of proteins to helper T-lymphocytes (T_H-cells). In the presence of cytokine such as interleukin-1 (IL-1) and IL-12 that are produced by macrophages and dendritic cells, the presented antigen activates T_H-cells with a surface receptor that recognizes the same peptide. Meanwhile, free peptides and protein structures that bind to the antigen receptor on the surface of B-cells activate amplification and secretion of specific antibodies. The translation of an innate response programmed to respond to a diverse array of pathogens into an exquisitely specific response directed to individual proteins of a single pathogen is an elegant and distinguishing feature of animal immunity. One additional adaptive immune cell, the cytotoxic T-lymphocyte (T_C-cell) is activated by engagement of specific viral peptides on the surface of virus-infected cells with the antigen receptor on T_C-cells. The selective expansion and activation of T_H-, T_C-, and B-cells that are specific for the proteins of an infecting virus enables the adaptive immune system to respond to individual agents in a controlled fashion. Figure 1 summarizes the key elements of antiviral immunity in swine, showing the rapid innate response and the later—antigen-specific—adaptive response.

Because of this specificity that is directed to individual proteins and because viruses may express hundreds of proteins, the adaptive immune system is able to respond simultaneously to a wide variety of stimuli. Because the proliferation of antibodies and cytotoxic T-cells requires a week or more to be fully elaborated, it is important that innate immune mechanisms work effectively. However, the great advantage of the adaptive response is that a portion of specific B- and T-cells displaying receptors that recognize the pathogen, circulate as memory cells in blood for long periods of time. In the event of a second infection, these cells mobilize a very rapid and specific response that combines with innate immune mechanisms to provide highly effective protection. Vaccines rely on

Figure 1. Anti-viral immunity in swine—innate responses in the infected cell occur within hours, adaptive responses require days to weeks



this memory aspect of adaptive immunity for their efficacy.

In recent years it has become apparent that antigen-specific helper T-cells can display different patterns of cytokine expression that are associated with two different patterns of resistance. The patterns are known as type I and type II, and the T_H-cells associated with each are known as TH1- and TH2-cells. In numerous studies using the mouse as a model system, it has been shown that type I helper T (TH1) cells secrete interferon-γ (IFN-γ), whereas type II helper T (TH2)-cells secrete IL-4. Type I responses are associated with IFN-γ secretion, macrophage activation, and cytotoxic T-cell activation. These responses are particularly effective against intracellular infections in which the pathogen, including bacteria and viruses, is inaccessible but the infected cell can be recognized and destroyed. For this reason, type I responses are often assumed to be evidence of a successful anti-viral immune response. Type II responses, mediated by TH2-cells, are associated with IL-4 stimulation of B-cell proliferation and differentiation and the production of neutralizing antibodies that bind to and inactivate extracellular bacteria and viruses. Thus, type II responses are particularly effective against free viral particles in the circulation and could play an important role in limiting viral shedding and transmission. The TH1/TH2 paradigm has influenced infectious disease immunology thinking enormously, and stimulated research into development of vaccines that differentially stimulate the type of immunity, which is predicted to most effectively control and eliminate the target pathogen. However, at times it is forgotten that a great many pathogens, including viruses, have both intracellular and extracellular phases in their life cycles, and that induction of efficient means to recognize and destroy all life cycle forms is key to the control, elimination, and prevention of viral infection.

Swine immunologists in general have adopted the TH1/TH2 paradigm for viral disease research even though in many cases they lack the reagents and tools for proper hypothesis testing. A key element of the paradigm is that cytokine expression patterns are determined in individual antigen-specific helper T-cells that have been isolated and expanded in cell culture. In swine, individual antigen-specific helper T-cells cannot be isolated and expanded in cell culture. Therefore, at this time it is not possible to directly test whether or not swine T_H-cells exhibit the same two cytokine expression patterns that murine T_H-cells possess. Instead, cytokine expression profiles are examined in peripheral blood leukocyte cultures, which are a complex mixture of many cell types, only a minority of which are helper T cells, which possess a vast array of antigen specificities. The principal type I cytokine, IFN-γ, can be quantified accurately and reliably in swine, but assays that detect IL-4, the principal type II cytokine, have

not been widely used and the expression of IL-4 in swine lymphocytes has not been characterized yet. Because of the significant limitations in both cell culture and cytokine assay methods in swine, the TH1/TH2 paradigm has not yet helped to understand the porcine adaptive immune response to viral infection.

Throughout much of the history of immunology the immune response to infection has been studied by looking through the window of blood. The response of pigs to viral infection has been inferred from changes in serum antibodies and in peripheral blood lymphocytes. More recently we have learned that the immune response at mucosal surfaces is quite different that the systemic immune response. Antigens that are administered orally and stimulate immune responses in the gut, for example, elicit strong intestinal secretory IgA production and low levels of IgG in blood. Intramuscular or subcutaneous administration of the same antigen elicits high levels of IgG in blood but virtually no secretory IgA antibody. These observations help to explain the difficulty in develop vaccines that provide protection against a variety of mucosal viruses of the respiratory and enteric systems. Those vaccines that are effective tend to induce high levels of neutralizing antibodies that remove extracellular forms of the pathogen from blood. Perhaps protection against cell-associated viruses that infect at mucosal surfaces is more difficult because traditional intramuscular routes of administration fail to produce immunity at the mucosal surface.

