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COMPARATIVE PATHOGENESIS OF ENTERIC VIRAL INFECTIONS OF SWINE

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1. SUMMARY

At least 11 enteric viruses belonging to 6 distinct families (*Adenoviridae*, *Astroviridae*, *Caliciviridae*, *Coronaviridae*, *Parvoviridae*, and *Reoviridae*) cause diarrhea in swine mainly during the nursing and immediate post-weaning period. Most infect the small intestinal enterocytes, inducing various degrees of villous atrophy and subsequently a malabsorptive, maldigestive diarrhea. In addition rotaviruses possess an enterotoxin (NSP4) which induces a secretory diarrhea in mice. These viruses have distinct predilections for different vertical (villus/crypt) and horizontal (duodenum, jejunum, ileum, colon) replication sites in the intestine and the diarrhea intensity is often related to the extent of viral replication at these sites. In addition concurrent infections with multiple enteric viruses can produce synergistic or additive effects leading to more extensive villous atrophy throughout the intestine and more severe and prolonged diarrhea. Knowledge of enteric viral replication sites and comparative mechanisms of diarrhea induction may lead to new or improved vaccine strategies or therapeutic approaches for the prevention or treatment of these viral diarrheas.

2. INTRODUCTION

Eleven enteropathogenic viruses belonging to 6 families are associated with diarrheal infections of swine as summarized in Table 1. Most have been identified only within the past 2 decades and some, such as a porcine torovirus (Kroneman et al., 1998) and a porcine enteric parvovirus (Yasuhara et al., 1995), have only recently been further characterized following their initial detection (Scott et al., 1987; Yasuhara et al., 1989).

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Multiple serogroups (A,B,C,E) of porcine rotaviruses exist with multiple serotypes within each serogroup (A,C). Distinct serogroups and serotypes do not elicit cross-protection (Theil, 1990; Paul and Stevenson, 1992; Saif and Jiang, 1992; Saif, Rosen, and Parwani, 1994). Multiple serotypes also exist for porcine adenoviruses, but it is unclear if any cause diarrhea other than porcine adenovirus type 3 (Coussement et al., 1981; Benfield, 1990). The existence of multiple serogroups/serotypes complicates diagnosis and vaccine strategies by necessitating serogroup specific reagents and inclusion of multiple serogroups/serotypes for effective vaccines. This question of antigenic diversity has not been adequately explored for the other swine enteric viruses (caliciviruses, astroviruses, enteric parvoviruses, and porcine toroviruses).

Both enveloped and non-enveloped viruses replicate in the intestine and cause diarrhea. Interestingly, the enveloped enteric viruses also infect the upper respiratory tract to various degrees which may contribute to their pathogenesis by increasing the viral dose swallowed (Saif and Wesley, 1992). In addition both adenovirus (Coussement et al., 1981) and porcine enteric parvovirus (Yasuhara et al., 1995) infect the respiratory tract; the latter virus was also isolated from multiple organs after intranasal but not oral administration confirming its ability to also induce systemic infections.

These enteric viruses possess unique characteristics related to their intestinal tropism and replication (Saif, 1990). They are stable to low pH and proteolytic enzymes, factors important for their replication and survival in the intestine and their eventual adaptation to passage in cell culture (Saif, 1990; Theil, 1990). In fact, the porcine enteric calicivirus, the only enteric calicivirus adapted to cell culture, remains refractory to serial passage in cell culture unless the culture medium is supplemented with intestinal contents from uninfected gnotobiotic pigs (Flynn, Saif, and Moorhead et al., 1988). Most of the enteric viruses are heat labile, which may explain the prevalence of viral diarrheas during winter. Whereas coronaviruses and toroviruses are enveloped and sensitive to inactivation by common disinfectants, the other enteric viruses are non-enveloped and highly resistant to many disinfectants and environmental conditions.

