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Enteric Viral Infections of Pigs and Strategies for Induction of Mucosal Immunity

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I. Introduction and Background

Enteropathogenic viruses, such as transmissible gastroenteritis virus (TGEV) and rotavirus, replicate and induce lesions only in the gastrointestinal tract. The susceptible target cell is the villous enterocyte (Saif, 1990). Thus active immunity against enteropathogenic viral infections depends on stimulation of local immune responses within the intestine. To date, only limited success has been achieved in the development of oral vaccines to prevent neonatal viral diarrheas, and commercial vaccines show limited efficacy in the field (Saif and Jackwood, 1990). Although use of live attenuated poliovirus is often cited as

a model for an effective oral vaccine, the mechanism of viral pathogenesis and hence protective immunity differs from that needed to prevent viral diarrheas. Poliovirus undergoes primary replication in Peyer's patches or intestinal lymphoid cells (not epithelial cells), but the target cell for disease induction is the neuron (Melnick, 1990). Thus stimulation of circulating antibodies using either live oral or inactivated poliovirus vaccines can prevent the systemic spread of poliovirus to the central nervous system and the paralytic disease.

Currently only oral vaccines containing live replicating organisms have been highly effective in inducing mucosal immune responses, especially secretory, (S)IgA antibodies. The oral administration of soluble or killed antigens generally induces immunity of short duration or even systemic tolerance (reviewed in Mowat, 1994). Whether the problems encountered with oral administration of soluble protein antigens can be overcome by the use of improved mucosal adjuvants [muranyl dipeptide, immune stimulating complexes (ISCOMs), cholera or *Escherichia coli* enterotoxins, avridine, proteosomes, cytokines, etc.] or new and novel delivery systems (liposomes, live recombinant vectors, microspheres, DNA plasmids, virus-like particles) requires further investigation, and specific examples are given in this review.

Coronaviruses and rotaviruses are well-characterized enteropathogens that account for a high percentage of the viral diarrheas in many animals (Saif and Wesley, 1992; Saif *et al.*, 1994a). In addition, rotaviruses are the leading cause of dehydrating diarrhea in young children worldwide (Kapikian and Chanock, 1990). Thus these viruses serve as important models to study mucosal immunity to enteric viruses. In this review, the impact of the site of viral replication (intestine vs respiratory tract), vaccine dose, and type (attenuated, inactivated) on the isotype, level, and distribution of virus-specific antibody-secreting cells (ASCs) and protection against viral challenge in pigs is summarized and discussed.

II. Characteristics of Enteropathogenic Viruses

Enteropathogenic viruses belonging to at least five different families have been associated with diarrhea in pigs (Table I, Saif, 1990). Each of these viruses infects mainly the villous enterocytes of pigs and, with the possible exception of astroviruses (Saif *et al.*, 1980), induces villous atrophy and a malabsorptive diarrhea (Saif, 1990). None of these viruses causes systemic infections; hence, the localized nature of these intestinal viral infections is of prime consideration for designing effective strategies to induce mucosal immunity. A potential explanation for

TABLE I

CLASSIFICATION AND CHARACTERISTICS OF PORCINE ENTEROPATHOGENIC VIRUSES

Family/virus	Size (nm)	Nucleic acid	Discovery		Intestinal replication site	
			Year	Investigator	Villous	Crypt
<i>Enveloped</i>						
<i>Coronaviridae</i> / transmissible gastroenteritis virus (TGEV)	60–220	ssRNA	1946	Doyle and Hutchings (Solf and Wesley, 1992)	+	–
<i>Nonenveloped</i>						
<i>Reoviridae</i> /rotavirus (group A)	55–70	dsRNA	1976	Woode <i>et al.</i>	+	–
Rotavirus (Group B)	55–70	dsRNA	1980	Bridger	+	–
Rotavirus (Group C)	55–70	dsRNA	1980	Saif <i>et al.</i>	+	–
Rotavirus (Group E)	55–70	dsRNA	1986	Chasey <i>et al.</i>	?	?
<i>Caliciviridae</i> /calicivirus	30–40	ssRNA	1980	Bridger	+	–
			1980	Saif <i>et al.</i>		
<i>Astroviridae</i> /Astrovirus	28–30	ssRNA	1980	Bridger	+	–
			1980	Saif <i>et al.</i>		
<i>Adenoviridae</i> /Adenovirus	70–90	DNA	1981	Coussement <i>et al.</i>	+	±

the localized nature of many enteric viral infections was highlighted in a recent study (Rossen *et al.*, 1996). The authors found that TGEV enters and exits from polarized epithelial cells *in vitro* via the apical surface; in contrast, another coronavirus, mouse hepatitis virus (MHV), enters the same cells apically but exits basolaterally. The investigators speculated that similar differences in the mode of release of coronaviruses from infected host cells could contribute to the nature of the localized intestinal infections induced by TGEV (released apically into the gut lumen) or the systemic infections associated with MHV (released basolaterally into the blood and lymph).

