Review Articles

Negative effects of porcine endemic pathogens on the immune system

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Abstract

The immune system is made of an intricate, yet well-organized, cellular network that operates to protect and defend the host against infections. The successful immune function relies on the proliferation and differentiation of numerous lymphocyte populations, which participate in immune mediated activities, and the healthy lymphoid microenvironments. Thus, any factors affecting the functions of the cells and/or organs of the immune system, can eventually lead to immunosuppression (i.e. secondary immunodeficiency). Several porcine endemic pathogens are known to affect the function(s) of the host immune system, which lead to various patterns of disease complications. Some pathogens possess more than one immunomodulatory activities and can suppress the immune system at different levels. More importantly, the immunosuppressive effects caused by these pathogens are not pathogen-specific. Thus, even the pathogens causing low mortality or subclinical infections with immunosuppressive pathogens can substantially impair the host immune system that resulted in secondary immunodeficiency. This article will discuss the roles of some important porcine endemic pathogen, particularly in the South East Asia region, on an induction and the outcome of the pathogen-induced immunosuppression.

Keywords: immunosuppression, pigs, endemic pathogens, immune system

Introduction: the immune system

The immune system is made of an intricate, yet well-organized, cellular network that operates to protect and defend the host against infections. Sensing of the presence of pathogens (or pathogen-associated molecular patterns) through numerous Pattern Recognition Receptors (PRRs), e.g. Toll like receptors (TLR), Nucleotide Oligomerizing Domain protein (NOD), Scavenger receptors etc., by sentinel cells leads to activation of several innate defense mechanisms in order to activate the early defense mechanisms and phagocytic cells (i.e. dendritic cells, macrophages and neutrophils) in nearby tissue. These include production of a wide range of molecules such as antimicrobial peptides, acute phase proteins, type I interferons (IFNs), chemokines, and pro-inflammatory cytokines (e.g. interleukin (IL)-1, IL-6 and tumor necrosis factor (TNF)-αetc.) by various cells at the infected sites. This so-called innate immunity can be initiated immediately after sensing the danger, i.e. pathogen invasion, and typically leads to 1) an inflammatory reaction, mediated by pro-inflammatory cytokines, at the infected site and 2) departure of mature antigen-presenting cells (APCs), carrying the antigen and co-stimulatory signals required for the activation of antigen-specific lymphocytes, to the draining lymphoid tissues. In the lymphoid tissues, where porcine lymphocytes mainly reside, APCs provide both the antigen and co-stimulatory signals to the specific T-lymphocytes and direct the development of protective specific-immunity, according the information obtained from the APCs. The late adaptive immunity (>96 hr post infection) depends on activation and clonal expansion of the pathogen-specific lymphocytes in secondary lymphoid tissues. Antigen-specific lymphocytes are selected to expand and differentiate either to the effector lymphocytes or, in the later stage, to the long-lived, antigen-specific memory populations. The effector B- and T- lymphocytes initiate humoral and cell-mediated immunity, respectively, during the activation phase of an adaptive immunity. Once the elimination of the pathogens (or threats) is achieved, the effector cells are deleted or functionally suppressed by several mechanisms, including the induction of apoptosis or the production of immunosuppressive cytokines such as IL-10 and/or transforming growth factor (TGF)-β. Subsequently, the immunological activities decline and the body resumes its homeostasis, leaving just the reserved memory populations.

Memory T cells can be broadly divided into central memory and effector memory subsets. The central memory T cells (T_{CM}) mainly reside secondary lymphoid tissues, exhibit limited effector function, but can rapidly proliferate and become effector cells upon antigenic stimulation. Whereas, the effector memory T cells (T_{EM}) home to peripheral tissues, rapidly resume effector function upon antigen re-exposure, with limited proliferative ability. It should be noted that memory T cells can persist for a lifetime in the absence of antigen and even MHC molecules. A noteworthy example is the finding that more than 90% of volunteers vaccinated against smallpox 25-75 years ago maintain viral-specific, cellular immunity, which declines slowly with a half life of 8-15 years (Hammarlund et al. 2003). Interestingly, continual antigenic stimulation induces continuous proliferation and differentiation of all T cells into effector cells, but not the memory populations. The high numbers of effector cells are believed to maintain for some time to mediate effective protection from a challenge. However, this type of cellular differentiation prevents development of the memory T cells (Lanzavecchia & Sallusto 2005). The finding implies that persistent antigenic stimulations by chronic infection may impair the host ability to induce pathogen-specific memory T cell population.