The enteric viruses described in Table 1 commonly occur as enzootics in seropositive herds, most frequently in 2–3-week-old pigs and within 1–2 weeks post weaning (Saif, 1990). However, transmissible gastroenteritis virus (TGEV) and porcine epidemic diarrhea virus (PEDV) also occur as epizootics causing diarrhea in swine of all ages, but with the most severe disease and diarrhea mortality occurring in neonates (Pensaert and deBouck, 1978; Pensaert, 1992; Saif and Wesley, 1992). Because group B and C rotaviruses are less widespread than group A rotaviruses based on seroprevalence studies, they may also cause epizootic infections (Saif and Jiang, 1994). The epidemiology of TGEV infections in Europe consists mainly of enzootic infections since the appearance of the TGEV deletion mutant, PRCV; however, in North America, widespread epizootic outbreaks of TGEV continue (Saif and Wesley, 1992).

For viral diarrheas, the incubation periods are usually short, the viruses are excreted in feces in large numbers and spread to susceptible pigs via the fecal-oral route or possibly aerosols occurs rapidly. Systemic infections are generally not reported for these enteric viruses with the exception of enteric parvovirus (Yasuhara et al., 1995). Thus the localized nature of most infections is of major consideration for the design of effective vaccines and intervention strategies. One possible explanation for the localized nature of these enteric viral infections was suggested from recent in vitro studies of TGEV using polarized epithelial cells (Rossen et al., 1996). TGEV entered and exited these cells via the apical surface; in comparison, a murine coronavirus associated with systemic infections entered the same cells apically, but exited basolaterally. Whether similar effects occur in vivo in the intestine, and may account for enteric virus

Table 1. Characteristics of porcine enteropathogenic viruses

Family/Virus	Size	Nucleic acid	Discovery	
			Year	Investigator*
Enveloped				
Coronaviridae/				
Transmissible gastroenteritis virus (TGEV)	60–220 nm	ssRNA	1946	Doyle & Hutchings
Porcine epidemic diarrhea virus (PEDV)	60–220 nm	ssRNA	1978	Pensaert & Debouck and Chasey & Cartwright
Porcine torovirus	70–240 nm	ssRNA	1987	Scott et al.
Nonenveloped				
Reoviridae/				
Rotavirus (group A)	55–70 nm	dsRNA	1975	Rodger et al.
Rotavirus (group B)	55–70 nm	dsRNA	1980	Bridger et al.
Rotavirus (group C)	55–70 nm	dsRNA	1980	Saif et al.
Rotavirus (group E)	55–70 nm	dsRNA	1986	Chasey et al.
Caliciviridae/				
Calicivirus	30–40 nm	ssRNA	1980	Bridger et al., Saif et al.
Astroviridae/				
Astrovirus	28–30 nm	ssRNA	1980	Bridger et al., Saif et al.
Adenoviridae/				
Adenovirus	70–90 nm	DNA	1981	Coussement et al.
Parvoviridae/				
Enteric Parvovirus	18–26 nm	DNA	1989	Yasuhara et al.

*See references for literature citations.

localization in villous or crypt enterocytes or systemic spread, remains speculative. Although viruses that cause localized infections of villous enterocytes do so via the luminal surface, viruses that infect primarily crypt enterocytes (parvovirus) may do so only after systemic infection followed by hematogenous (or via infected lymphoid cells) dissemination of virus to the basolateral surface of crypts.

3. COMPARATIVE PATHOGENESIS OF THE PORCINE ENTEROPATHOGENIC VIRUSES

The enteric viruses have predilections for replication in distinct vertical and longitudinal regions of the small intestine and the lesions induced are most pronounced at these sites (Table 2, Saif, 1990). Moon (1978, 1994) and Saif (1990) drew a corollary between the extent of viral replication vertically in villous enterocytes and the severity of enteric viral infections as summarized in Table 2. For example, TGEV infects and destroys absorptive cells in multiple stages of differentiation along the entire villus causing pronounced villous atrophy and often fatal diarrhea. Rotaviruses and astroviruses infect the distal tips to two-thirds of the villus, causing less severe villous atrophy

and diarrhea. Group B rotaviruses infect cells on the villus tips and induce syncytia, a pathognomonic lesion distinctive of group B rotaviruses (Saif and Jiang, 1994). Adenoviruses, PEDV and caliciviruses infect enterocytes at the base and sides of the villus inducing moderate villous atrophy and diarrhea (Benfield, 1990; Bridger, 1990; Coussement et al., 1981; Flynn, Saif, and Moorhead, 1988; Pensaert, 1992). Enteric parvoviruses infect crypt enterocytes inducing severe villous atrophy, mucosal collapse, and severe hemorrhagic and often fatal diarrhea (Yasuhara, 1995).

