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Immunity to transmissible gastroenteritis virus and porcine respiratory coronavirus infections in swine

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Abstract

Despite the pioneering efforts to identify correlates of passive immunity to transmissible gastroenteritis virus (TGEV), effective vaccines for the control of TGE in suckling pigs have remained elusive. The initial concept of an enteromammary immunologic axis in monogastrics originated from studies of lactogenic immunity to TGEV in swine. These studies revealed that infection of pregnant swine with virulent TGEV stimulated high titers of SIgA antibodies in milk which correlated with protection of suckling pigs against TGE; parenteral or oral inoculation with live attenuated or killed TGEV vaccines induced mainly IgG antibodies in milk which generally provided poor protection to suckling pigs. The recent appearance of PRCV infections in swine and continuing studies of TGEV infections, present a unique model for further studies of mucosal immunity. Research using these viruses has increased our understanding of the various components of the common mucosal immune system and their interactions. Although the most important consideration in designing an effective vaccine for TGEV is the stimulation of GALT through intestinal virus replication, studies addressing the contribution of BALT to immunity to TGEV and PRCV may provide insights for alternative vaccine approaches. The mechanism by which exposure to PRCV elicits a variable degree of immunity to TGEV challenge is unknown. Virus replication in the gut or respiratory tract is a major factor affecting the magnitude of the immune response at the respective site and may be necessary for the recruitment of specific immune cells from other mucosal inductive sites, i.e., GALT to BALT and BALT to GALT migration. Further studies on the induction and immune regulation of specific responses to TGEV and PRCV that affect the distribution patterns of IgM-, IgG- and IgA-antibody-secreting cells (ASC) and T lymphocytes should provide valuable insights for optimizing vaccine regimens to elicit the highest mucosal immune responses and optimal protection against TGEV challenge.

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1. Introduction

Transmissible gastroenteritis virus (TGEV) and a recently identified variant, porcine respiratory coronavirus (PRCV), are antigenically related coronaviruses with distinct enteric or respiratory tissue tropisms, respectively (Pensaert and Cox, 1989; Saif and Wesley, 1992). Both viruses contain three major structural proteins: a nucleocapsid protein (N), and two surface glycoproteins, the membrane (M) and the spike (S) (Laude et al., 1990). The latter protein functions in cell attachment, membrane fusion and induction of neutralizing antibodies (Welch and Saif, 1988; Enjuanes et al., 1990; Laude et al., 1990). Although a high degree of nucleotide sequence homology exists (96.5%), the genome of PRCV differs from that of TGEV by deletions (672–681 nucleotides) in the 5′ region of the S gene and by point mutations or other deletions in nonstructural protein A gene (Rasschaert et al., 1990; Wesley et al., 1991b). It is hypothesized that such differences may contribute to the distinct tissue tropisms of the two coronaviruses. cDNA probes to TGEV which map in the deletion region of the S gene of PRCV effectively distinguish TGEV from PRCV strains (Bae et al., 1991; Wesley et al., 1991a). Similarly although TGEV and PRCV strains are indistinguishable by conventional serologic assays, the two viruses can be antigenically differentiated by using monoclonal antibodies (mAb) reactive to TGEV, but nonreactive to PRCV, that map in the deletion region (antigenic sites B or D) of the S protein of PRCV (Callebaut et al., 1988; Enjuanes et al., 1990; Laude et al., 1990; Simkins et al., 1992, 1993). Recent studies using mAb to TGEV have shown that antigenic site A of the S protein is highly conserved on TGEV and PRCV strains and is immunodominant, eliciting high titers of neutralizing antibodies (Laude et al., 1990; DeDiego et al., 1992; Simkins et al., 1992, 1993).

Since the first report of the disease in 1946, TGE continues to cause major economic losses to the swine industry, and to date effective vaccines or treatments are not available (Saif and Wesley, 1992). Exposure of pigs to TGEV or PRCV results in different disease patterns related to the distinct tissue tropisms of the two viruses (Pensaert and Cox, 1989; Van Nieuwstadt and Pol, 1989; Halbur et al., 1993; Van Cott et al., 1993). In the epizootic form of TGE, viral exposure of newborn pigs results in infection of villous enterocytes leading to vomiting, diarrhea, dehydration and mortality approaching 100% in newborn seronegative pigs (Saif and Wesley, 1992). Although TGEV, especially attenuated TGEV strains that cause mild or no diarrhea in neonatal pigs, infects the respiratory tract (Furuuchi et al., 1979; Van Cott et al., 1993; Brim et al., 1994b) and may be isolated from the lungs of chronically infected, older swine (Underdahl et al., 1975), TGEV does not cause clinical pneumonia. Adult seronegative pigs are susceptible to TGEV infections, but may remain asymptomatic while serving as reservoirs for enzootic TGE. Enzootic TGE persists in swine in continuous farrowing herds and although the disease is less severe, the economic impact is significant (Hill, 1989). In contrast PRCV strains replicate almost exclusively