A. COMPARATIVE PATHOGENESIS OF VIRULENT AND ATTENUATED TRANSMISSIBLE GASTROENTERITIS, PORCINE RESPIRATORY CORONAVIRUS, AND ROTAVIRUS

Exposure of pigs to attenuated TGEV (TGEV-A), virulent TGEV (TGEV-V) or porcine respiratory coronavirus (PRCV) results in distinct disease patterns related to differences in virulence and tissue tropisms between the viruses (Table II) (Pensaert and Cox, 1989; Saif and

TABLE II

VERTICAL AND LONGITUDINAL SITES OF REPLICATION AND VILLOUS ATROPHY IN THE INTESTINE FOR PORCINE CORONAVIRUSES AND GROUP A ROTAVIRUSES

Virus	Diarrhea	Longitudinal		Vertical small intestine (site)		Villous atrophy small intestine		Respiratory tract		Reference
		Small intestine	Colon	Villous	Crypt	Site	Extent	Upper	Lower	
<i>Coronavirus</i>										
Virulent TGEV	Severe	D,J,I	-	+ Entire	-	D,J,I	Severe	±	±	Frederick <i>et al.</i> (1976)
Attenuated TGEV	Mild/ none	J,I	-	+ Entire	-	J,I/ none	Mild	+	±	Frederick <i>et al.</i> (1976) Furuuchi <i>et al.</i> (1979)
PRCV	None	-	-	- NA	-	None	None	+	+	Pensaert and Cox (1989)
<i>Rotavirus</i>										
Group A	Mild- severe	D,J,I	±	+ Entire	-	J,I	Moderate- severe	-	-	Theil <i>et al.</i> (1978)

Wesley, 1992; Saif *et al.*, 1994b). Virulent TGEV replicates in villous epithelial cells throughout the small intestine, inducing severe villous atrophy and a malabsorptive diarrhea leading to nearly 100% mortality in seronegative, neonatal pigs. Attenuated strains of TGEV replicate in scattered villous epithelial cells in the distal portion of the small intestine of neonatal pigs and induce mild or no diarrhea (Frederick *et al.*, 1976). They also replicate more extensively in the respiratory tract compared to virulent TGEV strains (Furuuchi *et al.*, 1979). In contrast, PRCV strains replicate in the upper and lower respiratory tract, with little or no replication in the intestine, and generally cause subclinical infections or mild respiratory disease (Pensaert and Cox, 1989). TGEV infections remain a leading cause of piglet diarrhea and mortality in swine herds in North America, and commercial vaccines, even live attenuated oral TGEV vaccines, are of limited efficacy in the field (Saif and Wesley, 1992). In previous studies, PRCV induced partial protection against experimental challenge with TGEV (Van Nieuwstadt *et al.*, 1989; Cox *et al.*, 1993), but the mechanisms involved were not elucidated. These three antigenically related porcine coronaviruses with distinct differences in virulence and tissue tropisms (enteric TGEV-A or TGEV-V or respiratory PRCV) provided an ideal model to study interactions between bronchus-associated lymphoid tissues (BALT) and gut-associated lymphoid tissues (GALT) in the induction of mucosal immunity and protection against virulent TGEV challenge (Brim *et al.*, 1995; Saif, 1996; VanCott *et al.*, 1993, 1994).

By comparison, porcine group A rotaviruses also replicate throughout the small intestine and occasionally the colon, inducing moderate to severe villous atrophy in the distal small intestine (Table II, Theil *et al.*, 1978). Similar to enzootic infections with TGEV-V in seropositive herds, rotaviruses are a frequent cause of diarrhea in 2- to 3-week-old pigs with morbidity rates approaching 100%, but with lower mortality rates (5–20%) (Paul and Stevenson, 1990; Saif *et al.*, 1994a).

To date, commercial and experimental candidate vaccines have not been highly effective in preventing enteric viral infections and gastroenteritis in humans or animals (reviewed in Saif and Jackwood, 1990; Kapikian and Chanock, 1990). Poor efficacy has frequently been encountered in the field using live oral or parenterally administered vaccines to prevent coronavirus and rotavirus-induced diarrhea in swine (Saif and Jackwood, 1990). Likewise, clinical trials of candidate rotavirus vaccines in infants have often failed in various aspects of safety, immunogenicity, or efficacy, especially when tested in developing countries (Kapikian and Chanock, 1990). These results suggest that more research is needed to optimize enteric vaccines to induce local

mucosal immune responses that more closely mimic ones elicited after exposure to the virulent organism.

III. Mucosal Immunity to Enteropathogenic Viruses

A unique mucosal immune system separate from the systemic immune system has evolved to protect mucosal surfaces from pathogens and to exclude environmental antigens and foreign proteins, thereby preventing them from evoking systemic-type inflammatory immune responses (reviewed by Brandtzaeg, 1992; Husband, 1993; McGhee *et al.*, 1992; Mestecky, 1987). The mucosal immune system is characterized by a preponderance of SIgA antibodies selectively secreted onto mucosal surfaces by an active transport mechanism (polyimmunoglobulin receptor, PIgR). The SIgA antibodies play a major role in preservation of mucosal integrity by down-regulation of systemic-type immune responses, preventing invasion of pathogens from the mucosa by blocking of attachment or invasion, neutralization (in the lumen or intracellularly), and "immune exclusion." These functions are in contrast to systemically induced IgG antibodies that mediate inflammatory reactions leading to the killing and elimination of pathogens, thereby maintaining systemic sterility.

Although in earlier studies SIgA was envisioned to act mainly at the luminal mucosal surfaces, recent data suggest that dimeric IgA may bind antigens on the basolateral side of intestinal epithelial cells (Kaetzel *et al.*, 1992). These immune complexes would then be transported across the epithelial cell via the PIgR and secreted back into the intestinal lumen, thereby eliminating foreign antigens that have penetrated the epithelium. Other recent reports suggest that SIgA may function intracellularly in host defense by inhibiting viral replication or assembly *in vitro* (Armstrong and Dimmock, 1992; Marzanec *et al.*, 1992) and *in vivo* (Burns *et al.*, 1996). If further confirmed *in vivo*, such findings imply that SIgA can promote recovery from viral infections as well as initial protection.