Longitudinal segmentation of enteric viral replication sites and lesions also occurs in the intestine (Table 2, Saif, 1990). Generally, viruses that infect only limited, segmental portions of the intestine or restricted numbers of enterocytes cause mild or no villous atrophy, and diarrhea. An example is astrovirus which infects few enterocytes and causes mild or no villous atrophy and diarrhea in pigs (Saif et al., 1980; Bridger, 1990). In comparison, TGEV produces an almost continuous infection of whole villi throughout the entire small intestine which results in severe villous atrophy and often fatal diarrhea (Moon, 1994; Pensaert et al., 1970; Saif and Wesley, 1992). Other viruses produce intermediate degrees of villous atrophy and diarrhea intensity. Rotaviruses (groups A to C) generally replicate and cause villous atrophy in the distal small intestine, but usually not the colon (Paul and Stevenson, 1992; Saif and Jiang, 1994; Saif, Rosen, and Parwani, 1994; Theil, 1990). Groups A and C rotaviruses infect a higher percentage of cells than group B rotaviruses which produce scattered foci of infection in the villous tips of the distal small intestine (Saif and Jiang, 1994). Adenoviruses, PEDV and enteric parvovirus infections and lesions are restricted mainly to the jejunum and ileum, but PEDV also infects the colon (Benfield, 1990; Coussement et al., 1981; Pensaert, 1992; Yasuhara et al., 1995). In contrast to all other enteric viruses, calicivirus infections, and lesions occur mainly in the proximal small intestine (Bridger, 1990; Flynn, Saif, and Moorhead, 1988).

All the swine enteric viruses produce cytolytic infections of enterocytes leading to varying degrees of villous atrophy, crypt hyperplasia, and frequently villous fusion (Moon, 1978; 1994; Saif, 1990). Villus loss and fusion lead to reduced absorptive capacity in the small intestine and a malabsorptive, maldigestive diarrhea accompanied by dehydration and death in severe cases. Maldigestion results from loss of the digestive enzymes produced by the absorptive villous enterocytes; loss of the glucose coupled sodium transport mechanism results in malabsorption of nutrients (Argenzio, 1997; Moon, 1978; 1994). Replacement of absorptive cells by the incompletely differentiated crypt cells, which retain their secretory capacity, and the ensuing crypt hyperplasia, also lead to increased secretion in the intestine, further accentuating the diarrhea (Argenzio, 1997; Moon, 1978; 1994).

Although, it is well-established that enteric bacterial infections such as enterotoxigenic *Escherichia coli* (ETEC) induce a secretory diarrhea in pigs mediated by secretion of enterotoxins (Argenzio, 1997; Moon, 1978), recent evidence indicates that rotaviruses also possess an enterotoxin, the nonstructural protein NSP4 (Ball et al., 1996). The NSP4 and its synthetic peptides induced an age and dose-dependent diarrhea response in young rodents. Evidence indicates that NSP4 activates a signal transduction pathway that increases intracellular calcium and promotes chloride secretion (Dong et al., 1997). Although the enterotoxin potential of rotavirus NSP4 has not been confirmed in pigs, if documented, it could explain the early diarrhea seen in rotavirus-infected pigs prior to the detection of villous atrophy (Saif, Rosen, and Parwani, 1994; Theil, 1990). Furthermore, virulent and attenuated pairs of porcine rotaviruses (OSU, Gottfried) differ in their NSP4 nucleotide sequence (Zhang et al., 1998). Whereas NSP4

Table 2. The vertical and longitudinal sites of replication and villous atrophy in the intestine associated with infection by porcine enteropathogenic viruses

