Porcine Innate and Adaptative Immune Responses to Influenza and Coronavirus Infections

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ABSTRACT: **Both innate and adaptative immune responses contribute to the control of infectious diseases, including by limiting the spreading of zoonotic diseases from animal reservoirs to humans. Pigs represent an important animal reservoir for influenza virus infection of human populations and are also naturally infected by coronaviruses, an important group of viruses, which includes the recently emerged severe acute respiratory syndrome (SARS) virus. Studies on both innate and adaptative immune responses of pigs to influenza virus and coronaviruses contribute, therefore, to a better control of these infections in their natural hosts and will be briefly reviewed in this article. Pro-inflammatory cytokines, including type I interferon (IFN), tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6), were found in lung secretions of influenza virus infected pigs, and correlated with the intensity of clinical signs, whereas prior vaccination against influenza strongly reduced the production of infectious virus and cytokines in the lungs upon challenge, which was associated with clinical protection. An early type I IFN production was also found in coronavirus infected pigs, including at mucosal sites. IFN induction by coronavirus is shown to involve interaction between a viral glycoprotein and a leukocyte subset, likely equivalent to plasmacytoid dendritic cells, present in the mucosae and associated lymphoid tissues. Given the IFN mediated antiviral and immunomodulatory effects, the use of IFN or IFN inducers may prove an efficient strategy for a better control of influenza virus and coronavirus infections in pigs. Because influenza and coronaviruses target mucosal surfaces, adaptative immune responses have to be characterized at mucosal sites. Thus, nasal and pulmonary antibody responses were analyzed in influenza virus infected or vaccinated pigs showing short-lived, but potentially protective local IgA and IgG antibody (Ab) responses. Interestingly, primary influenza virus infection induced long-lived increase of lung $CD8^+$ T cells and local lymphoproliferative responses. Pigs infected by a respiratory coronavirus (PRCV) showed virus-specific IgG Ab-secreting cells**

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in the bronchial lymph nodes, whereas the transmissible gastroenteritis coronavirus (TGEV) induced more IgA Ab-secreting cells in gut tissues, which illustrates the importance of the route of antigen administration for inducing local immune effector mechanisms. Porcine viral infections provide, therefore, valuable models for evaluating the immune parameters that are important for controlling transmission of important viral zoonotic infections.

KEYWORDS: **immunology; pigs; interferon; influenza; coronavirus**

INTRODUCTION

Eleven of 18 emerging zoonotic infections listed over the last 5 years are caused by viruses, most of them being RNA viruses,[1] including major respiratory viral diseases, such as influenza or the recently emerged severe acute respiratory syndrome (SARS) virus. The easy and rapid airborne transmission of respiratory viruses between the natural host animals and the possibility for transmission from animals to humans point out the need for an efficient control of virus excretion at the site of replication, namely the respiratory tract mucosae. Such mechanisms for control involve therefore local, mucosa-associated, antiviral immune mechanisms.

Animals are both reservoirs and natural hosts of several important zoonotic respiratory viral infections, like influenza virus in birds, pigs, horses,[2] or SARS coronavirus in civets and other animals including pigs.[3–5] Pigs are also naturally infected by several coronaviruses, either respiratory (porcine respiratory coronavirus [PRCV]) or enteric (transmissible gastroenteritis virus, [TGEV]).[6] In addition, pigs show anatomical, physiological, and immunological similarities to humans. Pigs are, therefore, a relevant animal species for studying host responses and immune mechanisms to influenza and coronavirus infections.

The aim of this article is to briefly summarize our current knowledge about porcine immune responses, including innate and adaptative immunity, to both of these viral infections, with a special emphasis on effector mechanisms at mucosal sites.

Antiviral Innate Immune Mechanisms

The innate immune response to viral infections includes cell-mediated effector mechanisms such as natural killer (NK) activity, and soluble effectors among which type I interferons (IFN) play a major role. IFNs are a group of cytokines, initially identified by their ability to induce resistance to viral infection,[7] but also currently recognized as pro-inflammatory molecules and potent modulators of both innate and adaptative immune responses.[8,9] In the course of an experimental influenza infection of pigs, type I IFN is present in