With the help of helper T (Th) cells, antigen-specific B-lymphocytes differentiate into long-lived memory B cells that mostly remain in the lymphoid tissues. Upon the second antigenic stimulation, these memory B cells differentiate into plasmablasts and subsequently long-lived plasma cells. Most of the plasmablasts migrate either to inflamed tissue, or to the bone marrow. In the bone marrow, and to a lesser

degree in secondary lymphoid organs, long-lived plasma cells survive and continuously produce antigen-specific antibodies, providing a lifetime humoral memory (Radbruch et al. 2006).

As discussed above, the complicate functions of the immune system relies on interactions of the cells from different compartments that facilitate selection and expansion of the immune cells. There are, however, some weaknesses in this system. These are due to the vulnerability of the constantly proliferating and differentiating cells that participate in immune mediated activities and the requirement for proper lymphoid microenvironments for activations of the effector cells and/or maintaining of the memory populations. Therefore, any factors affecting the functions of the cells and/or organs of the immune system, can eventually lead to secondary immunodeficiency. Several pathogens are known to induce immunosuppression that leads to various patterns of disease complication in pigs. This article will discuss the roles of some important endemic pathogen, particularly in the South East Asia region, on induction of immunosuppression in pigs.

Pathogen-induced immunosuppression: destruction of the lymphocytes and lymphoid tissues

Healthy lymphocytes and lymphoid tissues are the fundamental requirement for the proper functions of the immune system. Thus, it is not surprised that most of the major immunosuppressive pathogens aim to kill the lymphocytes and destroy the lymphoid tissues. An example of the endemic pathogens causing severe generalized lymphoid destruction is classical swine fever virus (CSFV). During the early phase of CSFV infection, leucopenia, in particularly a decrease in lymphocyte numbers, is the distinctive clinical feature (Susa et al. 1992). Lymphocyte apoptosis and necrosis in the peripheral blood, as well as primary and secondary lymphoid tissues, are the unique pathological characteristics during an early phase of CSFV infection (Pauly et al. 1998; Summerfield et al. 1998; Summerfield et al. 2000; Summerfield et al. 2001). The permanent damage of the lymphoid structures caused by CSFV infection, subsequently leads to complications cause by secondary infections in the infected pigs.

Although, CSFV preferentially replicates in monocytes/macrophages and vascular endothelial cells, viral infection causes severe depletion of the lymphocytes (both B- and T-lymphocytes) from 1-4 days post infection, even before the onset of viraemia and independent of leukocyte infection (Summerfield et al. 2001). The severe changes in lymphoid tissues and circulating lymphocytes suggest the cytopathic effects are not a direct effect of the virus or viral proteins (Le Potier et al. 2006). Recent report suggests that lymphocyte depletion may relate to the induction of "cytokine storm" (Summerfield et al. 2006). During an acute phase of infection, CSFV stimulates natural interferon producing cells (NIPCs), also known as plasmacytoid dendritic cells (pDC), to produce large amount of IFN-α resulting in systemic hypercytokinemia. The anti-proliferative and pro-apoptosis effects of IFN-α are believed to be directly responsible for the depletion of the bystander lymphocytes, as the onset of IFN-α production in infected pigs correlated well to lymphocyte depletion (Summerfield et al. 2006).

During the last decade, there has been an increased incidence of subacute and chronic CSF outbreaks caused by the moderately virulent CSFV. Although the moderately virulent CSFV causes milder clinical symptoms, and the infected pigs may survive for a long period without any obvious clinical signs of CSF (Damrongwatanapokin et al. 2002; Parchariyanon et al. 1999), it should also be emphasized that both highly virulent and moderately virulent CSFV strains comparably induce lymphoid depletion in the infected pigs (Summerfield et al. 2001). This finding implies that, regardless of the clinical outcomes, the degrees of immunological damage are comparable between the 2 CSFV strains. This information highlights the importance of routine monitoring of the herd immune

status, e.g. serosurveillance, and routine pathological examination of the dead animals in the farm situated in the CSFV endemic area.