		Vertical			Longitudinal		Villous atrophy Small intestine	
Virus	Diarrhea	Villus	(Site)	Crypt	Small intestine	Colon	Site	Extent
I. *Infect villous enterocytes*								
Coronavirus–TGEV	Severe	+	Entire	–	D,J,I	–	D,J,I	Severe
Rotavirus								
Group A	Mild/severe	+	Entire	–	J,I	±	J,I	Moderate-Severe
Group B	Mild	+	(Tips)	–	D,J,I	–	D,J,I (syncytia)	Mild
Group C	Mild/severe	+		–	J,I	–	J	Mild/severe
Group E	Mild	+		–	?	–	?	?
SRSV								
Astrovirus	Mild/None	+	(Dome)	–	D,J,I	None	–	
Calicivirus	Moderate	+		–	D,J	D,J	Moderate	
II. *Infect villous & crypt enterocytes*								
Adenovirus	Moderate	+	(Intranuclear Inclusions)	±	J,I	+	J,I	Mild/Moderate
Coronavirus–PEDV	Moderate	+		±	D,J,I	+	J,I	Moderate
III. *Infect crypt enterocytes*								
Parvovirus	Severe/Hemorrhagic	?		+	J,I		J,I	Severe

from virulent OSU porcine rotavirus induced diarrhea in neonatal mice and increased intracellular calcium levels, NSP4 from attenuated OSU rotavirus or a mutated form of virulent NSP4 was avirulent in mice. The enterotoxigenicity of NSP4 has not yet been assessed in pigs.

Recovery from enteric viral infections depends on local immunity and regeneration of villi by absorptive cells from the undifferentiated crypt cell population. Villous enterocytes on the tips are continually replaced by progenitor cells originating in the crypts that differentiate and mature enzymatically as they migrate up the villus (Argenzio, 1997; Moon, 1978; 1994). The enterocyte turnover rates are slower in younger and gnotobiotic pigs, leading to less rapid repair of villous atrophy, which may contribute to the greater susceptibility of neonates to viral diarrheas (Moon, 1978; 1994). Replacement of damaged villous enterocytes by undifferentiated cells originating in crypts, that are refractory to infection by several viruses (Mebus and Newman, 1977; Pensaert et al., 1970) may partially explain the self-limiting nature of many enteric viral infections. However enteric viruses that also replicate in crypt cells may be able to replicate in the undifferentiated enterocytes which have characteristics more similar to crypt cells. Furthermore such infections may persist longer in the intestine. For example, PEDV and adenovirus both infect crypt enterocytes: PEDV appears capable of infecting regenerating cells (Pensaert, 1992); and adenovirus may persist in the intestine of infected pigs up to 45 days after exposure (Benfield, 1990; Coussement et al., 1981). In addition, the crypt hyperplasia which accompanies many enteric viral infections and increases crypt mitotic activity may predispose animals to viruses that require such rapidly dividing cells for infection.

Although these differences in viral pathogenicities can influence the severity of viral diarrheas, in the field, complex interactions among agent, host, and environment occur which further contribute to variation in the severity of enteric disease. Such factors (reviewed in Saif, 1990) include viral dose, host age, and immune status, diet, and nutrition, microbial flora, concurrent infections, hormonal influences, and environmental factors such as level of sanitation, age of weaning, level of supplemental feeding of suckling pigs, cold or heat stress, and numerous management variables. An example of a factor that influences viral pathogenicity in nursing pigs is the impact of variable levels of passive immunity on enzootic viral infections. In the field this was manifested by varied segmental distribution of villous atrophy with minimal lesions in pigs from TGEV seropositive herds with enzootic TGEV (Moxley and Olsen, 1989b). Experimentally in a pig suckling a sow previously infected with TGEV, villous atrophy was confined to the ileum and was not evident throughout most of the small intestine as seen in fully susceptible piglets (Moxley and Olsen, 1989b).

4. MULTIPLE ENTERIC VIRAL INFECTIONS

In suckling and postweaning pigs, dual or multiple enteric viral infections are common, and may be more frequent than single agent infections in post-weaning pigs (Bridger, 1990; Chu et al., 1985; Morin et al., 1983; Nagy et al., 1996; Saif et al., 1980; Theil and McCloskey, 1995). In addition dual infections with enteric viruses, and bacterial or parasitic pathogens are also frequent (Chu et al., 1985; Morin et al., 1983; Nagy et al., 1996) and in some studies such multiple infections were more common (59% of affected pigs) than single agent infections (33%) (Chu et al., 1985). Only limited data exists concerning the underlying interactive pathophysiologic mechanisms associated

with multiple enteric infections in pigs. Additive or synergistic effects presumably occur in the field and have been demonstrated in some experimental studies of combined rotavirus and ETEC infections (Benfield et al., 1988; Lecce et al., 1982; Tzipori et al., 1980). For example, gnotobiotic pigs dually infected with rotavirus and ETEC had more severe clinical disease than with either pathogen alone (Benfield et al., 1988). Similarly, rotavirus infections were shown to enhance ETEC infections in postweaning pigs (Lecce et al., 1982; Tzipori et al., 1980). Although the mechanisms involved were not delineated, both virus-induced malabsorption, and enterotoxin-induced secretion would be expected to compound the diarrhea severity.