Another unique feature of the mucosal immune system compared to the systemic immune system is the induction of antigen-specific B and T cells in IgA inductive organized lymphoid tissue (GALT, BALT, etc.) and their distribution to remote mucosal effector sites (i.e., lamina propria regions of the intestine, bronchi, genitourinary tract, and secretory glands). This cellular distribution pathway linking distant mucosal sites is referred to as the common mucosal immune system (Mes-

tecky, 1987). Thus antigen taken up (via M cells) and processed via GALT [Peyer's patches (PP) and aggregates of lymphoid tissue in the lamina propria] induces activated T and B cells which migrate from the PP through the MLN and via the thoracic duct into the systemic circulation, subsequently repopulating distant mucosal tissues. Maturation of these B cells into IgA plasma cells occurs within the mucosal effector sites in response to antigen, T cells, and cytokines (Lebman and Coffman, 1994). Key studies in rabbits confirmed that PP are an enriched source of IgA precursor cells which repopulate the lamina propria of the intestine and distant mucosal sites (Craig and Cebra, 1971).

Among the first reports to document that antigenic stimulation at one mucosal site (intestine) leads to SIgA antibody responses at a distinct mucosal site (mammary gland) were the studies of lactogenic immunity to TGEV in swine by Bohl *et al.* (1972) and Saif *et al.* (1972). The discovery and subsequent confirmation (Weisz-Carrington *et al.*, 1978) of the interrelationship between the SIgA system of the intestine and mammary gland was an important tenet of the common mucosal immune system, and this system was later confirmed in humans and other species (Mestecky, 1987; McGhee *et al.*, 1992). Thus antigen-specific B and T cells induced in IgA inductive lymphoid tissues (GALT, BALT, etc.) are disseminated to remote mucosal effector sites (i.e., lamina propria of the gut, mammary gland, bronchi, genitourinary tract, etc.).

Recent studies, including further studies of immunity to porcine coronaviruses (Van Cott *et al.*, 1993, 1994; Saif *et al.*, 1994b; Saif, 1996), have suggested that functional compartmentalization and limited reciprocity may exist within some components of the common mucosal immune system. For example, migration of cells from BALT is more limited than from GALT (Sminia *et al.*, 1989) and BALT exposure often leads to dissemination of non-IgA committed secondary B cells (Cebra *et al.*, 1984). In addition, IgA precursor cells derived from GALT more readily repopulate the gut lamina propria than distant mucosal sites (Cebra *et al.*, 1984; Brandtzaeg, 1992). Such observations have important implications for the design of effective mucosal vaccines, but information on effective and practical procedures to induce protective immunity at mucosal surfaces is lacking. In the following sections, our studies of the induction of mucosal immunity and protection using the antigenically related porcine coronaviruses, TGEV and PRCV, are reviewed as are results of studies comparing different types of rotavirus vaccines.

A. STUDIES OF ACTIVE IMMUNITY TO TRANSMISSIBLE GASTROENTERITIS VIRUS AND PORCINE RESPIRATORY CORONAVIRUS

To analyze the interrelationships between BALT and GALT related to protective immunity, we used as a model the three antigenically related porcine coronaviruses. Virulent TGEV replicates primarily in the intestine and induces diarrhea; attenuated TGEV replicates in the intestine and the upper respiratory tract but induces no diarrhea; and PRCV replicates in the upper and lower respiratory tract, but induces only a subclinical infection (Table II; Frederick *et al.*, 1976; Pensaert and Cox, 1989; Saif and Wesley, 1992). These questions were addressed: Is PRCV a more effective candidate vaccine for TGEV than attenuated TGEV? Does a high dose of attenuated TGEV administered orally induce greater ASC responses in GALT than a lower dose (comparable or higher virus titer than commercial TGEV vaccines)? What are the comparative IgA and IgG ASC responses induced in GALT and BALT and the level of protection after inoculation with PRCV, TGEV-A, or TGEV-V and challenge with TGEV-V? In pigs recovered from infection with TGEV-V and reexposed to TGEV-V, what are the correlates of protective immunity?

We first investigated the comparative immune responses to live PRCV versus TGEV-V, the degree of protection induced against TGEV-V challenge, and potential correlates of protection. Three groups of 11-day-old TGEV seronegative pigs were oronasally inoculated with virulent TGEV, PRCV, or mock-infected cell-culture fluids, respectively, and challenged 24 days later with virulent TGEV (Brim *et al.*, 1995; Saif *et al.*, 1994b; Saif, 1996; VanCott *et al.*, 1993, 1994). Immune responses in intestinal (gut lamina propria and mesenteric lymph nodes (MLNs) and respiratory (bronchial lymph nodes, BLN) lymphoid tissues were assessed at challenge and postchallenge day (PCD) 4 by enumeration of IgA and IgG TGEV-specific ASC by ELISPOT and by lymphoproliferative assays (LPAs) using inactivated TGEV as antigen. The major ASC responses and percent of pigs protected are summarized in Table III. All pigs inoculated with TGEV-V developed diarrhea, shed TGEV in feces, and recovered. The presence of high numbers of IgA-ASC in the gut lamina propria (LP) and high LPA responses in the MLN at challenge (PCD 0) was associated with 100% protection against diarrhea after TGEV challenge. No significant increases were observed in numbers of ASC or LPA responses in the gut LP or MLN, respectively, after TGEV challenge (PCD 4), reflecting the lack of viral replication associated with complete protection. In contrast, pigs inoculated with PRCV had no clinical disease and shed virus in nasal secre-

