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Review

Evidence for a common mucosal immune system in the pig☆

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ABSTRACT

The majority of lymphocytes activated at mucosal sites receive instructions to home back to the local mucosa, but a portion also seed distal mucosa sites. By seeding distal sites with antigen-specific effector or memory lymphocytes, the foundation is laid for the animal's mucosal immune system to respond with a secondary response should to this antigen be encountered at this site in the future. The common mucosal immune system has been studied quite extensively in rodent models but less so in large animal models such as the pig. Reasons for this paucity of reported induction of the common mucosal immune system in this species may be that distal mucosal sites were examined but no induction was observed and therefore it was not reported. However, we suspect that the majority of investigators simply did not sample distal mucosal sites and therefore there is little evidence of immune response induction in the literature. It is our hope that more pig immunologists and infectious disease experts who perform mucosal immunizations or inoculations on pigs will sample distal mucosal sites and report their findings, whether results are positive or negative. In this review, we highlight papers that show that immunization/inoculation using one route triggers mucosal immune system induction locally, systemically, and within at least one distal mucosal site. Only by understanding whether immunizations at one site triggers immunity throughout the common mucosal immune system can we rationally develop vaccines for the pig, and through these works we can gather evidence about the mucosal immune system that may be extrapolated to other livestock species or humans.

1. Introduction

The mucosal-associated immune system (MALT) includes the conjunctiva (conjunctiva-associated lymphoid tissue (CALT)), lacrimal duct-ALT (LDALT), larynx-ALT (LALT), salivary duct-ALT (SDALT), nasal-ALT (NALT), bronchus-ALT (BALT), gut-ALT (GALT) and vaginal (VALT) (Gebert and Pabst, 1999). Although the components of the MALT are anatomically and functionally distinct, they share traits such as organized inductive sites where T cells are presented antigen via antigen-presenting cells (APCs). As with other animals, the majority of porcine pathogens gain entry into the body through mucosal surfaces when ingested or inhaled from the feed, the environment or from fecal contamination. Systemic vaccinations (through intramuscular, intraperitoneal, subcutaneous routes, etc.) generally do not promote mucosal immunity and therefore the animal's immune system can only combat the pathogen after it has gained entry into the body (Mestecky, 1987; Mestecky et al., 1978; Murtaugh, 2014). Thus, because mucosal immunity has the potential to control pathogens at their point of entry, it would be advantageous to develop vaccines that trigger a strong mucosal and systemic immune response rather than simply stimulating the systemic immune system.

For induction of a local mucosal immune response, in the gut for example, antigen is taken up by intestinal DCs which migrate to the mesenteric lymph node (mLN) and lead to antigen-specific T and B lymphocytes activation (Annacker et al., 2005; Fujimoto et al., 2011; Johansson-Lindbom et al., 2005; Schulz et al., 2009). Upon activation, lymphocytes undergo proliferation and differentiation and, in most mammals, these activated clonal lymphocytes exit the mLN via the efferent lymph where they drain into the thoracic cavity and enter into the circulation. The pig has inverted lymph nodes and therefore immigration into the lymph node tissue takes place either by afferent lymph vessels or by high endothelial venules (HEV) and they emigrate directly into the circulation through HEV (Binns and Pabst, 1994; Rothkötter, 2009). Once in

☆ This article belongs to Non-rodent animal models.

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the circulation, the majority of activated lymphocytes home back to site where the antigen was initially encountered (Kiyono and Fukuyama, 2004; Kunisawa et al., 2008; Lefrancois et al., 1999; Svensson et al., 2002).

DCs play a critical role in regulating expression of homing molecules on the surface of activated lymphocytes (Johansson-Lindbom et al., 2003; Stock et al., 2013). In the intestine, migratory CD103 + CD11c + MHCII+ DCs produce retinoic acid (RA) which promotes the expression of α4β7 and CCR9 on activated lymphocytes in mice and humans (Campbell and Butcher, 2002; Johansson-Lindbom et al., 2005, 2003; Mora et al., 2003, 2006; Stagg et al., 2002; Stock et al., 2013; Yokota et al., 2009). The receptor for α4β7, MadCAM, is highly enriched on the endothelium of the vasculature supplying the small intestine such that lymphocytes bearing α4β7 undergo extravasation in these post-capillary venules (Berlin et al., 1993). CCR9+ lymphocytes in turn home to the small intestine epithelial cells that constitutively express the CCR9+ ligand, CCL25 (Kunkel et al., 2000; Lazarus et al., 2003). Homing of IgA producing B cells to diverse mucosal tissues appears to be mediated by CCR10 and the ligand CCL28 (Lazarus et al., 2003). In contrast, skin-derived DCs imprint expression of P- and E-selectin ligands and CCR10 on activated lymphocytes (Campbell and Butcher, 2002; Campbell et al., 2003; Schon et al., 1999). Lung DCs imprint the expression of CCR4 on lymphocytes which promote homing to the lung (Mikhak et al., 2013). Thus, the majority of activated lymphocytes home back to the site of antigen uptake by the DC.