in the respiratory tract of swine and generally cause subclinical infections (Pensaert and Cox, 1989; Van Cott et al., 1993), although exceptions (pneumonia and mortality) are reported, especially in pigs under 1 week of age (Van Nieuwstadt and Pol, 1989; Halbur et al., 1993). In the field, pigs become infected with PRCV at 5–10 weeks of age coincident with the loss of maternal immunity (Pensaert and Cox, 1989). Whereas PRCV infections are widespread in Europe, resulting in almost all swine in some countries becoming seropositive for antibodies to TGEV (Pensaert and Cox, 1989), there is little evidence to date for the spread of PRCV among swine in the US. In a 1989–1990 survey (Anonymous, 1991), only 36% of swine herds were seropositive for antibodies to TGEV. However, the lack of availability until recently (Simkins et al., 1993) of a routine diagnostic assay to serologically distinguish between TGEV and PRCV infections has hindered serologic surveys for PRCV in the US.

2. Passive immunity to TGEV and PRCV

Because TGEV infections are most severe and are often fatal in neonatal pigs, and because it is easier to vaccinate the sow than individual piglets in a litter, investigators have focused vaccination efforts on methods to enhance passive immunity to TGEV. Pioneering studies by Hooper and Haelterman (1966) elaborated the concept of lactogenic immunity and showed that milk antibodies from sows recovered from TGE, but not passively-derived circulating antibodies to TGEV, were protective. Subsequent research by Bohl et al. (1972) and Saif et al. (1972) expanded the concept of lactogenic immunity to TGEV to show the correlation between IgA predominance in the milk and protection of suckling piglets against TGE. They were the first to propose the concept of an enteromammary immunologic axis in swine and provide indirect evidence for its existence based on studies of lactogenic immunity to TGE in swine (reviewed in Saif and Wesley, 1992). Direct evidence for an immunologic link between the intestine and the mammary gland was provided by subsequent cell trafficking studies in mice (Weisz-Carrington et al., 1978) and experimental evidence for this concept has been extended to include other monogastrics such as humans. In pregnant or lactating sows, it is theorized that IgA precursor B cells, stimulated in the gut-associated lymphoid tissues (GALT) upon replication of virulent TGEV, migrate to the mammary glands and secrete SIgA antibodies to TGEV in milk. Thus stimulation of SIgA antibodies in milk correlated with virus virulence and the induction of optimal passive immunity to TGE (Saif and Wesley, 1992). Parenteral or oral inoculation with live attenuated or killed TGEV vaccines stimulated mainly IgG antibodies which did not persist at high levels in milk with the following exceptions. In pregnant sows injected intramammarily with live attenuated TGEV, persisting high levels of IgG neutralizing antibodies were induced in milk and provided partial passive protection (Bohl and Saif, 1975). By comparison, intramammary injection of lactating sows with live attenuated TGEV induced mainly IgA antibodies in milk, possibly related to the replication of TGEV in the mam-

mary tissue of lactating sows (Saif and Bohl, 1982). Partial passive protection was also observed in litters of pregnant sows orally inoculated with the attenuated Nouzilly TGEV, a strain resistant to acidity and intestinal proteases (Aynaud et al., 1991). However in the latter study, there was no correlation between neutralizing or ELISA IgA antibody titers in milk and the piglet protection rate.

Other studies have confirmed that porcine IgG or IgA TGEV-neutralizing antibodies or bovine hyperimmune colostral TGEV neutralizing antibodies (mostly IgG) were protective against TGE if provided in adequate titers on a frequent basis to artificially fed piglets (Stone et al., 1977; Mensik et al., 1978; Wesley et al., 1988). However, pigs fed antisera with neutralizing antibody titers from goats immunized against virulent TGEV (Woods and Wesley, 1992) or pigs fed neutralizing mAb to the M or S proteins of TGEV were not protected against TGEV challenge (Wesley et al., 1988).

Prior exposure of sows to PRCV induced a variable degree (44–53% mortality rates) of passive protection to TGEV in suckling pigs (Bernard et al., 1989; Paton and Brown, 1990; DeDiego et al., 1992). Variability in protection during TGE outbreaks was also noted in the field among litters of PRCV-exposed sows (Pensaert and Cox, 1989). Moreover, a decrease in clinical TGE has been observed in Europe concomitant with the appearance of PRCV, suggesting that prior PRCV infections moderate the severity of TGE in suckling pigs (Pensaert and Cox, 1989). Presumably this occurs via priming of the immune system by PRCV followed by a rapid anamnestic response in milk after TGEV infection (Callebaut et al., 1990).