TABLE III

COMPARISON OF INTESTINAL AND RESPIRATORY ASC RESPONSES AND PROTECTIVE IMMUNITY INDUCED BY TGEV AND PRCV STRAINS IN NEONATAL PIGS AT POSTCHALLENGE DAY (PCD) 0 AND 4^a

Virus inoculum group	Mean No. ASC/5 \times 10 ⁵ MNC at PCD 0						Mean No. ASC/5 \times 10 ⁵ MNC at PCD 4						Percent protection against TGEV challenge	
	Intestinal lamina propria			Bronchial lymph node			Intestinal lamina propria			Bronchial lymph node			Diarrhea (%)	Shedding (%)
	IgG	IgA	G/A ^b	IgG	IgA	G/A	IgG	IgA	G/A	IgG	IgA	G/A		
Virulent TGEV	109	620	0.18	25	1	25	15	109	0.14	300	94	3.2	100	80
PRCV	<1	1	UD	223	1	223	150	4	38	320	7	46	58	17
Controls	<1	<1	—	<1	<1	—	<1	<1	—	<1	<1	—	10	22

^aData summarized from VanCott *et al.* (1994).

^bG/A, ratio of IgG to IgA ASCs; UD, undetermined because numerator <1.

tions but not feces. At challenge (PCD 0), the PRCV-exposed pigs had mainly IgG ASC and high LPA responses in the BLN, but low ASC numbers and LPA responses in the intestine (gut LP or MLN, respectively). About 58% of the pigs were protected against diarrhea (compared to 10% of controls) and only 17% were protected against fecal TGEV shedding (comparable to controls). After TGEV challenge (PCD 4), the numbers of IgG-ASC and to a lesser extent IgA-ASC increased rapidly in the intestinal lamina propria of the PRCV-exposed pigs, suggesting that virus-specific IgG-ASC precursors derived in BALT or systemic lymphoid tissues of the PRCV-exposed pigs may migrate to the intestine in response to TGEV challenge and contribute to the partial protection observed. The higher numbers of IgA-ASC in BALT of TGEV-exposed pigs compared to PRCV-exposed pigs at PCD 4 probably reflects TGEV replication and restimulation in the gut resulting in trafficking of IgA precursor cells from GALT to BALT (Husband, 1994; Mestecky, 1987; McGhee *et al.*, 1992). Thus TGEV infections or vaccines that induce immunity via GALT and secondarily via BALT may prevent PRCV infections. Whether the more frequent use of live attenuated TGEV vaccines in the United States (which induce IgG ASC in BLN, Table IV) compared to Europe has had an impact on limiting the

TABLE IV

COMPARISON OF INTESTINAL AND RESPIRATORY PRIMARY AND MEMORY ASC RESPONSES INDUCED BY VIRULENT TGEV AND LOW VERSUS HIGH DOSES OF ATTENUATED TGEV IN NEONATAL PIGS^a

Virus inoculum group/response ^b		Mean No. ASC/5 × 10 ⁵ MNC					
		Mesenteric lymph node			Bronchial lymph node		
		IgG	IgA	G/A ^c	IgG	IgA	G/A ^c
Virulent TGEV	Primary	48	9	5	7	1	7
	Memory	5295	1159	5	2989	327	9
Attenuated TGEV							
Low dose (10 ⁶ pfu)	Primary	2	<1	UD	16	1	16
	Memory	60	<10	UD	866	34	25
High dose (10 ⁸ pfu)	Primary	9	1	9	28	1	28
	Memory	1133	79	14	4475	159	28

^aData summarized from VanCott *et al.* (1993).

^bPrimary immune responses were assayed by ELISPOT directly on mononuclear cells (MNCs) obtained from pigs at PID 12 and 24 and the mean numbers of ASC per 5 × 10⁵ MNC are shown. Memory or secondary immune responses were assayed by ELISPOT after *in vitro* TGEV stimulation (5 days) of MNC obtained from pigs at PID 24 and 40 and the mean numbers of ASC per 5 × 10⁵ MNC are shown.

^cG/A, ratio of IgG to IgA ASCs; UD, undetermined.

spread of PRCV infections in the United States is unknown, but at present PRCV infections appear to be less widespread among swine in the United States than in Europe. Thus our major conclusions were that functional compartmentalization exists in the BALT and GALT responses: immunization via BALT (PRCV infection) induced a systemic type of response (IgG-ASC) with low numbers of ASC and LPA responses in the gut and provided incomplete protection against TGEV-V. Immunization via GALT (TGEV-V infection) induced high numbers of IgA-ASC and high LPA responses in the gut and provided complete protection against TGEV-V induced diarrhea. Further studies on the induction and immune regulation of responses to TGEV and PRCV that affect the distribution of ASC and T lymphocytes should provide important insights to optimize oral vaccine regimens to elicit protective mucosal immune responses against enteric pathogens.