Importantly, a portion of activated lymphocytes seed mucosal tissues outside the local mucosa which is tremendously valuable as distal mucosal sites may also encounter the pathogen (i.e. the source of the antigen) in the future (Brandtzaeg et al., 1999; Campbell et al., 2003; Kunkel and Butcher, 2003). Adoptive transfer experiments in animals has shown that cells obtained from mucosal tissues that have been donated to syngeneic animals preferentially repopulate the recipient's mucosal tissues which is compelling evidence of a common mucosal immune system (Griscelli et al., 1969; Hall et al., 1977; McDermott and Bienenstock, 1979; Weisz-Carrington et al., 1979). This activation by antigen at a mucosal inductive site which leads to effector and/or memory T and B cells in distal mucosal sites is referred to as functional connectiveness and is the basis for the common mucosal immune system (Kiyono and Fukuyama, 2004; Kunisawa et al., 2008; McGhee et al., 1992) (Table 1).

Due to the hostile environment of the gastrointestinal tract and coupled with the propensity of the oral immune system to respond with tolerance to oral antigens, it is a substantial challenge to elicit protective mucosal immune responses in the gut using oral immunizations (Faria et al., 2003; Faria and Weiner, 2005; Strobel and Ferguson, 1985; Strobel and Mowat, 1998). Several physical barriers prevent antigen/pathogen contact with gut-associated lymphoid tissues (GALT) and penetration of the gut wall such as mucous production, peristaltic movement of the gut, secretion of natural antibacterial substances such as lysozyme and host defense peptides which protect the intestinal surface against bacterial penetration, and the extreme pH environment of the stomach and the protease rich environment of the small intestine which compromise the immunogenicity of ingested antigens (Medina and Guzmán, 2000; Pasetti et al., 2011). Also, antibodies or other components in maternal colostrum/milk may interfere with antigen uptake and/or function (Brandtzaeg, 2003; Snoeck et al., 2003). Therefore, if one could design a vaccine to activate the common mucosal immune system, it would be a tremendous advantage to initiate mucosal immunity to oral antigens at respiratory or genital mucosa where the activated lymphocytes would then migrate to the oral mucosa to protect the gastrointestinal tract.

The majority of mucosal vaccines are comprised of replicating, attenuated pathogens which, although effective, have the potential to revert to virulence (www.vetvac.org/index.php). In a disease such as Porcine Respiratory and Reproductive Syndrome Virus (PRRSV), which is economically devastating to a pig barn should an outbreak occur, live-attenuated vaccines are not administered to seronegative herds. Even though it is unlikely that the attenuated virus will revert to virulence, it is considered too great a risk to vaccinate proactively and therefore PRRSV vaccines are administered to pigs in barns that have had an outbreak, and thus these vaccines are not proactively administered (Botner et al., 1997; Hu and Zhang, 2014; Storgaard et al., 1999). One may speculate that the reason why attenuated pathogens are so effective as mucosal vaccines may be that in order to trigger an oral immune response instead of tolerance, the pathogen must traverse the gut wall and/or penetrate the epithelial cells lining the gut wall. However, some researchers have shown that subunit vaccines formulated with adjuvants such as cholera toxin (CT) can trigger mucosal immunity in pigs, and some of these works are described within (Foss and Murtaugh, 1999, 2000; Hyland et al., 2004; Verdonck et al., 2005a,b).

For this review, we present manuscripts with evidence that vaccinations and/or inoculation of pigs at one mucosal site triggers a measurable immune response within the local mucosa, within the blood and within at least one distal mucosal immune site. For example, research wherein pigs have been exposed to replicating but attenuated virus via the intranasal route and which showed virus-specific IgG or IgA antibody production or cell-mediated immune responses within the respiratory mucosa (i.e. local mucosa), oral or vaginal mucosa (distal mucosa) as well as in the blood (systemic immune response) would meet our criteria for reporting. If only a local mucosal immune site and/or serosal response is reported (i.e. intranasal vaccination of piglets reporting antibody production in the bronchoalveolar lavage fluid and/or blood only), the report will not be included in this review. As such, we intend this review to be a thorough examination of the literature that reports evidence for induction of the common mucosal immune response in pigs. We are aware, however, that our approach has several limitations which we will now outline. First, intranasal immunizations may indeed also be peroral if a portion of the vaccine is swallowed. If this is the case, we cannot interpret evidence of antibodies in the gut as truly an induction of a distal mucosal site because the GALT was directly stimulated and therefore should be considered local mucosa. Unfortunately, it is impossible to discern whether sufficient precautions were taken to ensure an exclusively intranasal immunization in these reports and we can only trust what was reported (i.e. that the route was intranasal). But for this reason, we are more confident that the common mucosal immune system was induced if any reports of intranasal immunization also report immunity at a distal site other than the oral mucosal immune system. Second, our approach will be to evaluate protection or immune response through induction of antigen-specific IgG or IgA titers, the presence of antibody-secreting cells (ASCs) and/or induction of antigen-specific cell-mediated immunity (such as induction of IFNγ expression or lymphocyte proliferation), if reported. Unfortunately, the majority of the manuscripts examined here only report antibody production without correlation with the presence of ASCs in that organ. We are thus aware that there is the possibility that IgA and IgG antibodies may be in the blood and transported to the distal mucosal site via receptors such as pIgR or FcRn, respectively (Kaetzel et al., 1991; Raghavan et al., 1993; Stirling et al., 2005). Thirdly, if the antigen is delivered as part of a replicating bacteria, yeast, or virus, the possibility exists that the pathogen/vector can disseminate (unless the pathogen has a strict tissue-tropism) and/or the antigen is presented to distal mucosal sites through the migration of activated DCs. A careful examination of the types of DCs that may take up the antigen