Apart from CSFV, there are also evidences of pathogen-induced killing of lymphocytes in pigs infected by porcine parvovirus (PPV), Porcine reproductive and respiratory syndrome virus (PRRSV), and Porcine circovirus-2 (PCV-2). The underlined mechanisms of lymphoid depletion by these viruses are different and has been extensively reviewed elsewhere (Charerntantanakul & Roth 2007). Regardless of the viruses, pigs infected with these pathogens demonstrated significantly reduction in the lymphocyte numbers, especially following acute infections. Interestingly, infection of an intracellular bacteria, *Lawsonia intracellularis*, also results in decreased numbers of $CD8^+$ T and B lymphocytes in the infected tissues. The finding suggests that infection with *L. intracellularis* may also lead to secondary immunodeficiency, particularly the immune mechanism against intracellular pathogens at the infected site (MacIntyre et al. 2003).

Unlike the generalized lymphoid depletion of the lymphoid organs observed following CSFV infection, the histopathological lesion of PCV-2 infection is characterized by granulomatous inflammation of several lymphoid tissues (Chae 2004). PCV-2 preferentially replicates in lymphocyte populations, resulting in significant depletion in lymphocyte, but not the monocyte, subpopulation (McCullough et al. 2007). On the other hand, monocytes and macrophages are drawn to the secondary lymphoid tissues, via the induction the viral-induced chemokine; monocyte chemoattractant protein-1 (MCP1) (Kim and Chae 2003). This leads to the permanent damage of the lymphoid tissue structure characterized by severe lymphoid depletion with loss of lymphoid follicles, with infiltrations of epithelioid cells and multinucleated giant cells (Chae 2004). At this stage, it is unlikely that the immune system of the infected host will be able to perform its normal functions. The notion that PCV-2 can cause secondary immunodeficiency has been raised and discussed by several research groups (McCullough et al. 2007; Segales et al. 2004; Zlotowski et al. 2006). Interestingly, an impaired humoral immune response, described by an inability to mount neutralizing antibodies against PCV-2, has been linked to pigs affected with postweaning multisystemic wasting syndrome (PMWS) (Fort et al. 2007).

Pathogen-induced immunosuppression: interfering with innate immunity

Suppression of the interferon system

The type I interferon system is a major innate defense mechanism against viruses. Virus-infected cells synthesize and secrete type I interferons (e.g. IFN-α,-β) which are potent antiviral cytokines and important modulators of the adaptive immune system. The secreted IFNs trigger susceptible cells to establish the"anti-viral state" that limits viral replication and spreading. Type I interferons are induced by viral infection, through the detection of double-stranded RNA (dsRNA), a by-product of viral replication, or by activation of the TLR signaling pathway. At present, there are at least two distinct cellular signaling pathways for detecting of the presence of viruses or viral products. Most cells of the body including fibroblasts, hepatocytes and conventional dendritic cells (cDCs) utilize the so-called classical pathway. During the infection, viral RNAs in the cytoplasm are detected by intracellular sensors (e.g. RIG-I, MDA5, PKR) and activate the main interferon regulatory transcription factors IRF-3 and NF-κB resulted in IFN-β gene expression. In addition, some cells of the haematopoietic systems, for example dendritic cells, recognize viruses and other dsRNA molecules in an endocytic compartment, via TLR3 engagement, and share the downstream classical signaling pathway (reviewed in Weber and Haller 2007). Infected cells secrete mainly IFN-β as an initial response to infection, but switch to IFN-α in the later phase (Marie et al. 1998). In contrast, plasmacytoid dendritic cells (pDCs, also

known as NIPC) use Toll-like receptors (TLRs), in particular TLR7, 8 and 9, for detection of viral genetic materials. TLR signaling of pDCs primarily involves the adaptor protein MyD88 and activates IRF-7 that serves as a master regulator of IFN-α/β gene expression (Honda et al. 2005). Upon activation, pDCs secrete high levels of IFN-α. Regardless of the cellular sources, bindings of the newly synthesized type I IFNs to their receptor on the cellular surface activate expression of several hundreds interferon stimulating genes (ISGs) via the JAK/STAT signaling pathway, resulting in productions of antiviral proteins (e.g. Mx, OAS/RNaseL, ISG20, PKR etc.), proteins related to class I antigen processing and presentation, and cytokines (reviewed in Haller et al. 2006).