the bronchoalveolar secretions together with tumor necrosis factor-α (TNF-α), and interleukins (IL-1 and IL-6).[10] The IFN response starts within 12 h post inoculation (PI) and lasts for several days. Peak cytokine titers occur within 18–24 h PI and they are correlated with the peak of virus replication, clinical signs, and infiltration of neutrophils in the bronchoalveolar lavage fluids. In addition, the level of lung pro-inflammatory cytokines is correlated with the intensity of clinical signs. Thus, prior vaccination of pigs against influenza strongly reduced the production of infectious virus and cytokines in the lungs upon challenge, which was associated with clinical protection.[11] IFN-α producing leukocytes have been detected in the bronchiolar epithelium of infected pigs, at the peak of virus replication by immunohistochemistry. The IFN-producing cells were found at very low numbers, and in close contact with influenza virus infected cells.[12] An early type I IFN production was also characterized in coronavirus infected pigs, including at mucosal sites. Within 24 h after an experimental respiratory coronavirus infection (PRCV) in pigs, type I IFN started to be produced in the lung secretions, for more than 4 days, in the absence of significant levels of other pro-inflammatory cytokines (TNF-α and IL-1). At the same time, there were few if any clinical signs and lung neutrophil infiltration was much less prominent than during a swine influenza infection, which suggests that lung type I IFN in itself is not involved in pro-inflammatory and harmful effects.[13] The coronavirus TGEV experimental infection in newborn piglets is also characterized by a high and early IFN-α production, in intestinal secretions, and in several other organs.[14,15] This coronavirus experimental infection model has generated original data pertaining to mechanisms of IFN-α induction by viral glycoproteins: thus, only one viral external glycoprotein, gM, was shown to play a major IFN inducing role,[16,17] accordingly, virus-like particles, made of only two TGEV proteins (M and E) and devoid of viral genome, were as effective as native virus to induce IFN-α production by porcine leukocytes.[18] The IFN-α producing cells, referred to as natural interferon producing cells (NIPC)[19] were recently identified as plasmacytoid dendritic cells (PDC), in both humans/mice[20,21] and pigs.[22] PDC are low-density cells, negative for CD11c and lineage markers (CD3, CD19, CD56, and CD14), but positive for MHCII, CD4, and CD123.[23] In the course of coronavirus induced *in vivo* IFN-α production in pigs, NIPC were detected in spleen and secondary lymphoid organs and shared several phenotypic features with PDC.[14,24] Regarding mucosal innate responses, IFN-producing cells were investigated *in situ* by immunohistochemical staining of duodenum, jejunum, ileum, mesenteric lymph node, popliteal lymph node, and spleen cryosections collected from TGEV-infected piglets at the time of highest IFN production. This showed that the vast majority of IFN-α producing cells were located in the small intestine (inside lamina propria and surrounding Peyer's patches) and accumulated in the mesenteric lymph nodes.[14] It was, therefore, concluded that most if not all circulating IFN-α in TGEV-infected piglets originates from gut and mesenteric lymph node. These intestinal IFN-producing cells are in contact

with but distinct from TGEV-antigen positive cells and express MHCII, therefore resembling potential intestinal porcine pDC. Nevertheless, the frequency of intestinal NIPC is very low compared to 'ordinary' DC that are extremely numerous, sometimes filling the whole lamina propria of a villus.[25] One can wonder about their function at such site: their small number makes them unlikely to be a major antigen-presenting DC subset. On the other hand, even a rare intestinal NIPC in mesenteric lymph node will flood the T cell area with IFN-α which is very likely to influence the outcome of the immune response. Contrary to their murine counterpart but in common with humans, porcine NIPC/pDC are the only DC subset able to bind bacterial/viral components via TLR9, TLR7 or yet unknown receptors.[26] Mucosal porcine NIPC/pDC could therefore be preferential targets for using natural ligands of TLR9 (bacterial and viral DNA or CpG-ODN[27]) as immunomodulators and IFN inducers. Given the potent IFN mediated antiviral and immunomodulatory effects,[7] the use of IFN or IFN inducers may prove an efficient strategy[28] for a better control of influenza virus and coronavirus infections in pigs. Besides type I IFN, other antiviral innate immune mechanisms include NK cell activity and both influenza virus and coronavirus were shown to activate porcine NK activity.[29]

