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COMPARATIVE STUDIES OF THE PATHOGENESIS, ANTIBODY IMMUNE RESPONSES, AND HOMOLOGOUS PROTECTION TO PORCINE AND HUMAN ROTAVIRUSES IN GNOTOBIOTIC PIGLETS

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SUMMARY

Gnotobiotic piglets serve as a useful animal model for studies of rotavirus pathogenesis and immunity. An advantage over laboratory animal models is the prolonged susceptibility of piglets to rotavirus-induced disease, permitting an analysis of cross-protection and active immunity. Studies from our laboratory of the pathogenesis of human rotavirus infections in gnotobiotic piglets have confirmed that villous atrophy is induced in piglets given virulent but not attenuated human rotavirus (Wa strain) and have revealed that factors other than villous atrophy may contribute to the early diarrhea induced. To facilitate and improve rotavirus vaccination strategies, it is important to identify correlates of protective immunity. Comparison of antibody immune responses induced by infection with virulent porcine and human rotaviruses (mimic host response to natural infection) with those induced by live attenuated human rotavirus (mimic attenuated oral vaccines) in the context of homotypic protection has permitted an analysis of correlates of protective immunity. Our results indicate that the magnitude of the immune response is greatest in lymphoid tissues adjacent to the site of viral replication (small intestine). Secondly there was a direct association between the degree of protection induced and the level of the intestinal immune response, with primary exposure to virulent rotaviruses inducing significantly higher numbers of IgA ASC and complete protection against challenge. These studies thus have established basic parameters related to immune protection in the piglet model of rotavirus-induced disease, verifying the usefulness of this model to apply new strategies for the design and improvement of rotavirus vaccines.

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INTRODUCTION

Group A rotaviruses cause diarrheal infections in young pigs and in infants and young children worldwide (Bohl et al, 1984; Hoshino and Kapikian, 1994; Theil et al, 1978). Vaccine development has focused on the use of live attenuated oral vaccines (Hoblet et al, 1986; Mebus et al, 1973), and in humans a "Jennerian" approach using heterologous animal strains or human/animal reassortants as candidate vaccines is being investigated (Hoshino and Kapikian, 1995; Kapikian and Chanock, 1990). Unfortunately, these candidate oral vaccines have often failed in various aspects of safety, immunogenicity or efficacy in clinical trials (DeLeeuw et al, 1980; Hoblet et al, 1986; Kapikian and Chanock, 1990).

The use of animal models can facilitate and improve vaccine development by providing a more comprehensive understanding of rotavirus pathogenesis and mucosal immunity to rotaviruses. Although laboratory animals (mice and rabbits) serve as useful models for evaluating immune responses to rotaviruses, especially using host-specific strains, these animal models are not conducive for studies of active immunity to clinical infections because older mice and rabbits are refractory to rotavirus disease (Conner et al, 1991; Ramig, 1988). Moreover human rotavirus infections in these species are usually subclinical (Conner et al, 1991; Ramig, 1988). In comparison, gnotobiotic piglets remain susceptible to infection and disease induced by virulent porcine and several human rotavirus strains for up to at least 6 weeks of age (Saif, LJ, unpublished). Other advantages of the gnotobiotic piglet model include: 1) as monogastrics, they closely resemble humans in gastrointestinal physiology and mucosal immune development (Kim, 1975; Phillips and Tumbleson, 1986); 2) the placenta of pigs acts as a barrier to the transfer of maternal antibodies; hence colostrum-deprived gnotobiotic pigs are devoid of rotavirus maternal antibodies but are immunocompetent at birth (Kim, 1975); and 3) the derivation and maintenance of piglets in a gnotobiotic environment assures that exposure to extraneous rotaviruses or other enteric pathogens is eliminated as a confounding variable.

The Pathogenesis of Group A Rotaviruses in Neonatal Gnotobiotic Piglets

In spite of the host-specificity of rotaviruses, researchers have found that gnotobiotic piglets are susceptible to infection and disease by heterologous rotaviruses, including human rotaviruses, under experimental conditions (Mebus et al, 1973; Middleton et al, 1975, Steel and Torres-Medina, 1984; Torres-Medina et al, 1976; Wyatt et al, 1980). This observation agrees with antigenic and genetic data showing a close relationship between certain human and animal rotaviruses, suggesting that interspecies transmission of rotaviruses may occur under certain poorly defined circumstances in nature (Urasawa et al, 1992).

The pathogenesis of virulent and attenuated strains of porcine and human rotaviruses has been studied in gnotobiotic pigs in our facility and comparative data is summarized (Table 1) (Chen et al, 1995; Hoshino et al, 1988, 1995; Saif et al, 1995; Theil et al, 1978; Ward, Yuan, Rosen, and Saif, unpublished).