The mechanism for the induction of IgA antibodies observed inconsistently in the milk of PRCV-exposed sows is unclear, as is the correlation of such antibodies with passive protection to TGEV challenge (Pensaert and Cox, 1989; Callebaut et al., 1990). After a primary exposure to PRCV, IgA antibodies occurred in the milk of only 30% of sows; this percentage increased to 84% in sows reinfected with PRCV. The variable IgA antibody responses observed in the milk of sows after primary exposure to PRCV could relate to the low numbers of virus-specific IgA antibody-secreting cells (ASC, three or less per 5×10^5 mononuclear cells, MNC) that were observed in bronchus-associated lymphoid tissue (BALT), GALT and mesenteric lymph nodes (MLN) of seronegative pigs exposed to 10^8 PFU of PRCV (Van Cott et al., 1993, 1994). Reinfections with PRCV may be necessary to boost numbers of IgA ASC in BALT and to increase the efficiency of a possible SIgA immunologic link between BALT and the mammary gland, if such a link exists in sows. Mainly virus-specific IgG-ASC (135–198 per 5×10^5 MNC) were observed in BALT after oral–nasal or aerosol inoculation of seronegative pigs with 10^8 PFU of PRCV (Van Cott et al., 1993). Following TGEV challenge, increased numbers of IgG-ASC (from less than three to 30–200 per 5×10^5 MNC) also appeared in GALT of the PRCV-exposed pigs (Van Cott et al., 1994). Because IgG antibodies are also prevalent in the milk of sows exposed to viruses that replicate in the respiratory tract, e.g. attenuated TGEV strains and pseudorabies virus (Saif and Bohl, 1977), it is conceivable that an IgG immunologic link may exist between BALT and the mammary gland in sows or that

alternatively, BALT stimulation contributes serum IgG antibodies which are subsequently transudated into the milk. Additional research is needed to elucidate if an immunologic link exists between BALT and the mammary gland using PRCV infections as a model and to explore alternative mechanisms for the induction of lactogenic immunity to TGEV.

3. Active immunity to TGEV and PRCV: antibody responses

Infection of pigs with virulent TGEV stimulates active immunity and confers protection to subsequent TGEV challenge (Cox et al., 1993; Brim et al., 1994b; Van Cott et al., 1994), although the duration of this immunity is unknown. This protective active immunity against TGEV correlates best with antigenic stimulation of IgA-inductive sites (Peyer's patches, etc.) in GALT following replication of TGEV in the intestinal tract. Stimulation of GALT in this manner is achieved most efficiently when swine are orally inoculated or naturally exposed to virulent strains of TGEV. In accordance, high numbers of TGEV-specific IgA-ASC (400–1400 per 5×10^5 MNC), as measured by an ELISPOT assay, and IgA-containing cells have been found to selectively accumulate in the proximal region of the gut lamina propria (duodenum and jejunum) following oral or oral–nasal exposure of pigs to virulent TGEV (Saif, 1976; Van Cott et al., 1994). Mononuclear cells from MLN of virulent TGEV-exposed pigs also contain TGEV-specific ASC of both the IgA and IgG isotype (9 and 48 per 5×10^5 MNC, respectively) and, after a secondary in vitro virus boost, produce measurable levels of IgA and IgG antibodies and enhanced numbers of IgA- and IgG-ASC (1160 and 5300 per 5×10^5 MNC, respectively; Wesley et al., 1986; Berthon et al., 1990; Van Cott et al., 1993). The presence of TGEV-specific IgA antibodies in serum and intestinal fluids of pigs exposed to virulent TGEV, but not to attenuated TGEV, likely reflects GALT-derived active mucosal immunity to TGEV (Kodama et al., 1980; Sprino and Ristic, 1982).