Multiple enteric viral infections are also common in individual pigs in the field. These include: coronaviruses (TGEV) and rotaviruses (Chu et al., 1985; Theil et al., 1979); caliciviruses and rotavirus (Saif et al., 1980); caliciviruses, rotaviruses, and astroviruses (Bridger, 1980; Theil and McCloskey, 1995); and coronaviruses (PEDV) and porcine reproductive, and respiratory syndrome virus (PRRSV) (Sueyoshi et al., 1996). In the latter outbreak of epidemic diarrhea in neonatal piglets, diarrhea, and dehydration were severe and piglet mortality was higher than expected with either agent alone. The PEDV antigens were detected in enterocytes of the small and large intestine and PRRSV antigens were present in macrophages in the lamina propria, Peyer's patches, and mesenteric lymph nodes of infected pigs. Although the interactive pathogenic mechanisms in these dual or multiple enteric viral infection were not elucidated, in the latter scenario PRRSV infection of intestinal macrophages in neonatal pigs may have destroyed these cells or compromised their function, thereby facilitating, and enhancing the severity of PEDV infection, leading to increased piglet mortality.

Because enteric viruses have predilections for different regions of the villus and small intestine, it is likely that dual or multiple viral infections will result in more extensive villous atrophy throughout greater regions of the intestine. Superimposing viruses that also infect crypt enterocytes (PEDV, adenovirus, parvovirus) with ones that infect villous enterocytes, results in destruction of villous and crypt enterocytes, impairing both the absorptive and regenerative capacity of the mucosa. More severe diarrhea and delayed clinical recovery is expected in such cases. Although multiple infections are common in the field, most of our understanding of disease mechanisms has been derived from studies of single agent infections. Thus there is a paucity of information regarding the pathophysiologic and interactive mechanisms contributing to diarrhea in multiple infections. This area should receive a higher research priority in the future to better address the field situation, especially as related to current high intensity swine production systems.

5. THERAPEUTIC APPROACHES TO ENHANCE MUCOSAL REPAIR AND RECOVERY FROM ENTERIC VIRAL INFECTIONS

New concepts of the pathophysiology of diarrheal diseases and potentially new therapeutic strategies are emerging based on recent knowledge of neuro-endocrine-immune communication in the intestine (Argenzio, 1997; Blikslager and Roberts, 1997). In response to enterotoxigenic bacteria and invasive pathogens, the host intestinal neuroimmune system and the mediators released (cytokines, prostaglandins, serotonin, VIP, etc) can directly and indirectly affect enterocytes and through the enteric nervous

system can amplify the range and magnitude of a local stimulus. For example, recent evidence indicates that at least 60% of the cholera toxin-induced secretory response is indirectly neurally mediated in vivo (Argenzio, 1997). Thus host factors produced in response to infection by an enteric pathogen can directly contribute to the diarrheal response.

Although the pathophysiologies of viral-induced diarrheas have been less studied than bacterial or parasite-induced diarrheas, several recent concepts have emerged with important implications for clinical treatment of viral diarrheas. For both TGEV and rotavirus infections of pigs, it was shown that the neutral NaCl absorptive mechanisms were preserved, in spite of villous atrophy, but substrate-linked absorptive processes (eg coupled Na-Glucose transport) were impaired (Homaidon et al., 1991; Rhoads et al., 1991). These results indicate that the immature, undifferentiated cells retain the NaCl absorptive process, but the substrate-linked absorptive processes of the mature villous enterocytes are lost. Thus oral rehydration fluids and treatments designed to optimize the residual NaCl absorption process (addition of glutamine to such fluids or treatment with calcium blocking agents) should be useful therapeutically to treat viral diarrheas (Homaidon et al., 1991; Rhoads et al., 1991). In addition glutamine, which is the major fuel of the small intestine also promotes enterocyte proliferation (Blikslager and Roberts, 1997).