Piglets orally inoculated with 10^5–10^6 focus-forming units (FFU) of virulent (pig-passaged) Wa rotavirus or 10% intestinal content suspensions of virulent OSU or SB1A rotavirus developed diarrhea within 12–18 post-inoculation (PI) hours which correlated with the presence of rotavirus antigen within villous epithelial cells. Moderate (Wa-inoculated pigs) or severe (OSU-inoculated pigs) villous atrophy was observed at PI hours

Table 1. Comparison of clinical signs, lesions, virus detection and seroconversion after oral inoculation and challenge of gnotobiotic piglets with OSU or SB1A porcine or Wa human rotavirus

	Primary inoculation[a]					Homologous challenge[a]	
Primary virus inoculum[a] (strain)	Diarrhea	Fecal virus shedding	Viral antigen in gut	Villous atrophy	Sero-conversion	Diarrhea	Virus shedding
Porcine							
Virulent (SB1A,OSU)	Yes	Yes	Yes	Yes	Yes	No	No
Attenuated (OSU)	Slight	Yes	NT[b]	NTb	Yes	No	No
Human							
Virulent (Wa)	Yes	Yes	Yes	Yes	Yes	No	No
Attenuated (Wa)	No	No[c]	No	No	Yes[c]	Partial[d]	Partial[d]
None	No	No	No	No	No	Yes	Yes

[a]n = 5-18 piglets used for analysis of each response; piglets were orally inoculated with virulent, attenuated or no rotavirus (controls) and orally challenged with the homologous virulent OSU SB1A or Wa rotavirus at post-inoculation day (PID) 14-22.

[b]NT = not tested.

[c]Only 6% of piglets shed virus but 96% seroconverted after inoculation with attenuated Wa rotavirus.

[d]56% of piglets developed diarrhea (compared to 83% of controls) and 81% shed virus (compared to 100% of controls) after virulent Wa rotavirus challenge.

24–72 coincident with the peak of virus replication, and was most pronounced in the caudal small intestine. Diarrhea and rotavirus shedding persisted between 4 to 7 PI days (PID). Recovery correlated with the presence of morphologically normal villi by PID 7. Thus the Wa human rotavirus induced lesions in gnotobiotic pigs, similar, but somewhat less severe than ones seen after infection with the host-specific OSU or SB1A porcine rotaviruses. The diarrhea induced (by PI hour 13) in the Wa rotavirus-inoculated pigs preceded the detection of villous atrophy, suggesting that factors other than villous atrophy may contribute to this early diarrhea.

No histologic changes were observed in the stomach, MLN, colon, kidney, liver, lung or spleen of the rotavirus-inoculated or control pigs. However rotaviral antigen was observed in the colon and MLN of the virulent OSU and Wa rotavirus-inoculated pigs. Piglets inoculated with attenuated (cell-passaged) strains of OSU or Wa rotavirus showed only slight or no diarrhea, respectively (Table 1) (Bohl et al, 1984; Saif et al, 1995; Ward, et al, unpublished; Yuan et al, 1995). Whereas fecal shedding of rotavirus was detected in most pigs inoculated with attenuated OSU rotavirus, only 6% of pigs inoculated with attenuated Wa rotavirus shed detectable virus in feces. Viral antigens were not detected in the intestine or any other tissues of the attenuated Wa rotavirus-inoculated pigs or controls, nor was villous atrophy evident in any of these pigs (Table 1).

Active Immunity to Human and Porcine Rotaviruses in a Gnotobiotic Piglet Model of Disease

Previous studies of porcine rotavirus infections in gnotobiotic piglets confirmed that rotaviruses which share common VP4 (P) and VP7 (G) serotypes induced a high degree or complete cross-protection against challenge with rotavirus strains bearing the common P or G types (Bohl et al, 1984; Chen et al, 1995; Hoshino et al, 1988). Little or no cross-protection was evident in the piglets inoculated and challenged with heterotypic (in both G and P type) serotypes (Bohl, 1984). We have expanded these studies to identify and compare correlates of homotypic (common G and P types) protection in the gnotobiotic piglet model of porcine and human rotavirus-induced diarrhea (Saif et al, 1995; Yuan et al, 1995). In these studies, 3- to 5-day-old piglets were orally inoculated with the virulent (stool-passaged) SB1A porcine or Wa human rotavirus or the attenuated Wa human rotavirus (cell culture-passaged) and challenged at ~PID 21 with the homologous virulent SB1A or Wa rotavirus. These viruses were selected to mimic natural infection with virulent rotavirus or oral inoculation with a live attenuated Wa rotavirus vaccine. Piglets were examined for clinical signs and rotavirus shedding by ELISA (Hoblet et al, 1986) and cell culture immunofluorescence assays (Bohl et al, 1984) after inoculation and challenge and intestinal lesions were evaluated in selected pigs (Ward et al, unpublished; Table 1). Correlates of protective immunity were determined by ELISPOT [Chen et al, 1995; Saif et al, 1995; VanCott et al, 1994; Yuan et al, 1995, (B cell responses) using intestinal (gut lamina propria; mesenteric lymph node) and systemic (blood; spleen) lymphoid tissues collected at various PID (Table 2).