Eleven-day-old seronegative pigs oral–nasally exposed to a US strain of PRCV, which showed no tropism for the intestinal tract, produced few or no TGEV-specific IgA- and IgG-ASC in GALT (three or less per 5×10^5 MNC) and 42% of the pigs remained susceptible to TGEV-induced diarrhea when challenged at 5 weeks of age (Van Cott et al., 1994). The European strains of PRCV also failed to elicit fully protective active mucosal immunity to TGEV in 9–10 week old pigs that were exposed to PRCV 4 weeks earlier (Van Nieuwstadt et al., 1989; Cox et al., 1993). However, in the latter study, the duration of TGEV excretion in feces was decreased in the PRCV-immune pigs compared with the seronegative control pigs. In all three studies, TGEV infection or villous atrophy was detected in the small intestine of the PRCV-exposed pigs after TGEV challenge, and a rapid secondary neutralizing antibody response occurred in the serum, confirming a lack of complete protection after TGEV challenge. Moreover in a recent study, Simkins et al. (1993) further confirmed a rapid secondary rise in blocking antibodies to S protein site A (conserved on PRCV and TGEV) but not S protein site D (con-

served on TGEV but not on PRCV) in the serum of PRCV-exposed pigs after challenge with TGEV. Such results suggest the importance of rapid secondary immune responses to provide a degree of protection against TGEV challenge. More IgG- and IgA-ASC were induced in MLN by high doses of attenuated TGEV than by comparable doses of PRCV (Van Cott et al., 1994). Based on this data, it is possible that increased vaccine doses of attenuated TGEV would enhance protective immunity to TGEV to a greater degree than similar doses of PRCV.

We observed extensive virus replication in the respiratory tract along with high numbers (135–198 per 5×10^5 MNC) of virus-specific, IgG-ASC in BALT (bronchial lymph nodes) of 11-day-old pigs following inoculation of 10^8 PFU of PRCV oral–nasally or by aerosol (Van Cott et al., 1994). The few IgA-ASC (three or less per 5×10^5 MNC) found in BALT and GALT of the PRCV-exposed pigs may explain why PRCV did not elicit complete protective active immunity against TGEV challenge. The partial protection observed correlated with the rapid appearance and accumulation of IgG-ASC, most likely BALT-derived, in the gut lamina propria of these pigs following TGEV challenge (Van Cott et al., 1994). In a study of 10-week-old pigs, Cox et al. (1993) observed TGEV-specific IgA antibodies in the feces of PRCV-exposed pigs, but only after TGEV challenge; however, the occurrence of IgG TGEV antibodies was not monitored concurrently in feces. More research is needed to address the role of IgG and IgA TGEV antibodies induced in PRCV-exposed pigs during secondary mucosal immune responses, in protection against TGEV challenge.

The importance of ASC responses to TGEV in BALT of virulent TGEV-inoculated pigs may have limited impact on the outcome of TGEV infections because of the large number of IgA-ASC (up to 1400 per 5×10^5 MNC) elicited in GALT as a direct result of extensive virus replication in the gut. One study demonstrated TGEV-neutralizing IgG and IgA antibodies in the lung washings of pigs inoculated oral–nasally with virulent TGEV (Sprino and Ristic, 1982). We found higher numbers of IgA-ASC in BALT of TGEV-exposed pigs, than in PRCV-exposed pigs after TGEV challenge even though TGEV replicated much less in the respiratory tract than PRCV (Van Cott et al., 1994). These results suggest that enteric TGEV replication enhanced IgA-ASC responses in BALT via the localization of GALT-derived virus-sensitized IgA B cells to BALT. Therefore, one might speculate that vaccines for TGEV which induce immunity in GALT, and subsequently BALT, may also prevent PRCV infections. Whether the more common use of live attenuated TGEV vaccines in the US compared with Europe has had an impact on limiting the spread of PRCV infections among swine in the U.S. is unknown. However, this information is important to develop strategies for the control of infections by more pathogenic strains of PRCV (Van Nieuwstadt and Pol, 1989; Halbur et al., 1993).

4. Active immunity to TGEV and PRCV: cell-mediated immunity (CMI)

The mechanisms of active CMI to TGEV infections have been studied less than humoral immunity and, to our knowledge, there have been no published studies

on CMI to PRCV infections. The clarification of cellular immune responses to TGEV is important because CMI may play a direct role in protection and recovery from infection and the production of antibodies is regulated by various cytokines derived from activated MNC during an immune response. Antigen-specific CMI mechanisms that have been described in pigs infected with TGEV include antibody-dependent cell-mediated cytotoxicity (ADCC), T-lymphocyte cytotoxicity, and lymphocyte proliferation. Antibodies participate in CMI through ADCC in which specific antibody acts in cooperation with macrophages, neutrophils or lymphocytes to directly kill antibody-coated virus-infected cells. ADCC against TGEV-infected targets has been reported in swine (Cepica and Derbyshire, 1984a). Another mechanism of specific antiviral immunity is major histocompatibility antigen-restricted killing by cytotoxic T lymphocytes of infected target cells that express viral antigens on their surface. A study on the cytotoxic T cell response to TGEV in 2–3 month old pigs showed that oral priming of enteric mucosa will stimulate virus-specific T-lymphocyte cytotoxicity at the sites examined (Peyer's patches, MLN, spleen and peripheral blood) (Shimizu and Shimizu, 1979a), although the role of these cells in recovery from TGE in young pigs has not been elucidated.