Altered intestinal ion transport is also mediated by the host inflammatory response (Argenzio, 1997). Inflammation induced by enteric viral infections is less pronounced than after many bacterial/parasite infections (Saif, Rosen, and Parwani, 1994; Sueyoshi et al., 1996; Theil, 1990) and it may also be a secondary consequence of the massive cytolytic destruction of villous enterocytes and secondary bacterial infections. The impact of cytokines released by inflammatory cells on stimulation of prostaglandins and their direct, and indirect effects on increased chloride-secretion by enterocytes (secretory diarrhea) has not been examined for enteric viral infections. Moreover inflammation can also disrupt mucosal integrity and create leaky membranes, further allowing translocation of bacteria and toxins across the intestinal epithelial barrier and initiation of systemic infections (Blikslager and Roberts, 1997). In this regard, transient increases in macromolecular permeability were observed in piglets infected with rotavirus or coronavirus (Moon, 1994) and in vitro studies showed that rotavirus infection led to enhanced toxin uptake into cells (Liprandi et al., 1997).

A number of potential therapeutic agents have been proposed as aids to enhance intestinal mucosal repair, but their impact on recovery from enteric viral infections has not been widely assessed. These include polyamines and growth factors (TGFα, EGF) to stimulate epithelial restitution and proliferation and prostaglandins to stimulate closure of tight junctions (Argenzio, 1997). In a recent study by (Zijlstra et al., 1994) supraphysiological doses of human recombinant EGF were beneficial in stimulating recovery of intestinal epithelium in rotavirus-infected pigs, but only in the proximal mid small intestine.

6. IMMUNIZATION APPROACHES TO CONTROL ENTERIC VIRAL INFECTIONS

The localized nature of most enteric viral infections is of major consideration for designing effective immunization strategies to induce intestinal immunity. However, only limited success has been achieved in the development of vaccines to prevent viral

diarrheas (Saif and Jackwood, 1990; Saif and Wesley, 1992; Saif, 1996; 1998; Saif and Fernandez, 1996; Saif et al., 1997; Yuan et al., 1997). Commercial vaccines show limited efficacy in the field, including oral modified live or parenterally-administered vaccines to prevent coronavirus and rotavirus-induced diarrhea in swine (Saif and Jackwood, 1990; Saif and Wesley, 1992; Saif, 1996; 1998, Saif and Fernandez, 1996; Saif et al., 1997; Yuan et al., 1997). The existence of multiple serogroups and serotypes of porcine rotaviruses further complicates vaccine design (Paul and Stevenson, 1992; Saif and Jiang, 1994; Saif, Rosen, and Parwani, 1994; Theil, 1990).

A unique mucosal immune system, distinct from the systemic immune system has evolved to protect mucosal surfaces from pathogens (Reviewed in McGhee et al., 1992; Saif, 1996; 1998; Walker, 1994). The mucosal immune system is characterized by a ponderance of secretory (S) IgA antibodies in mucosal secretions produced by underlying plasma cells in the lamina propria. The dimeric IgA antibodies produced are selectively transported via the polymeric immunoglobulin receptor (secretory component) which is produced by crypt epithelial cells and expressed on their basolateral surface. The transported SIgA antibodies are then secreted onto mucosal surfaces. In addition, in the process of transport of dimeric IgA through epithelial cells via the polymeric Ig receptor, the IgA may function to transport viruses as immune complexes back to the intestinal lumen (Kaetzel et al., 1992) and may also inhibit intracellular viral replication or assembly (Armstrong and Dimmock, 1992; Marzanec et al., 1992; Burns et al., 1996). Although potential mechanisms for intracellular inhibition or neutralization of viral replication by SIgA are poorly understood, these findings if confirmed in vivo imply that SIgA might also promote recovery from viral infections as well as protection from reinfection. Another hallmark of the common mucosal immune system is the induction of immune responses at one mucosal site and the trafficking of effector mucosal lymphoid cells to distant mucosal sites as well as back to the site of origin (McGhee et al., 1992; Saif, 1996; 1998; Walker, 1994). For the intestine, M cells overlying Peyer's patches are specialized epithelial cells for transporting antigens from the lumen to the follicle underneath, a major inductive site for IgA responses. However compartmentalization exists within this system such that antigen stimulation at one mucosal site does not always lead to optimal protection at a distant mucosal site. This concept was confirmed by recent studies showing that use of respiratory PRCV vaccines induced poor intestinal immune responses and only partial protective immunity to enteric TGEV challenge (Saif, 1996; 1998).