As induction of IFN system can significantly inhibit viral growth at an early stage of infection, the viruses have to develop several strategies to cope with the host IFN system. Most of the pathogenic viruses have evolved at least one mechanism to interfere or shut down the interferon response circuit. Numerous evidences have demonstrated that viruses can interfere pathways related to the interferon response circuit at different levels, starting from interference with induction of IFNs, or interferon-activated signaling pathway, and inhibition of the interferon-induced effector proteins (reviewed in Haller et al. 2006; Weber & Haller 2007). Several porcine endemic viruses have also been known to have mechanism(s) to suppress the IFN responses. For example, Leader proteinase (L^{pro}) of foot and mouth disease virus (FMDV), a papain-like proteinase, blocks host cell metabolism by inhibition of host cap-dependent mRNA translation and, therefore, shut down protein synthesis in the host cells (reviewed in Mason et al. 2003). In addition, the L^{pro} protein appears to control the transcription of gene involved in the IFN circuit as well (de Los Santos et al. 2006). The N-terminal protease (N^{pro}) of CSFV inhibits IFN production by induction of proteasome degradation of the IRF3, the main interferon regulatory transcription factors (Bauhofer et al. 2005; Bauhofer et al. 2007; Ruggli et al. 2005). The EP0 protein of pseudorabies virus (PRV) inhibits the IFN signaling pathways, in particular the phosphorylation of STAT1 (Brukman and Enquist 2006; Brukman and Enquist 2006). Recently, it has been shown that PCV-2 infection also results in inhibition of IFNs and tumor necrosis factor (TNF) productions in the infected NIPCs (Vincent et al. 2007). Furthermore, PRRSV can inhibit IFN-α production in infected pigs by an unknown mechanism (Albina et al. 1998; Royaee et al. 2004; Van Reeth et al. 1999). These results imply that active infections of the endemic viruses can significantly impair the host immune system in sensing and fighting against other viral infection.

Destruction and/or Interfering of antigen presenting cells

T lymphocytes, the key players of the cell-mediated immunity, recognize antigenic peptides presented in the context of Major Histocompatibility Complex (MHC) molecules that are expressed on the cellular surface of antigen presenting cells (APCs). During the priming phase, activation of the naïve antigen-specific T lymphocyte population requires both antigen signal and co-stimulatory signal provided by the professional APCs (i.e. dendritic cells, macrophages and B lymphocytes). The signals provided by the professional APCs are essential for clonal expansion of the antigen-specific T cell clones and, more importantly, direct the development of the appropriate protective immunity against the pathogens.

As cells of the monocyte/macrophage lineage are essential for the host immunocompetency, it is not surprised that these cells are targeted for interference and/or destruction by several endemic pathogens. For example, *Actinobacillus pleuropneumoniae* produces a cytotoxin that is toxic for alveolar macrophages (Tarigan et al. 1994). The PRV and PPV have been shown to replicate in monocyte and alveolar macrophages and impair their immune functions. Swine influenza virus (SIV) and PRRSV replicate in alveolar macrophage and lyse the

infected macrophages (reviewed in Roth and Thacker 2006). Recent evidence also demonstrates that PRRSV infection of monocyte-derived DCs resulted in reduced phagocytic activity, antigen presentation ability, and the ability to induce Th1 development (Wang et al. 2007). Thus, PRRSV suppresses the innate immune response in several ways including direct cytolysis, altering the cytokine pattern of macrophages and dendritic cells, suppressing the phagocytic and antigen presentation activities (reviewed in Mateu and Diaz 2007).

Pathogen-induced immunosuppression: interfering with adaptive immunity and immune regulation

T cell suppression

T lymphocytes play important role in governing the immune responses within the infected host. During the activation phase, clonal activation and expansion of the antigen-specific cells are the key events in specific immune responses. Apart from the cytolytic effect on the lymphocyte (see above), there are also other mechanisms that can interfere with an induction of the specific immune responses. Pathogen-induced suppression of T cell activation and proliferation has been reported in PRRSV, CSFV, PPV, PRV and *Mycoplasma hyopneumoniae* infections. The mechanisms of immunosuppression by these pathogens are still unclear (reviewed in Charerntantanakul & Roth 2007).

