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Mucosal immunity: an overview and studies of enteric and respiratory coronavirus infections in a swine model of enteric disease

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Abstract

Based on the tenet of a common mucosal immune system, antigenic stimulation at one mucosal site results in the distribution of antigen-specific IgA precursor cells to distant mucosal sites. However, recent studies suggest that functional compartmentalization and limited reciprocity may exist within some components of the common mucosal immune system. Although oral immunization is often very effective in inducing immunity to respiratory pathogens, the converse (respiratory immunization to prevent enteric diseases) may not be as effective. To address this question and to study interactions between the bronchus-associated (BALT) and gut-associated (GALT) lymphoid tissues related to protective immunity, we used as a model two antigenically related porcine coronaviruses which replicate primarily in the intestine (transmissible gastroenteritis virus, TGEV) or respiratory tract (porcine respiratory coronavirus, PRCV). The tissue distribution and magnitude of the antibody secreting cell (ASC) responses (measured by ELISPOT) and cell-mediated immune responses (measured by lymphoproliferative assays, LPA) coincided with the viral tissue tropisms. Immunization via GALT (gut infection with TGEV) elicited high numbers of IgA ASC and high LPA responses in GALT (gut lamina propria, LP or mesenteric lymph nodes, MLN), but lower responses in BALT (bronchial lymph nodes, BLN) and induced complete protection against enteric TGEV challenge. In contrast immunization via BALT (respiratory infection with PRCV) elicited systemic type responses (high numbers of IgG ASC in the BLN), but few ASC and low LPA responses in the gut LP or MLN and induced only partial protection against enteric TGEV challenge. Thus administration of vaccines intranasally may not be optimally effective for inducing intestinal immunity in contrast to the reported efficacy of oral vaccines for inducing respiratory immunity.

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1. The Mucosal Immune System

A unique mucosal immune system independent of 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 in Newby, 1984; Mestecky, 1987; Brandtzaeg, 1992; McGhee et al., 1992; Husband, 1993). The mucosal immune system is characterized by a preponderance of secretory (S) IgA 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 which mediate inflammatory reactions leading to the killing and elimination of pathogens, thereby maintaining the sterility of systemic organs and tissues.

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 through the epithelium. At least two other recent reports have suggested that SIgA may function intracellularly in host defense by the inhibition of viral replication or assembly in vitro (Armstrong and Dimmock, 1992; Marzanec et al., 1992) and in vivo (Burns et al., 1996). 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 tissues (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 (Mestecky, 1987). Although originally it was proposed that activated IgA-committed B cells and Thelper (Th) cells traffic equally well to all secretory tissues independent of origin (Mestecky, 1987), it is becoming recognized that compartmentalization or regionalization occurs within the common mucosal immune system. For example, IgA precursor cells derived from GALT more readily repopulate the gut lamina propria than distant mucosal sites (Cebra et al., 1984; Brandtzaeg, 1992). Also the distant 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). Similarly, immunization via the lower intestinal tract may lead to greater mucosal immune responses in the genital tract than immunization via the upper intestinal tract (Kagnoff, 1993). Such observations have important implications for the design of effective mucosal vaccines as demonstrated in comparative studies of mucosal immunity induced by infection of pigs with enteric versus respiratory coronaviruses (Brim et al., 1994,

Brim et al., 1995; Van Cott et al., 1993, Van Cott et al., 1994). These latter results will be discussed in greater detail in a subsequent section.

2. Oral immunization

Most pathogens enter or infect the host by way of mucosal surfaces making local mucosal immune responses of paramount importance for host defense. A number of investigations in a variety of species have provided evidence that induction of IgA-precursor cells in GALT by orally administered antigens leads to dissemination of effector cells or molecules to intestinal and remote mucosal tissues or secretions (Reviewed in Mestecky, 1987 and McGhee et al., 1992). 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). Thus key studies in rabbits confirmed that PP are an enriched source of IgA precursor cells which repopulate the lamina propria of the intestine (Craig and Cebra, 1971).

Although general concepts related to mucosal immunity as elaborated above have been established, information on effective and practical procedures to induce protective immunity at mucosal surfaces is lacking. In particular oral immunization with non-replicating or soluble antigens has induced poor SIgA responses of short duration (Newby, 1984) or led to oral tolerance (Andre et al., 1973; Reviewed in Mowat, 1994). Current hypotheses to explain these results and oral tolerance suggest that if antigens such as soluble, non-replicating antigens are presented and processed via normal villous epithelial cells, mainly suppressor T cells are induced leading to the suppression of immune responses (Bland and Warren, 1986; Mayer and Shlien, 1987).

