Mini-Review

Neonatal Gnotobiotic Pig Models for Studying Viral Pathogenesis, Immune Responses, and for Vaccine Evaluation

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Abstract | The definition of gnotobiotic pig is a pig in which only certain known strains of virus or bacterium are present through deliberate inoculation with a particular virus strain or bacterial species and being maintained free of unwanted microbiota in sterile isolators. The sterile surgical derivation and gnotobiotic status of the pigs allow studies of the infection, disease and immune responses caused by a specific pathogen in the absence of interfering maternal antibodies, other maternal immune regulators, and intestinal and environmental microbes. Due to the similarities between pigs and humans in terms of genetics, physiology, intestinal anatomy, and immune system, pig is particularly a suitable animal species for the modeling of human enteric pathogen infections, immune responses and for vaccine evaluations. These are the basis for the successful establishment of the infection and disease models for human rotavirus, human norovirus and human enterovirus 71 using neonatal gnotobiotic pigs. The differences between germ-free and normal animals in the maturation status of immune systems caused by the lack of gut microbiota can be minimized by establishing human-gut-microbiota transplanted gnotobiotic pig models. Given the advantages of gnotobiotic pig models, it is expected that they will be used more widely in biomedical research for studies of human viruses and other human diseases.

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In a recent study published in the May issue of the Emerging Microbes and Infections journal (Yang, et al., 2014) we demonstrated that neonatal gnotobiotic (Gn) pigs infected orally or combined oral-nasally with non-pig adapted human enterovirus 71 strain BJ110 (C4 genotype) resulted in the virus shedding pattern, clinical signs, pathology, and immune responses similar to those seen in human patients. Virus shedding in the fecal samples was detected from post-inoculation day (PID) 1 to PID 21. Fever, one of the most common clinical signs in human patients, was induced in the combined oral-nasally infected pigs on PID 4 and PID 6. High titers of neutralizing serum antibodies against EV71, and strong IFN-γ producing CD4+ and CD8+ T cell responses were generated. Although no severe pathology was observed in tissues of intestines, respiratory and central nervous systems, notable respiratory and neurological signs were present in the infected neonatal Gn pigs, especially those infected through combined oral-nasal infection route. In particular, in contrast to other animal models for the human enterovirus 71 infections in mice and monkeys, Gn pigs were infected through the natural route of infection, namely, oral route, by an original human enterovirus 71 strain isolated from human patients and produced the

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clinical signs seen only in mice or monkeys infected through non-natural routes of infections (Zhang, et al., 2011), in immunocompromised animals (Caine, et al., 2013), transgenic animals (Fujii, et al., 2013; Lin, et al., 013), or using adapted virus strains (Wang, et al., 2011; Wang, et al., 2004). Based on our results, we concluded that neonatal Gn pig model for human enterovirus 71 represents an excellent alternative animal model to the current mice and monkey models for virus pathogenesis study and vaccine development.

Pigs are increasingly being used to study various human diseases. Given their similarities to humans in terms of genetics, physiology, intestinal anatomy, and immune system, pigs are particularly good animal species for the modeling of human enteric pathogen infections and immune responses (Meurens, et al., 2012). The sterile surgical derivation and gnotobiotic status of the pigs allow studies of the infection, disease and immune responses caused by a specific enteric pathogen in the absence of interfering maternal antibodies, other maternal immune regulators, and intestinal and environmental microbes. These are the basis for us to successfully establish the pig models for human rotavirus, human norovirus and human enterovirus 71 infection and disease using neonatal gnotobiotic pigs (Bui, et al., 2013; Kocher, et al., 2014; Ward, et al., 1996; Yang, et al., 2014; Yuan, et al., 2002). Gn pigs are excellent model for the study of viral pathogenesis. Due to the similarities of pigs and humans as well as the lack of confounding commensal microbes that are present in all specific pathogen free (SPF) animals, Gn pigs can be used to identify the pathological changes directly as the result of a particular virus infection. Virus replication, spread and the resulting tissue damages and clinical signs can be more accurately recapitulated compared to the SPF animal models. The mechanisms of the host responses and the pathology associated with the virus infection can also be more clearly identified. Thus, Gn pigs are ideal for the identification of causative agents of viral diseases and mechanisms of viral pathogenesis.