Interference with host immune regulation and induction of immunosuppressive cytokines

In general, APCs recognize "molecular patterns" of the invading pathogens through their PRRs, and translate the information into the cellular signals that govern the development of the antigen-specific, T lymphocytes. Information provided by the APCs is crucial for the development of adaptive immunity. For example, sensing the presence of virus or bacteria preferentially induce the development of antigen-specific Th1 cells, leading to the induction of cell-mediated immunity. In contrast, intestinal parasitic infection preferentially induces the development of antigen-specific Th2 cells that mediate induction of IgE and other phagocytic independent immune mechanisms. It is also known that both Th1 and Th2 cells can significantly interfere the development and function of the counterpart Th cells. Th1 cells produced IFN-γ to inhibit the differentiation of Th2 cells, while the Th2 cells produce IL-4 to suppress the development of the Th1 cells (Abbas et al. 2000). Theoretically, it will be difficult to induce the proper development of Th1 response in the presence of an overwhelmed Th2 cytokines in the microenvironment. For example, infection with *Ascaris suum* induces a predominant Th2 responses in the infected pigs (Dawson et al. 2005), that significantly compromises the efficacy *M. hyopneumoniae* vaccine (Urban et al. 2007). In some cases, the pathogen preferentially induces of the less effective Th cells, which, in turn, help repressing the development of the protective Th cells. For example, PRRSV and *M. hyopneumoniae* can enhance IL-10 production by the tracheal epithelial cells and could potential shift of the host immune response away from a Th1-type response (Thanawongnuwech et al. 2004).

During the immune response, cytokines play crucial roles in facilitating cellular communications at different levels. As the main purpose of immune activation is to eliminate the invading pathogens and quickly resume the homeostasis, therefore, depending on the phase of immune activation, cytokines can have either positive or negative immunomodulatory effects on the cells of the immune system. Among the vast array of cytokines, IL-10 has been known to have a potent immunosuppressive activity to both innate and adaptive immunity. Interleukin-10 has been reported to suppress pro-inflammatory cytokine production and differentiation of a Th1 response (reviewed in Moore et al. 2001). In pigs, IL-10 also inhibits the TNF-α and IFN-γ, a Th1 cytokine, productions (Charerntantanakul et al. 2006). It is believed that IL-10 plays a significant role in controlling the immunological activities during the late phase of immune responses, when the pathogens are

already cleared. However, premature or prolonged production of IL-10 following pathogen infection can significantly interfere the ability of the host to mount the immune responses against the invading pathogens. In fact, several pathogens induce IL-10 production during their active phase of infection for enhancing their survival within the host (reviewed in Redpath et al. 2001).

Interestingly, PRRSV significantly induces IL-10 production in infected pigs (Chung and Chae 2003; Royaee et al. 2004; Suradhat and Damrongwatanapokin 2003; Van Gucht et al. 2004). Both virulent US and EU genotypes significantly induced IL-10 production by porcine peripheral blood mononuclear cells (PBMCs) (Suradhat and Thanawongnuwech 2003). These findings are consistent by poor innate immune responses (Van Reeth et al. 1999; Van Reeth and Nauwynck 2000), delayed and inefficient specific immune responses (Meier et al. 2003), and declined local lung defenses, leading to secondary bacterial infections that observed in the PRRSV infected pigs (Halbur 1998; Lager and Mengeling 2000). Moreover, PRRSV could significantly interfere with the immune responses to the recall antigen, in particular CSFV, possibly via the viral-induced IL-10 (Suradhat et al. 2003). Immunization against CSFV during an acute phase of PRRSV infection resulted in vaccination failure (Suradhat et al. 2006). The evidence that PRRSV infection interferes the efficacy of vaccines against other pathogens has been reported by several groups of researchers (De Bruin et al. 2000; Li and Yang 2003; Thacker et al. 2000). Interestingly, some strains of modified live PRRSV vaccines also induced IL-10 production in vaccinated pigs (Diaz et al. 2006; Suradhat et al. 2006). The findings from these studies suggest that vaccination during the active PRRSV infection should be avoided. In addition, pigs subclinically infected with PCV-2 developed a transient IL-10 response during the viremic phase of infection (Darwich et al. 2007). In addition, abnormal cytokine productions following antigenic stimulation were observed in PCV-2 infected pigs (Darwich et al. 2003; Darwich et al. 2003; Darwich et al. 2004; Sipos et al. 2004, 2005). The data suggest that PCV-2 infection can impose significant effect on immune regulation within the infected pigs.