In a similar series of experiments, we also examined the effect of the dose (10^6 versus 10^8 pfu) of live TGEV-A administered oronasally to 11-day-old TGEV seronegative pigs, on primary and memory ASC responses in the MLN and BLN (Saif *et al.*, 1994b; Saif, 1996; VanCott *et al.*, 1993). Our findings (summarized in Table IV) revealed that the high dose of TGEV-A (10^8 pfu) induced 2–4 times more primary IgG ASC and about 5–20 times more memory IgG ASC in the MLN and BLN than the lower dose. Only the high dose of TGEV-A elicited low numbers of primary or memory IgA ASC in the MLN, but numbers were 9–15 times lower than after inoculation with TGEV-V. Of interest were the two- to fourfold higher numbers of primary and memory IgG ASC induced in BALT by the high-dose TGEV-A compared to TGEV-V consistent with reports that attenuated strains of TGEV replicate more extensively in the respiratory tract compared to virulent TGEV strains (Furuuchi *et al.*, 1979). Thus the high degree of attenuation of TGEV vaccines leading to reduced viral replication in the intestine of the sow (Saif and Jackwood, 1990; Saif and Wesley, 1992) and the use of low doses ($\leq 10^6$ pfu/ml) of live attenuated TGEV vaccines orally in piglets (Saif *et al.*, 1994b; VanCott *et al.*, 1993) were major determinants in their failure to induce SIgA antibodies in sow's milk or IgA ASC in the piglets' intestines, respectively. Such factors presumably contribute to the corresponding lack of efficacy of TGEV vaccines in the field.

B. NEW VACCINE APPROACHES TO INDUCE ACTIVE IMMUNITY TO TGEV

Several new potential vaccine approaches to induce immunity to TGEV have recently been reported based on delivery of antigenic peptides of the TGEV S protein in orally administered live bacterial vec-

tors (Der Vartanian *et al.*, 1997; Smerdou *et al.*, 1996). In studies by Der Vartanian *et al.*, (1997), two antigenic peptides of the TGEV S protein, TGEV S_A and S_C, were tandemly inserted (25 amino acids) into the major CIPG subunit of the CS 31A fibrillae of *Escherichia coli* K-12 strain. The responses of mice to these constructs were as follows: (1) The two TGEV epitopes were immunogenic when injected intraperitoneally (IP) into mice as hybrid CIPG subunits, chimeric CS31A polymers, or recombinant bacteria; (2) the chimeric CS31A fibrillae elicited TGEV antibodies in the serum of mice reactive with TGEV peptides and native TGEV; and (3) mice inoculated orally with the recombinant bacteria produced IgA intestinal antibodies reactive against the CS31A fibrillae and TGEV S_C peptide.

In another approach, a recombinant live attenuated *Salmonella typhimurium* was used for oral delivery of a TGEV peptide vaccine in rabbits (Smerdou *et al.*, 1996). An antigenic peptide of the TGEV S protein (S_D, amino acids 378–395) was expressed as a fusion protein with *E. coli* LT-B in the Salmonella. The rationale for fusion with LT-B was to enhance the immunogenicity of the bivalent vaccine since LT-B also functions as an oral adjuvant. Studies of immune responses of rabbits inoculated with purified LT-B/S_D fusion products expressed from Salmonella or the recombinant Salmonella revealed that neutralizing antibodies to TGEV were induced by the purified LT-B/S_D and TGEV antibodies were elicited in serum and intestinal secretions after oral inoculation with the recombinant Salmonella. Thus, if similar TGEV neutralizing IgA antibody responses can be induced in the intestines of pigs by the recombinant bacterial vaccines, such vaccines warrant further study to access their ability to induce protective immunity to TGEV in pigs.

In our laboratory, we are currently exploring optimal oral adjuvants and delivery systems for recombinant TGEV S and M protein vaccines. Preliminary data indicate S and M protein vaccines administered IP with incomplete Freund's adjuvant (IFA) induced higher numbers of memory ASCs in GALT than an inactivated TGEV vaccine administered IP (Sestak *et al.*, 1997).

C. STUDIES OF ACTIVE IMMUNITY TO GROUP A ROTAVIRUS

The gnotobiotic piglet model of porcine and human rotavirus-induced diarrhea has been used to further evaluate the influence of vaccine type (attenuated or binary-ethyleneimine inactivated) compared to wild-type virus infection on induction of intestinal ASC responses and protective immunity (Chen *et al.*, 1995; Saif *et al.*, 1996; Yuan *et*

al., 1996, 1998). Results of oral or IM inoculation of 3- to 5-day-old pigs with Wa human rotaviruses (G1, P1A) and homologous virulent rotavirus oral challenge at postinoculation day (PID) 21 are summarized in Table V. B-cell responses (ASC) were measured by ELISPOT for intestinal (lamina propria) and systemic (peripheral blood lymphocytes, PBL) lymphoid tissues at challenge (PID 21). The major findings were that the numbers of IgA ASCs in the intestinal lamina propria and PBL were significantly greater in virulent-rotavirus inoculated pigs (mimic natural infection) than in the other groups (attenuated, inactivated, controls) and were correlated ($r = 0.9$) with the high degree of protection against diarrhea (89% of piglets protected). The transient appearance of IgA ASC in the blood mirrored the IgA ASC responses in the gut and could serve as an indicator for IgA ASC intestinal responses after rotavirus infection. Piglets inoculated with attenuated rotavirus had partial protection against diarrhea (44% protected) and the second highest numbers of IgA and IgG ASC in the intestinal lamina propria. Interestingly pigs inoculated IM or perorally (PO) with inactivated rotavirus in IFA had a very high number of IgG ASCs in PBL, but few IgG or IgA ASCs in the intestinal lamina propria and, like pigs given inactivated virus PO, minimal protection (0–17%) against diarrhea. Thus, the vaccine type influenced the site, isotype,

TABLE V

COMPARISON OF MUCOSAL AND SYSTEMIC ASC RESPONSES AND PROTECTIVE IMMUNITY INDUCED BY VIRULENT, ATTENUATED AND INACTIVATED ROTAVIRUS VACCINES IN NEONATAL PIGS AT PCD 0 (PID 21)^a

Virus inoculum group	Mean No. ASC/5 \times 10 ⁵ MNC						Percent protection against rotavirus challenge	
	Intestinal lamina propria			Peripheral blood lymphocytes			Diarrhea	Shedding
	IgG	IgA	G/A ^b	IgG	IgA	G/A ^b		
<i>Live</i>								
Virulent rotavirus (PO)	64	53	1.2	2	6	0.3	89%	100%
Attenuated rotavirus (PO)	41	6	6.8	2	1	2	44%	19%
<i>Inactivated</i>								
Rotavirus (PO)	0.7	5	0.14	88	1	88	0%	0%
Rotavirus (IM)	4	3	1.3	237	2	119	17%	0%
Controls	<1	<1		<1	<1		14%	0%

^aData summarized from Yuan *et al.*, (1996, 1998); Saif *et al.*, (1996, p. 199).