Antiviral Adaptative Immune Mechanisms at Respiratory Surfaces

Antiviral adaptative immune mechanisms involve neutralizing antibody (Ab), including secretory IgA at mucosal surfaces, and cytotoxic T lymphocytes (CTL). Because influenza virus and coronaviruses target mucosal surfaces, adaptative immune responses have to be specifically characterized at mucosal sites. Thus, nasal and pulmonary antibody responses were analyzed in influenza virus infected or vaccinated pigs, showing short-lived IgG, but more durable IgA local Ab responses,[30,31] which resulted in protection to virus challenge.[32] Interestingly, primary influenza virus infection in pigs induced local antigen-specific lymphoproliferative responses[33] and a long-lived increase of lung $CD8^+$ T cells which could play a role in the broad-spectrum immune protection to heterotypic virus strains.[34] Pigs infected by a PRCV showed virus-specific IgG Ab-secreting cells and lymphoproliferative responses in the bronchial lymph nodes with low Ab responses in the gut, whereas the TGEV induced more IgA Ab-secreting cells in gut tissues and T cell responses in mesenteric lymph nodes, which illustrates the importance of the route of antigen or vaccine administration for inducing local immune effector mechanisms.[35,36]

These results show that specific antiviral effector mechanisms, including IgG and IgA Ab production, lymphoproliferative responses and $CD8^+$ T cell (presumably CTL) recruitment, are induced at mucosal sites of virus replication following infection and/or vaccination, with either influenza virus or coronavirus, in the pig respiratory tract. Local immunity is more appropriate for

controlling virus spreading and airborne transmission from animals to animals and from animal reservoirs to human targets.

CONCLUSION

In conclusion, pigs are valuable animal models for evaluating the respiratory mucosal immune parameters, and the preventive or therapeutic strategies, that are important for controlling the spreading of zoonotic viral respiratory infections.

REFERENCES

1. VAN DER GIESSEN, J.W.B., L.D. ISKEN & E.W. TIEMERSMA. 2004. Zoonoses in Europe: a risk to public health. RIVM Rapport.
2. PALESE, P. 2004. Influenza: old and new threats. Nat. Med. **10:** S82–S87.
3. CHEN, W. *et al*. 2005. SARS-associated coronavirus transmitted from human to pig. Emerg. Infect. Dis. **11:** 446–448.
4. TU, C. *et al*. 2004. Antibodies to SARS coronavirus in civets. Emerg. Infect. Dis. **10:** 2244–2248.
5. PEIRIS, J.S., Y. GUAN & K.Y. YUEN. 2004. Severe acute respiratory syndrome. Nat. Med. **10:** S88-S97.
6. LAUDE, H., K. VAN REETH & M. PENSAERT. 1993. Porcine respiratory coronavirus: molecular features and virus-host interactions. Vet. Res. **24:** 125–150.
7. PESTKA, S., C.D. KRAUSE & M.R. WALTER. 2004. Interferons, interferon-like cytokines, and their receptors. Immunol. Rev. **202:** 8–32.
8. LE BON, A. & D.F. TOUGH. 2002. Links between innate and adaptive immunity via type I interferon. Curr. Opin. Immunol. **14:** 432–436.
9. TUDOR, D. *et al*. 2001. Type I IFN modulates the immune response induced by DNA vaccination to pseudorabies virus glycoprotein C. Virology **286:** 197–205.
10. VAN REETH, K. 2000. Cytokines in the pathogenesis of influenza. Vet. Microbiol. **74:** 109–116.
11. VAN REETH, K., S. VAN GUCHT & M. PENSAERT. 2002. Correlations between lung proinflammatory cytokine levels, virus replication, and disease after swine influenza virus challenge of vaccination-immune pigs. Viral. Immunol. **15:** 583–594.
12. VAN REETH, K. *et al*. 2001. Characterization of the IFN-a producing cell in the lung of swine influenza virus-infected pigs. Symposium Towards Understanding Microbial Interactions. Louvain-la-Neuve.
13. VAN REETH, K. & H. NAUWYNCK. 2000. Proinflammatory cytokines and viral respiratory disease in pigs. Vet. Res. **31:** 187–213.
14. RIFFAULT, S. *et al*. 2001. Interferon-alpha-producing cells are localized in gut-associated lymphoid tissues in transmissible gastroenteritis virus (TGEV) infected piglets. Vet. Res. **32:** 71–79.
15. LA BONNARDIÈRE, C. & H. LAUDE. 1981. High interferon titer in newborn pig intestine during experimentally induced viral enteritis. Infect. Immun. **32:** 28–31.