A number of strategies have been proposed to overcome the inherent down-regulatory mechanisms associated with induction of mucosal immunity via oral immunization. These include improvements in antigen delivery systems such as use of biodegradable microspheres to protect antigens from low pH and target them to mucosal inductive sites such as PP (Eldridge et al., 1991). Live recombinant vectors which replicate in mucosal sites and express the recombinant antigens of interest are also under development. They include avirulent strains of *Salmonella* (Curtiss et al., 1989) and *Escherichia coli* (Hale, 1991), as well as indigenous strains of *Lactobacillus* (Gerritse et al., 1990). Various attenuated viruses are also under study including recombinant adenoviruses (Morin et al., 1987) and vaccinia virus (Rupprecht et al., 1986; Ramsay et al., 1994). Coexpression of cytokine genes with the influenza virus HA gene in the latter vector led to enhanced IgA antibody responses to the influenza virus HA in the respiratory tract (Ramsay et al., 1994). A number of adjuvants have also been shown effective for enhancing mucosal immune responses including cholera toxin (Tamura et al., 1988; Israel et al., 1992), liposomes (Husband, 1993), avridine (Anderson et al., 1987), and muranyl dipeptide (McGhee et al., 1992, Husband, 1993).

To date, successful strategies for induction of protective immunity via oral immunization have mainly relied on the use of live replicating vaccines or the aforementioned special adjuvants or delivery systems. There are a number of studies in various species demonstrating the efficacy of oral immunization for induction of protective immunity at remote mucosal sites. Examples of oral immunization to induce protection of the respiratory tract include the following. For protection against bacterial infections, the oral delivery of culture supernatants of *Pasteurella haemolytica* in poly hydrogels in cattle resulted in reduced pneumonic lesions upon challenge (Bowersock et al., 1994), and the oral inoculation of humans with *Hemophilus influenza* resulted in decreased colonization and incidence of respiratory infections by this organism (Clancy et al., 1989). In the case of viral infections, oral administration of adenovirus vaccines has been used to prevent human respiratory adenovirus infections (Dudding et al., 1973; Schwartz, 1974). Protection of other distant mucosal surfaces has also been achieved by oral immunization: inoculation of rats with *Streptococcus mutans* prevented dental carries (Michalek et al., 1976) and exposure of swine to the enteropathogenic transmissible gastroenteritis virus (TGEV) induced passive protection in piglets mediated by SIgA antibodies in milk (Bohl et al., 1972; Saif et al., 1972).

Although immunization via GALT effectively generates protective immune responses against certain pathogens at distant mucosal sites, less success has been achieved in the development of oral vaccines to actively protect the target intestinal epithelial cells from infection by enteropathogenic viruses. Use of live attenuated poliovirus is often cited as a model for an effective oral vaccine, but the mechanism of viral pathogenesis and hence protective immunity differs from that needed to prevent viral diarrheas. Poliovirus undergoes primary replication in PP 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. Because enteropathogenic viruses infect epithelial cells and many induce lesions only in the intestine, stimulation of local immune responses within the intestine is needed to most effectively prevent gastrointestinal disease.

To date, however, commercial and experimental candidate vaccines have not been highly effective in preventing enteric viral infections and gastroenteritis in humans or animals (Reviewed in Kapikian and Chanock, 1990; Saif and Jackwood, 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 and cattle (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). Studies of active or passive immune responses and protective immunity induced using live-attenuated, oral TGEV vaccines in swine have revealed that a high degree of attenuation leading to reduced viral replication in the intestine (of the sow) (Saif and Jackwood, 1990; Saif and Wesley, 1992) and the use of low to moderate oral doses of live attenuated TGEV vaccines ($< 10^6$ PFU ml^{-1}) in piglets (Van Cott et al., 1993; Brim et al., 1994; Saif et al., 1994) were major determinants in their failure to induce SIgA antibodies in sow's milk or IgA ASC in the piglets' intestine, respectively and

their corresponding lack of efficacy. 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). Animal studies of rotavirus-induced disease revealed similar findings: induction of intestinal IgA antibody responses or ASC and high local lymphoproliferative responses were positively associated with protection against rotavirus diarrhea (Feng et al., 1994; Saif et al., 1996). These results suggest that more research is needed to optimize enteric vaccines to induce local mucosal immune responses which more closely mimic ones elicited after exposure to the virulent organism.