^bG/A, ratio of IgG to IgA ASCs.

and level of the ASC response and, similar to the results of the TGEV studies, the degree of protection was correlated with the numbers of IgA ASCs induced in the intestine.

Similarly, in studies of natural rotavirus infections in children, higher fecal IgA antibody titers to rotavirus were associated with protection against infection and illness (Matson *et al.*, 1993). Mouse studies of rotavirus-induced infection revealed similar findings: induction of intestinal IgA antibody responses were positively associated with protection against rotavirus shedding (Feng *et al.*, 1994).

D. NEW VACCINE APPROACHES TO INDUCE IMMUNITY TO ROTAVIRUS

Although not yet evaluated in swine, a new strategy for rotavirus vaccines is the creation of recombinant virus-like particles (VLPs) produced by the coexpression of the four rotavirus capsid genes (VP2/4/6/7) in a baculovirus expression system (Crawford *et al.*, 1994). The VLP vaccines administered with IFA have been tested in rotavirus seronegative mice and rabbits (Conner *et al.*, 1996) and as a maternal vaccine to enhance passive immunity in rotavirus seropositive cows (Fernandez *et al.*, 1996). The VLP vaccines were shown to be noninfectious (no RNA), stable, antigenically authentic, and highly immunogenic in the above species. They induced protective immunity against rotavirus shedding in vaccinated mice and rabbits (Conner *et al.*, 1996) and passive immunity against rotavirus diarrhea in calves fed colostrum from the VLP-vaccinated cows (Fernandez *et al.*, 1998). Thus VLP vaccines show promise as novel vaccines designed to induce mucosal immunity against rotavirus. Further research is needed to identify the optimal delivery systems and mucosal adjuvants for use with the VLP vaccines to most effectively stimulate mucosal immunity.

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REFERENCES

- Armstrong, S. J., and Dimmock, N. J. (1992). Neutralization of influenza virus by low concentrations of hemagglutinin-specific polymeric immunoglobulin A inhibits viral fusion activity, but activation of the ribonucleoprotein is also inhibited. *J. Virol.* **66**, 3823-3832.

- Bohl, E. H., Gupta, R. K. P., Olquin, M. V. F., and Saif, L. J. (1972). Antibody responses in serum, colostrum and milk of swine after infection or vaccination with transmissible gastroenteritis virus. *Infect. Immun.* **6**, 289–301.
- Brandtzaeg, P. (1992). Humoral immune response patterns of human mucosae: Induction and relation to bacterial respiratory tract infections. *J. Infect. Dis.* **165**, S167–S176.
- Bridger, J. C. (1980). Detection by electron microscopy of caliciviruses, astroviruses and rotavirus-like particles in the faeces of piglets with diarrhoea. *Vet. Rec.* **107**, 532.
- Brim, T. A., VanCott, J. L., Lunney, J. K., and Saif, L. J. (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.
- Burns, J. W., Siadat-Pajouh, M., Krishnaney, A. A., and Greenberg, H. B. (1996). Protective effect of rotavirus VP6-specific IgA monoclonal antibodies that lack neutralizing activity. *Science* **272**, 104–107.
- Cebra, J. J., Fuhrman, J. A., Griffin, P., Rose, F. V., Schweitzer, P. A., and Zimmerman, D. (1984). Changes in specific B cells and the dissemination of the primed state in vivo following antigenic stimulation by different mucosal routes. *Ann. Allergy* **53**, 541–549.
- Chasey, D., Bridger, J. C., and McCrae, M. A. (1986). A new type of atypical rotavirus in pigs. *Arch. Virol.* **89**, 235.
- Chen, W. K., Campbell, T., VanCott, J. L., and Saif, L. J. (1995). Enumeration of isotype-specific antibody-secreting cells derived from gnotobiotic piglets inoculated with porcine rotaviruses. *Vet. Immunol. Immunopathol.* **45**, 265–284.
- Conner, M. E., Zarley, C. D., Hu, B., Parsons, S., Drabinski, D., Greiner, S., Smith, R., Jiang, B., Corsaro, B., Barniak, V., Madore, H. P., Crawford, S. E., and Estes, M. K. (1996). Virus-like particles as a rotavirus subunit vaccine. *J. Infect. Dis.* **174**, S88–S92.
- Coussement, W., Ducatelle, R., Charlier, G., and Hoorens, J. (1981). Adenovirus enteritis in pigs. *Am. J. Vet. Res.* **42**, 1905–1911.
- Cox, E., Pensaert, M. B., and Callebaut, P. (1993). Intestinal protection against challenge with transmissible gastroenteritis virus of pigs immune after infection with the porcine respiratory coronavirus. *Vaccine* **11**, 267–272.
- Craig, S. W., and Cebra, J. J. (1971). Peyer's patches: An enriched source of precursors for IgA-producing immunocytes in the rabbit. *J. Exp. Med.* **134**, 188–200.
- Crawford, S. E., Labbe, M., Cohen, J., Burroughs, M., Zhou, Y. J., and Estes, M. K. (1994). Characterization of virus-like particles produced by the expression of rotavirus capsid proteins in insect cells. *J. Virol.* **68**, 5945–5952.
- Der Vartanian, M., Girardeau, J.-P., Martin, C., Rousset, E., Chavarot, M., Laude, H., and Contrepolis, M. (1997). An *E. coli* CS31A fibrillum chimera capable of inducing memory antibodies in outbred mice following booster immunization with the enteropathogenic coronavirus transmissible gastroenteritis virus. *Vaccine* **15**, 111–120.
- Feng, N., Burns, J. W., Bracy, L., and Greenberg, H. B. (1994). Comparison of mucosal and systemic humoral immune responses and subsequent protection in mice orally inoculated with a homologous or a heterologous rotavirus. *J. Virol.* **68**, 7766–7773.
- Fernandez, F. M., Todhunter, D., Parwani, A. V., Crawford, S. E., Conner, M. E., Smith, K. L., Estes, M. K., and Saif, L. J. (1996). Isotype-specific antibody responses to rotavirus in cows inoculated with subunit vaccines comprised of recombinant SA11 rotavirus corelike particles (CLP) or virus-like particles (VLP). *Vaccine* **14**, 1303–1312.
- Fernandez, F. M., Conner, M. E., Parwani, A. V., Nielsen, P. R., Hodgins, D., Crawford, S. E., Estes, M. K., and Saif, L. J. (1998). Passive immunity to bovine rotavirus in new