16. LAUDE, H. *et al*. 1992. Single amino acid changes in the viral glycoprotein M affect induction of alpha interferon by the coronavirus transmissible gastroenteritis virus. J. Virol. **66:** 743–749.
17. RIFFAULT, S. *et al*. 1997. Reconstituted coronavirus TGEV virosomes lose the virus ability to induce porcine interferon-alpha production. Vet. Res. **28:** 105–114.
18. BAUDOUX, P. *et al*. 1998. Coronavirus pseudoparticles formed with recombinant M and E proteins induce alpha interferon synthesis by leukocytes. J. Virol. **72:** 8636–8643.
19. FITZGERALD-BOCARSLY, P. 1993. Human natural interferon-α producing cells. Pharmacol. Ther. **60:** 39–62.
20. ASSELIN-PATUREL, C. *et al*. 2001. Mouse type I IFN-producing cells are immature APCs with plasmacytoid morphology. Nat. Immunol. **2:** 1144–1150.
21. SIEGAL, F.P. *et al*. 1999. The nature of the principal type I interferon-producing cells in human blood. Science **284:** 1835–1837.
22. SUMMERFIELD, A. *et al*. 2003. Porcine peripheral blood dendritic cells and natural interferon-producing cells. Immunology **110:** 440–449.
23. LIU, Y.J. 2005. IPC: professional type I interferon-producing cells and plasmacytoid dendritic cell precursors. Annu. Rev. Immunol. **23:** 275–306.
24. RIFFAULT, S. *et al*. 1997. In vivo induction of interferon-alpha in pig by non-infectious coronavirus: tissue localization and in situ phenotypic characterization of interferon-alpha-producing cells. J. Gen. Virol. **78:** 2483–2487.
25. HAVERSON, K. & S. RIFFAULT. 2006. Antigen presenting cells in mucosal sites of veterinary species. Vete. Res. **37:** 339–358.
26. GUZYLACK-PIRIOU, L. *et al*. 2004. Type-A CpG oligonucleotides activate exclusively porcine natural interferon-producing cells to secrete interferon-alpha, tumour necrosis factor-alpha and interleukin-12. Immunology. **112:** 28–37.
27. ROTHENFUSSER, S. *et al*. 2002. Plasmacytoid dendritic cells: the key to CpG. Hum. Immunol. **63:** 1111–1119.
28. CHINSANGARAM, J. *et al*. 2003. Novel viral disease control strategy: adenovirus expressing alpha interferon rapidly protects swine from foot-and-mouth disease. J. Virol. **77:** 1621–1625.
29. CHARLEY, B. *et al*. 1983. Myxovirus and coronavirus induced "in vitro" stimulation of spontaneous cell-mediated cytotoxicity by porcine blood leukocytes. Ann. Virol. **134:** 119–126.
30. CHARLEY, B. & G. CORTHIER. 1977. Local immunity in the pig respiratory tract. II. – Relationship of serum and local antibodies. Ann. Microbiol. (Paris) **128B:** 109–119.
31. CORTHIER, G., B. CHARLEY & J. FRANZ. 1980. Local immunity in the pig respiratory tract. III. Immunoenzymatic determination of local immunoglobulin classes sharing anti-influenza activity. Ann. Virol. (Inst. Pasteur) **131:** 355–363.
32. LIM, Y.K. *et al*. 2001. Mucosal vaccination against influenza: protection of pigs immunized with inactivated virus and ether-split vaccine. Jpn J. Vet. Res. **48:** 197–203.
33. CHARLEY, B. 1977. Local immunity in the pig respiratory tract. I. – Cellular and humoral immune responses following swine influenza infection. Ann. Microbiol. (Paris) **128B:** 95–107.
34. HEINEN, P.P., E.A. DE BOER-LUIJTZE & A.T. BIANCHI. 2001. Respiratory and systemic humoral and cellular immune responses of pigs to a heterosubtypic influenza A virus infection. J. Gen. Virol. **82:** 2697–2707.

35. SAIF, L.J., J.L. VAN COTT & T.A. BRIM. 1994. Immunity to transmissible gastroenteritis virus and porcine respiratory coronavirus infections in swine. Vet. Immunol. Immunopathol. **43:** 89–97.
36. BRIM, T.A. *et al*. 1995. Cellular immune responses of pigs after primary inoculation with porcine respiratory coronavirus or transmissible gastroenteritis virus and challenge with transmissible gastroenteritis virus. Vet. Immunol. Immunopathol. **48:** 35–54.