Earlier studies demonstrated that MNC from GALT (Peyer's patches), MLN and systemic sites (spleen and peripheral blood) of pigs orally infected with virulent TGEV gave significant proliferative responses upon in vitro stimulation with viral antigen; only MNC isolated from systemic sites of pigs orally inoculated with attenuated TGEV gave positive responses (Shimizu and Shimizu, 1979b; Welch et al., 1988). Our recent work, however, has shown that MNC from MLN of pigs inoculated oral–nasally with a high dose of attenuated TGEV gave proliferative responses comparable to those from virulent TGEV-inoculated pigs (Brim et al., 1994a). Oral–nasal inoculation of pigs with PRCV induced high proliferative responses in BALT (bronchial lymph nodes), but did not result in intestinal replication of the virus, stimulation of significant virus-specific lymphocyte proliferation in MLN, or complete protection against virulent TGEV challenge (Brim et al., 1994b). High doses of attenuated TGEV were much more effective than PRCV in eliciting proliferative responses in MLN, but the immunological significance of these observations for improved vaccination strategies has not yet been assessed.

The effect of TGEV exposure on MNC subset composition from MLN, bronchial lymph nodes, spleens, and the duodenal and ileal lamina propria was another aspect of CMI addressed by this laboratory (Brim et al., 1994b; Van Cott et al., 1994). Flow cytometric analysis revealed that virulent TGEV exposure resulted in increased percentages of $CD2^+$, $CD4^+$, and $CD8^+$ T cells and decreased percentages of sIg^+ B cells in all lymphoid tissues but the duodenum. The expansion of T cell subsets was coincident with the demonstration of virus-specific lymphocyte proliferation by cells of the same origin (Brim et al., 1994b), although it is likely that TGEV-specific T cells were only a small part of the total T-cell population. The increase in duodenal sIg^+ B cells corresponded to a vigorous TGEV-specific IgA-ASC response in the duodenal lamina propria (Van

Cott et al., 1994). Overall, the effect of virulent TGEV exposure on T- and B-cell populations was most pronounced in GALT and MLN, lymphoid cells bordering the primary TGEV replication site in villous enterocytes of the small intestine (GALT) or in the draining lymph nodes for GALT (MLN).

Other mechanisms of antiviral immunity act in a nonspecific manner to limit virus spread in a host before replicating virus reaches the antigenic threshold and elicits a specific immune response. These innate mechanisms include the type I interferons (IFN) that are produced by MNC, epithelial cells, and fibroblasts (reviewed in La Bonnardiere et al., this issue) after contact with viruses, and natural killer (NK) cells that show spontaneous cytotoxicity for virus-infected cells and, unlike cytotoxic T lymphocytes, are neither major histocompatibility antigen-restricted nor sensitized by prior antigen exposure (Koren and Herberman, 1983). TGEV is a potent inducer of IFN-α, both in newborn pigs and in vitro, and IFN-α induction appears to result from the interaction of MNC with the TGEV transmembrane glycoprotein M (Laude et al., 1992). In newborn pigs during the first week of life, NK cell cytotoxicity against TGEV-infected targets was absent in peripheral blood MNC (Charley et al., 1987) and small intestine intraepithelial lymphocytes (Cepica and Derbyshire, 1984a). Adoptive transfer of peripheral blood MNC from an adult pig to SLA matched neonatal recipients conferred NK cell activity in the neonates' peripheral blood MNC and intestinal intraepithelial lymphocytes and increased the recipient pigs' resistance to virulent TGEV challenge as evidenced by a delayed onset of clinical signs and milder diarrhea compared with the control pigs (Cepica and Derbyshire, 1984b). NK cell cytotoxicity in peripheral blood MNC of newborn pigs against TGEV-infected cells was augmented by in vitro treatment with porcine IFN; furthermore, a synthetic IFN inducer administered to 2-day-old pigs stimulated IFN production and NK cell activity, resulting in a delayed onset of clinical signs after exposure to virulent TGEV (Lesnick and Derbyshire, 1988). Deficiencies in NK cell responses may contribute to the severity of TGE in the neonate. Other cytokines are important in modulating the antiviral functions of macrophages, such as macrophage inhibition factor which is produced by TGEV-sensitized T lymphocytes from the intestine, spleen (Frederick and Bohl, 1976), and peripheral blood (Woods, 1977).

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