- born calves fed colostrum supplements from cows immunized with recombinant SA11 rotavirus core-like particles (CLP) or virus-like particles (VLP) vaccines. *Vaccine* **16**, 507–516.
- Frederick, G. T., Bohl, E. H., and Cross, R. F. (1976). Pathogenicity of an attenuated strain of TGE virus for newborn pigs. *Am. J. Vet. Res.* **37**, 165–169
- Furuuchi, S., Shimizu, Y., and Kumagai, T. (1979). Multiplication of low and high cell culture passaged strains of TGE virus in organs of newborn piglets. *Vet. Microbiol.* **3**, 169–178.
- Husband, A. J. (1993). Novel vaccination strategies for the control of mucosal infection. *Vaccine* **11**, 107–112.
- Kaetzel, C. S., Robinson, J. K., Chintalacharuvir, K. R., Vaerman, J. P., and Lamm, M. E. (1992). The polymeric immunoglobulin receptor secretory component mediates transport of immune complexes across epithelial cells a local defense function for IgA. *Proc. Natl. Acad. Sci. USA* **88**, 8796–8800.
- Kapikian, A. Z., and Chanock, R. M. (1990). Rotaviruses. In “Virology” (B. N. Fields *et al.*, eds.), pp. 1353–1404. Raven Press, New York.
- Lehman, D. A., and Coffman, R. L. (1994). Cytokines in the mucosal immune system. In “Handbook of Mucosal Immunology” (P. L. Ogra *et al.*, eds.), pp. 243–250. Academic Press, San Diego, CA.
- Marzanec, M. B., Kaetzel, C. S., Lamm, M. E., Fletcher, D., and Nedrud, J. G. (1992). Intracellular neutralization of virus by immunoglobulin A antibodies. *Proc. Natl. Acad. Sci. USA* **89**, 6901–6905.
- Matson, D. O., O’Ryan, M. L., Herrera, I., Pickering, L. K., and Estes, M. K. (1993). Fecal antibody responses to symptomatic and asymptomatic rotavirus infections. *J. Infect. Dis.* **167**, 577–583.
- McGhee, J. R., Mestecky, J., Dertzbaugh, M. T., Eldridge, J. H., Hirasawa, M., and Kiyono, H. (1992). The mucosal immune system: from fundamental concepts to vaccine development. *Vaccine* **10**, 75–88.
- Melnick, J. L. (1990). Enteroviruses: Polioviruses, coxsackie viruses, echoviruses and new enteroviruses. In “Virology”, (B. N. Fields *et al.*, eds.), pp. 549–606. Raven Press, New York.
- Mestecky, J. (1987). The common mucosal immune system and current strategies for induction of immune responses in external secretions. *J. Clin. Immunol.* **7**, 265–276.
- Mowat, A. M. (1994). Oral tolerance and regulation of immunity to dietary antigens. In “Handbook of Mucosal Immunology” (P. L. Ogra *et al.*, eds.), pp. 185–201. Academic Press, San Diego, CA.
- Paul, P. S., and Stevenson, G. W. (1992). Rotavirus and reovirus. In “Diseases of Swine” (A. D. Leman *et al.*, eds.), pp. 331–348. Iowa State Univ. Press, Ames.
- Pensaert, M. B., and Cox, E. (1989). Porcine respiratory coronavirus related to transmissible gastroenteritis virus. *Agri-Practice* **10**, 17–21.
- Rossen, J. W. A., Bekker, C. P. J., Strous, G. J. A. M., Horzinek, M. C., Dveksler, G. S., Holmes, K. V., and Rottier, P. J. M. (1996). A murine and a porcine coronavirus are released from opposite surfaces of the same epithelial cells. *Virology* **224**, 345–351.
- Saif, L. J. (1990). Comparative aspects of enteric viral infections. In “Viral Diarrheas of Man and Animals” (L. J. Saif and K. W. Theil, eds.), pp. 9–31. CRC Press, Boca Raton, FL.
- Saif, L. J. (1996). Mucosal immunity: An overview and studies of enteric and respiratory coronavirus infections in a swine model of enteric disease. *Vet. Immunol. Immunopathol.* **54**, 163–169.
- Saif, L. J., and Jackwood, D. J. (1990). Enteric virus vaccines: Theoretical consider-