3. Compartmentalization within the common mucosal immune system: studies of the TGEV / PRCV coronavirus model in swine.

Two antigenically related porcine coronaviruses with distinct tissue tropisms (enteric TGEV or respiratory PRCV) provided an ideal model to study interactions between BALT and GALT in the induction of mucosal immunity and protection against the enteropathogenic TGEV strain (Brim et al., 1994, Brim et al., 1995; Saif et al., 1994; Van Cott et al., 1993, Van Cott et al., 1994). Exposure of pigs to TGEV or PRCV results in distinct disease patterns related to differences in tissue tropism between the two viruses (Pensaert and Cox, 1989; Saif and Wesley, 1992; Saif et al., 1994). TGEV replicates in small intestinal villous epithelial cells, inducing villous atrophy and a malabsorptive diarrhea leading to nearly 100% mortality in seronegative, neonatal pigs. The PRCV strains replicate in the respiratory tract, with little or no replication in the intestine, and generally cause subclinical infections or mild respiratory disease. TGEV infections remain a leading cause of piglet diarrhea and mortality in swine herds and commercial vaccines, even live attenuated oral 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. Therefore, we sought to explore the feasibility of using PRCV as a candidate vaccine to prevent TGEV and to use this enteric disease model to delineate the interactions between BALT and GALT in the induction of protective mucosal immunity. 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., 1994, Brim et al., 1995; Saif et al., 1994; Van Cott et al., 1993, Van Cott et al., 1994). Immune responses in intestinal (gut lamina propria and mesenteric lymph nodes, MLN), respiratory (bronchial lymph nodes, BLN) and systemic (spleen) lymphoid tissues were assessed at challenge and various post-challenge days by enumeration of IgA and IgG TGEV-specific antibody secreting cells (ASC) by ELISPOT and by lymphoproliferative assays (LPA) using inactivated TGEV as antigen. Our major findings were as follows. All pigs inoculated with TGEV developed diarrhea, shed TGEV in feces and recovered. The presence of high numbers of IgA–ASC in the gut lamina propria and high LPA responses in the MLN at challenge correlated with complete protection against TGEV challenge. No significant increases were observed in

numbers of ASC or LPA responses in the gut LP or MLN, respectively, or neutralizing antibody titers in serum after TGEV challenge, reflecting a lack of viral replication associated with complete protection. In contrast, pigs inoculated with PRCV had no clinical disease and shed virus in nasal secretions but not feces. At challenge, 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). Only partial protection against diarrhea (42% developed diarrhea) and fecal TGEV shedding was observed. After TGEV challenge, the numbers of IgG–ASC and to a lesser extent, IgA–ASC increased rapidly in the BLN of the PRCV-exposed pigs, suggesting that virus-specific IgG–ASC precursors derived in BALT of the PRCV-exposed pigs may migrate to the intestine in response to TGEV challenge and contribute to the partial protection observed. Interestingly, we found higher numbers of IgA–ASC in BALT of TGEV-exposed pigs than in PRCV-exposed pigs after TGEV challenge, consistent with previous observations indicating trafficking of IgA precursor cells from GALT to BALT (Mestecky, 1987; McGhee et al., 1992; Husband, 1993). Thus TGEV replication in the gut may increase the numbers of TGEV-specific IgA–ASC in BALT via trafficking of GALT-derived IgA cells to BALT. One might speculate that TGEV infections or vaccines which induce immunity via GALT and secondarily BALT, may prevent PRCV infections. Whether the more frequent use of live attenuated TGEV vaccines in the US compared with Europe has had an impact on limiting the spread of PRCV infections in the US is unknown, but at present PRCV infections appear to be much more widespread among swine in Europe than in the US. 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 ASC and LPA responses in the gut and provided incomplete protection against an enteric pathogen. Immunization via GALT (TGEV infection) induced high numbers of IgA–ASC and high LPA responses in the gut and provided complete protection against enteric disease. Further studies on the induction and immune regulation of responses to TGEV and PRCV that affect the distribution patterns of ASC and T-lymphocytes should provide important insights to optimize oral vaccine regimens to elicit protective mucosal immune responses against enteric pathogens.

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