Immunization strategies to induce passive and active immunity to enteric viruses in pigs have utilized porcine coronaviruses (TGEV and PRCV) and rotaviruses as models (Saif and Jackwood, 1990; Saif and Wesley, 1992; Saif, 1996; 1998, Saif and Fernandez, 1996; Saif et al., 1997; Yuan et al., 1997). Such studies revealed that the highest level of passive or active protective immunity against TGEV in pigs was achieved by oral immunization of sows or pigs with virulent TGEV and was correlated with the induction of SIgA antibodies in milk (passive immunity) or IgA antibody secreting cells (ASC) in the intestine (active immunity) (Saif and Jackwood, 1990; Saif and Wesley, 1992; Saif, 1996; 1998). The live respiratory coronavirus (PRCV) and a modified live TGEV vaccine induced only low levels of IgA antibodies to TGEV in the milk of vaccinated sows, few IgA ASC in the intestine of vaccinated pigs and only partial protection against TGEV challenge of piglets. A high dose of attenuated TGEV vaccine (10^8 PFU), much greater (3–4 logs) than used in commercial vaccines, was required to induce even low numbers of IgA ASC in the intestine (Saif, 1996; 1998) and this finding may partially explain the failure of modified live TGEV vaccines in the field (Saif

and Wesley, 1992; Moxley and Olson, 1989a). Likewise for immunity to rotavirus, attenuated or inactivated rotavirus vaccines administered orally or parenterally to pigs induced few or no intestinal IgA ASC, and only partial or little protective immunity, respectively (Yuan et al., 1997). Complete protection was achieved only by virulent rotavirus and was correlated with induction of high numbers of intestinal IgA ASC (Saif and Fernandez, 1996; Saif et al., 1997; Saif, 1998).

Thus to date live oral vaccines (which presumably increase vaccine dose by intestinal replication) have been more effective than parenterally administered, or killed or subunit vaccines to induce intestinal immunity to enteric viruses, but remain less effective than the enteropathogenic viruses (which are often more stable and replicate more extensively in the intestine). Live vaccines including live recombinant organisms, because of the amplification of dose and potential targeting to intestinal M cells are likely to remain promising candidates for oral vaccines to induce mucosal immunity. However new technologies are under development to overcome at least two problems specific to oral immunization, especially using non-living vaccines: delivery of intact antigens to key mucosal lymphoid tissues (M cells of Peyer's Patches) and enhancement of immune responses within these tissues. New technologies for oral antigen delivery include use of adhesion molecules or antigens conjugated to adhesion molecules [bacterial pili, *E. coli* LT or cholera toxin (CT), etc], microspheres, liposomes, and rotavirus-like particles (McGhee et al., 1992; Saif, 1996; 1998; Saif and Fernandez, 1996; Walker, 1994). Mucosal adjuvants include avridine (a lipoidal amine), proteosomes, muramyl dipeptide, LPS, and lipid A, selected cytokines, immune stimulating complexes (ISCOMS), CT, and LT enterotoxins (McGhee et al., 1992; Saif, 1996; 1998; Saif and Fernandez, 1996; Walker, 1994). These new delivery systems and adjuvants may provide an effective means for delivery and enhanced intestinal immune responses to future recombinant vectored viral vaccines or subunit viral vaccines administered orally.

An exciting new approach with the potential to create transgenic sows which would secrete TGEV antibodies in milk and provide passive immunity against TGEV to suckling pigs has recently been proposed by Castilla, et al. (1998). This concept involves creating transgenic animals secreting a recombinant monoclonal antibody (MAb) neutralizing TGEV into the milk. To date this TGEV MAb gene has been successfully expressed in the milk of transgenic mice (Castilla et al., 1998). However, further work is needed to create transgenic lines of swine stably expressing the recombinant MAb in milk and to confirm the efficacy of this engineered milk for conferring lactogenic immunity to TGEV in suckling pigs (Saif and Wheeler, 1998).

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