The results from Gn pig models are generally comparable to SPF animal models, even though some discrepancies do exist. The presence of certain microbial species in the intestinal environment may impact the infectivity of viruses. We found that human rotavirus replicates to higher titers in Gn pigs colonized with *Lactobacillus acidophilus and L. reuteri* compared to germ-free pigs (Zhang, et al., 2008a; 2008b). Several other studies have also shown that gut microbiota enhanced the replication or virus entry of enteric viruses and their pathogenesis (Kane, et al., 2011; Kuss, et al., 2011; Uchiyama, et al., 2014) and that elimination of microbiota delayed rotavirus infection and significantly reduced rotavirus infectivity in mice (Uchiyama, et al., 2014). On the other hand, certain species of the normal gut microbes can be protective against virus-induced tissue damages and clinical signs. *Lactobacillus rhamnosus* GG (LGG) has been found to reduce human rotavirus diarrhea in Gn pigs by protecting against virus induced tissue injury in the intestine (Liu, et al., 2013).

Discrepancies between the Gn pigs and SPF animal models mainly results from the lack of gut microbiota. It is well known that gut microbiota interact with host immune system and modulate their responses to viral infections. Such discrepancies can be minimized by establishing human gut microbiota (HGM) transplanted Gn pig models (Wen, et al., 2014). Similar to rotavirus infection of the *Lactobacillus* spp monoassociated Gn pigs, HGM Gn pigs shed higher titer of the virus after human rotavirus inoculation compared to the germ-free pigs (Wen, et al., 2014). Because gut microbiota play significant roles in shaping neonatal immune system and host susceptibility to enteric pathogens (Chung, et al., 2012), HGM transplanted Gn pigs may be a better animal model for certain human infectious diseases than germ-free and SPF animal models.

Gn pigs are particularly suited for the study of specific immune responses to virus stimulus. Gn pigs have been widely used to study immune responses to a variety of enteric viruses, including rotavirus (Azevedo, et al., 2012; Liu, et al., 2014; Nguyen, et al., 2003; Nguyen, et al., 2006a; Nguyen, et al., 2006b, Parreno, et a., 1999, Wen, et al., 2009; Wen et al., 2012a; 2012b; Wen, et al., 2014; Yuan, et al., 2005; 2004a; 2001; 2004b; 2002; 2008; 2013; Zhang, et al., 2008a; 2008b; 2008c) norovirus (Kocher, et al., 2014; Souza, et al., 2007), and enterovirus 71 (Yang, et al., 2014). These models have been extremely useful in understanding both innate and adaptive immune responses and for evaluations of the immunogenicity and protective efficacy of various vaccine formulations, adjuvants and immunization routes. Again, HGM transplanted Gn pigs can be used to introduce the effects of gut microbiota on the host immune responses to viruses and vaccines. Using HGM Gn pigs, our previous study

has shown that transplanted human gut bacteria can modulate host immune responses to human rotavirus infection and vaccination (Wen, et al., 2014).

In conclusion, given the advantages of Gn pig models, it is expected that Gn pigs will be used more widely for the study of viral pathogenesis and immune responses that are specific to a virus. Similarly, Gn pigs can also be used to study pathogenesis or immune responses to other types of stimulus, be it microbes, chemical, or physical. Currently, the applications of Gn pigs in modeling human diseases are limited by the cost and relative lack of research infrastructure and reagents. However, these limitations are becoming less prominent as the scientific community is increasingly interested in the Gn pig models and these models become more commonly used in biomedical researches.

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