Piglets inoculated with virulent SB1A or Wa rotavirus developed diarrhea and villous atrophy within 24–72 PI hours (Chen et al, 1995; Saif et al, 1995; VanCott et al, 1994; Yuan et al, 1995, Ward et al, unpublished, Table 1). All piglets shed virus in feces and seroconverted with neutralizing antibodies to the homologous rotavirus. Upon challenge with the homologous virulent SB1A or Wa rotavirus, all piglets were protected from virus shedding and severe to moderate diarrhea. Piglets given attenuated Wa rotavirus did not develop diarrhea or villous atrophy (Table 1). Fecal shedding was detected in only 6%

Table 2. Comparison of peak ASC responses to SB1A porcine and Wa human rotavirus in intestinal lamina propria and spleen lymphoid tissues of gnotobiotic pigs at ~21 days after oral inoculation

Virus inoculum (Strain)[a]	Intestinal lamina propria			Spleen		
	Mean (±SEM)[b] No. ASC[b]/5 X 10^5 MNC[b]			Mean (±SEM[b]) No. ASC[b]/5 X 10^5MNC[b]		
	IgG	IgA	IgG/IgA[c]	IgG	IgA	IgG/IgA[c]
Porcine						
Virulent (SB1A)	13(±6)	73(±30)	0.2	9(±6)	6(±5)	1.5
Human						
Virulent (Wa)	64(±26)	53*[d](±28)	1.2	3(±3)	4(±5)	0.8
Attenuated (Wa)	41(±26)	6*(±4)	6.8	2(±2)	1(±0.7)	2.0

[a]Gnotobiotic piglets were orally inoculated with virulent or attenuated Wa human rotavirus or virulent SB1A porcine rotavirus at 3-5 days of age.

[b]SEM = standard error of the mean; ASC = antibody secreting cells; MNC = mononuclear cells.

[c]IgG/IgA = ratio of rotavirus-specific IgG ASC to IgA ASC based on mean numbers of ASC per 5 X 10^5 MNC.

[d]"*" denotes significantly different ($p<0.05$) numbers of ASC between the virulent and attenuated Wa rotavirus-inoculated groups.

of the pigs, but 96% of the pigs seroconverted to Wa rotavirus. However, piglets were only partially protected from diarrhea (56% with diarrhea) and virus shedding (81% shed virus) after challenge exposure.

Assessment of the immune responses in these pigs revealed that the highest numbers of antibody secreting cells (ASC) were in intestinal tissues (adjacent to the site of rotaviral replication) of the rotavirus-inoculated pigs (Table 2) (Chen et al, 1995; Saif et al, 1995; Yuan et al, 1995). The numbers of ASC in the intestinal LP were 1.4–21-fold higher than ASC numbers in the spleen at challenge (PID 21). The numbers of IgA ASC were significantly higher ($p<0.05$) at PID 21 in the intestinal lymphoid tissues of the virulent-Wa rotavirus-inoculated pigs compared to the attenuated Wa rotavirus-inoculated pigs (Table 2). Moreover the mean IgG/IgA ratios were lower in the lymphoid tissues of the virulent rotavirus-inoculated pigs, than in the attenuated Wa rotavirus-inoculated pigs, reflecting the greater predominance of IgG ASC in the latter group of pigs. Pigs infected with virulent SB1A porcine rotavirus had slightly greater numbers of IgA ASC in the intestinal lamina propria, and proportionately fewer IgG ASC compared to pigs infected with virulent Wa human rotavirus. After challenge of the virulent Wa rotavirus-inoculated pigs, only transient low (≤2 fold) or no increases occurred in IgA and IgG ASC numbers reflecting the limited viral replication and antigenic stimulation which coincided with complete protection (Saif et al, 1995; Yuan et al, 1995). The significantly lower numbers of IgA ASC seen in the attenuated Wa rotavirus-inoculated pigs at challenge exposure (PID 21) correlated with induction of only partial protection against diarrhea and virus shedding after challenge (Table 1). Furthermore, these pigs developed greatly increased ASC numbers (6–7-fold) after challenge, consistent with virus infection (Saif et al, 1995; Yuan et al, 1995). Thus it appears that the magnitude of the immune response is greatest in lymphoid tissues adjacent to the site of rotavirus infection and tissue damage (small intestine) and that the level (and antibody isotype) of the local antibody immune response correlate with the degree of protection induced.

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