- ations, current status and future approaches. In "Viral Diarrheas of Man and Animals" (L. J. Saif and K. W. Theil, eds.), pp. 313–329. CRC Press, Boca Raton, FL.
- Saif, L. J., and Wesley, R. (1992). Transmissible gastroenteritis virus. In "Diseases of Swine" (A. D. Leman *et al.*, eds.), 7th ed., pp. 362–386. Iowa State University Press, Ames.
- Saif, L. J., Bohl, E. H., and Gupta, R. K. P. (1972). Isolation of porcine immunoglobulins and determination of the immunoglobulin classes of transmissible gastroenteritis viral antibodies. *Infect. Immun.* **6**, 600–609.
- Saif, L. J., Bohl, E. H., Theil, K. W., Cross, R. F., and House, J. A. (1980). Rotavirus-like, calicivirus-like, and 23-nm virus-like particles associated with diarrhea in young pigs. *J. Clin. Microbiol.* **12**, 105–111.
- Saif, L. J., Bohl, E. H., Theil, K. W., Kohler, E. M., and Cross, R. F. (1980). 30 nm virus-like particles resembling astrovirus in intestinal contents of a diarrheic pig. *Proc. Conf. Res. Workers Anim. Dis.* Chicago, 1980, Abstr. No. 149.
- Saif, L. J., Rosen, B., and Parwani, A. (1994a). Animal rotaviruses. In "Virus Infections of the Gastrointestinal Tract" (A. Z. Kapikian, ed.), 2nd ed., pp. 279–367. Dekker, New York.
- Saif, L. J., VanCott, J. L., and Brim, T. A. (1994b). Immunity to transmissible gastroenteritis virus and porcine respiratory coronavirus infections in swine. *Vet. Immunol. Immunopathol.* **43**, 89–97.
- Saif, L. J., Ward, L. A., Rosen, B. I., and To, T. L. (1996). The gnotobiotic piglet as a model for studies of disease pathogenesis and immunity to human rotaviruses. *Arch. Virol.* **12**, 153–161.
- Sestak, K., Lewis, P., Gillespie, A., and Saif, L. J. (1997). Immune responses in pigs intraperitoneally (IP) inoculated with baculovirus-expressed transmissible gastroenteritis virus S and M proteins. *Conf. Res. Workers Anim. Dis.*, Chicago, 1997, Abstract.
- Smerdou, C., Anton, I. M., Plana, J., Curtiss, R., III, and Enjuanes, L. (1996). A continuous epitope from transmissible gastroenteritis virus S protein fused to *E. coli* heat-labile toxin B subunit expressed by attenuated *Salmonella* induces serum and secretory immunity. *Virus Res.* **41**, 1–9.
- Sminia, T., van der Brugge-Gamelkoom, G. J., and Jeurissen, S. H. M. (1989). Structure and function of bronchus-associated lymphoid tissue (BALT). *Crit. Rev. Immunol.* **9**, 119–150.
- Theil, K. W., Bohl, E. H., Cross, R. F., Kohler, E. M., and Agnes, A. G. (1978). Pathogenesis of porcine rotaviral infection of experimentally-inoculated gnotobiotic pigs. *Am. J. Vet. Res.* **39**, 213–220.
- VanCott, J. L., Brim, T. A., Simkins, R. A., and Saif, L. J. (1993). Antibody-secreting cells to transmissible gastroenteritis virus and porcine respiratory coronavirus in gut- and bronchus-associated lymphoid tissues of neonatal pigs. *J. Immunol.* **150**, 3990–4000.
- VanCott, J. L., Brim, T. A., Lunney, J., and Saif, L. J. (1994). Contribution of antibody secreting cells induced in mucosal lymphoid tissues of pigs inoculated with respiratory or enteric strains of coronavirus to immunity against enteric coronavirus challenge. *J. Immunol.* **152**, 3980–3990.
- Van Nieuwstadt, A. P., Zetstra, T., and Boonstra, J. (1989). Infection with porcine respiratory coronavirus does not fully protect pigs against intestinal TGE virus. *Vet. Rec.* **125**, 58–60.
- Weisz-Carrington, P., Roux, M. E., McWilliams, M., Phillips-Qualiata, J. M., and Lamm, M. E. (1978). Hormonal induction of the secretory immune system in the mammary gland. *Proc. Natl. Acad. Sci. USA* **75**, 2928–2932.

- Woode, G. N., Bridger, J., Hall, G. A., Jones, J. M., and Jackson, G. (1976). The isolation of reovirus-like agents (rotaviruses) from acute gastroenteritis of piglets. *J. Med. Virol.* **9**, 203–209.
- Yuan, L., Ward, L. A., To, T. L., and Saif, L. J. (1996). Systemic and intestinal antibody-secreting cell responses to inactivated human rotavirus in a gnotobiotic pig model of diarrheal disease. *J. Virol.* **70**, 3075–3083.
- Yuan, L., Kang, S.-Y., Ward, L. A., To, T. L., and Saif, L. J. (1998). Antibody-secreting cell responses and protective immunity assessed in gnotobiotic pigs inoculated orally or intramuscularly with inactivated human rotavirus. *J. Virol.* **72**, 330–338.