The goal of anti-viral vaccination is to induce an adaptive immune response to a specific virus without causing disease. Because antigen-specific adaptive immunity includes a memory response, pigs are protected from a future challenge with the same virus. Vaccination, therefore, is an ideal tool for prevention of infection and disease. Two general approaches are used to induce protective immunity in the absence of disease, inactivation and attenuation. Inactivation is the process in which the virus is killed so that it cannot replicate, but its antigenic nature is preserved. In principle, an inactivated viral preparation is both safe and efficacious. In practice, inactivation frequently results in some loss of antigenicity because protein structure is altered and unique substances that stimulate innate immunity are lost. Also, nonreplicating antigens are not amplified in the pig so that the antigenic mass does not increase after administration. Thus, the level of immunity induced by inactivated viruses is frequently quite low. Adjuvants, substances that stimulate immune responses without being antigenic, are used to overcome low levels of immunity by stimulating innate immune responses and inflammation. The goal of adjuvants is to make a killed organism appear to the immune system as if it were live. Efficacious adjuvants are the same substances, such as double-stranded RNA, that trigger innate anti-bacterial and anti-viral responses. Nevertheless, even with potent adjuvants, the efficacy of inactivated vaccines is usually less than that which is obtained by live forms of the agent.

Attenuation of virulent viruses so that they retain their immunogenic properties without being pathogenic often results in more efficacious vaccines. Avirulent forms of viral pathogens fully possess all the antigenic properties of the virulent form, including stimulation of innate immunity in the appropriate cell and tissue location, induction of cytokines, activation of T-cells and B-cells, biological amplification, and persistence of antigen. Thus attenuated vaccines are expected to provide high levels of immunity for prolonged periods of time. The primary disadvantage of attenuated vaccines is reduced safety relative to killed vaccines.

To achieve the goal of complete safety and complete efficacy great effort has been expended to identify key protective antigens, present them to the immune system in the appropriate immunostimulatory context, and induce the correct immune response, either type I or type II. To date the goal has been difficult to achieve for a variety of reasons. Nevertheless, the increased knowledge of how innate and adaptive immunity function in swine provides a theoretical basis for improving anti-viral vaccine efficacy and safety.

The efficacy of vaccines is related to vaccine dose. Larger amounts of antigen and repeated administrations increase the level and duration of immunity. Similarly, the level of immunity achieved by attenuated vaccines appears to be related to their ability to grow in the host. Host factors also determine the efficacy of vaccines, just as host factors influence the pathogenicity of an infection. Examination of anti-influenza vaccine efficacy in humans has revealed a normal distribution of variation in intensity of immunity such that the large majority of the population has some level of protection, while a small proportion of individuals is highly protected and another small number remain susceptible. In swine it is not always apparent whether cases of "vaccine failure" can be attributed to the vaccine or simply represent the variation in animal immune responsiveness.

The swine industry has changed dramatically in recent decades, especially in the size of swine herds, increased density, confinement rearing, genetic selection, and intensive management to maximize growth and reproductive rates. Changes in health management practices include the widespread use of multivalent vaccines and feed-grade antibiotic growth promotants. The question is whether all of these factors have combined to overburden the immune system, resulting in suboptimal health. The answer to this question is, almost certainly, no. Pigs, like all animals, have always been exposed to a vast assortment of antigens in the environment, including soil, air, feed, and other pigs. As new management strategies cause

pigs to be exposed potentially to new antigens, they also reduce exposure to others. Housing of pigs in confinement may result in constant exposure to airborne antigens, but it also has greatly reduced exposure to soil microbes and enteric parasitic infections. Mixing weaned pigs from numerous farms is likely to cause physiological stress, but stress alone is not an unusual or unhealthy condition. Much more serious is the probability that diseased pigs from one farm will distribute virulent viruses to nonimmune, susceptible pigs. The danger to pig health is due to the management of animals that combines infected animals with susceptible animals, not to changes in the immune system of pigs and its capacity to resist infection and disease.

The application of immunological principles and practices to vaccine development and the improvement of swine health is critically dependent on diagnostics. Viral diseases that are easy to treat and prevent with vaccines and management are no longer significant threats to the swine industry. The viral diseases that remain are of recent origin. The interactions of the causative agent with a pig are not well understood, the pathogens are highly adapted, or they have acquired new characteristics in the pig and are able to subvert immune defenses. Diagnostic methods that assess humoral and T cell immune responses to individual proteins, and that quantitatively measure microbial loads in target tissues of the pig provide essential immunological information to guide the development of control and prevention strategies. Vaccines are a proven, powerful tool in disease prevention and control strategies for swine. Specific and detailed knowledge about immune function and immune responses in pigs to their pathogens is the key to development of safer and more efficacious vaccines.