Final remarks

Endemic pathogens can directly damage the host immune systems through there cytotoxic activities. Some endemic pathogens, particularly those that persist in the host, have evolved various immunomodulatory mechanisms for their immune evasion. As discussed above, endemic pathogens can suppress the immune system at different levels. Some pathogens possess more than one immunomodulatory activities (Fig. 1). It should be emphasized that the immunosuppressive effects caused by these pathogens are not pathogen-specific. Thus, infections with the immunomodulatory pathogens pose serious threat to the overall health of the animals. Secondary immunodeficiency results in several clinical findings including 1) illness from organisms of normally low pathogenic or from attenuated live vaccine, 2) recurrent illnesses that are usually difficult to control, 3) failure to respond adequate to vaccination, 4) unexplained neonatal illness and death affecting more than 1 animal in a litter, and 5) a variety of disease syndromes occurring concurrently in the herd (Roth and Thacker 2006). Apart from infectious agents, physical or psychological distress, inadequate nutrition and immunotoxic substances are also known to cause secondary immunodeficiency in pigs.

The economic loss from disease outbreaks is usually calculated from the direct loss of animal succumbed to the illness and expenses that are related to prevention and control of the particular disease. Most of the time, it is very difficult to project the loss from secondary immunodeficiency caused by persistently infected pathogens, as the outcome of the immunodeficiency can be varied depending on the pathogen(s) circulated in the farm. It should be emphasized that the degree of immuno-suppression induced by the pathogen may not be correlated to the mortality rate caused by such pathogen.

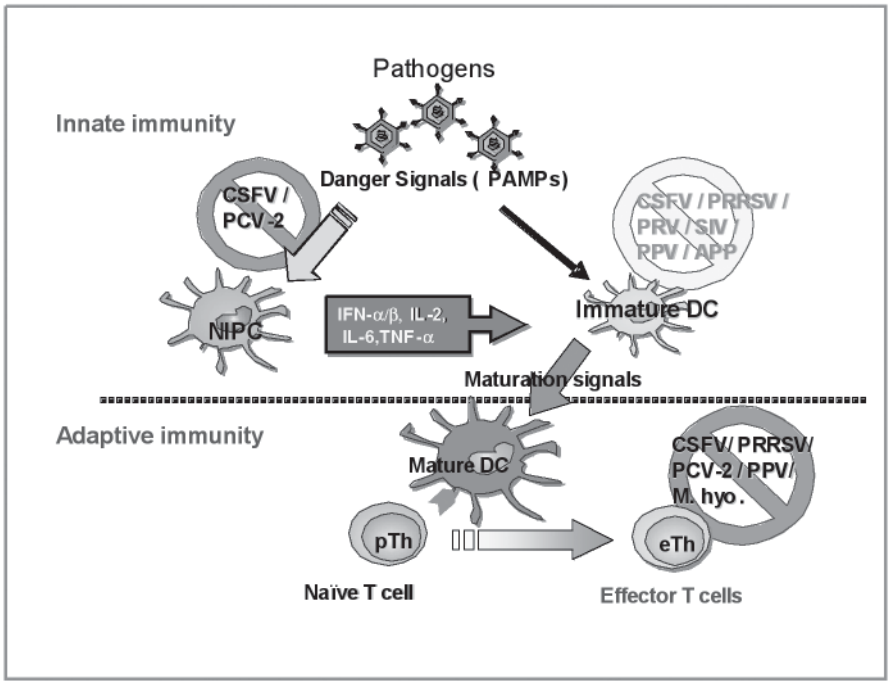

Figure 1 The immunosuppressive mechanisms of porcine endemic pathogens.
Schematic of the interaction between innate and adaptive immune responses and the mechanisms of immunosuppression by the porcine endemic pathogens (adapted from McCullough et al. 2007).

For example, infection of PRRSV, without other complications, generally does not result in high mortality. However, as discussed above, the virus appears to interact and efficiently modulate the host immune system at almost every level. This article argues that even the pathogens causing low mortality or subclinical infections can substantially impair the host immune system. Apart from the direct immunosuppressive effect, persistent infections greatly affect the general performance of the infected animals. This is due to the tight connection between the immune system and the neuroendocrine network. Systemic pro-inflammatory cytokines production has been known to link with an enhanced endogenous corticosteroid production that negatively affects the general performance of the infected animals (reviewed in Suradhat 2006).

As this article has explored the immunosuppressive effects of the important endemic pathogens on the pig immune system, it is perhaps ideal to minimize the pathogen loads and other immunosuppressive factors in the farms. Biosecurity and, in some cases, proper vaccination programs will be crucial for disease control and eradication. When infection does occur, understanding of pathogenesis and the subsequent immunomodulatory effects of the pathogens will be essential for managing the disease outbreak and prevention of the possible undesirable outcomes.

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