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Colostral antibody-mediated and cell-mediated immunity contributes to innate and antigen-specific immunity in piglets

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ABSTRACT

Immunoglobulins and immune cells are critical components of colostral immunity; however, their transfer to and function in the neonate, especially maternal lymphocytes, is unclear. Cell-mediated and antibody-mediated immunity in sow blood and colostrum and piglet blood before (PS) and after (AS) suckling were assessed to investigate transfer and function of maternal immunity in the piglet. CD4, CD8, and γδ lymphocytes were found in sow blood and colostrum and piglet blood PS and AS; each had a unique T lymphocyte profile. Immunoglobulins were detected in sow blood, colostrum, and in piglet blood AS; the immunoglobulin profile of piglet serum AS mimicked that of sow serum. These results suggest selectivity in lymphocyte concentration into colostrum and subsequent lymphocyte transfer into the neonate, but that immunoglobulin transfer is unimpeded. Assessment of colostral natural killer activity and antigen-specific proliferation revealed that colostral cells are capable of influencing the innate and specific immune response of neonatal pigs.

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1. Introduction

Maternal immunity is transferred to offspring *in utero* across the placenta or after birth via the ingestion of mammary secretions. Neonatal piglets first receive maternal immunity in the form of colostrum since the porcine placenta prohibits transfer of immunity *in utero*. Therefore, successful absorption of colostrum is essential for disease prevention and growth of healthy pigs. Immunomodulatory and antimicrobial factors including antibodies and a variety of cells are integral parts of colostrum (reviewed in Wagstrom et al., 2000; Salmon et al., 2009). Maternal immunoglobulins are concentrated from the blood into colostrum in the mammary gland via an Fc-receptor dependent mechanism (Hammer and Mossmann, 1978). Lymphocytes derived from the common mucosal immune system migrate to the mammary gland (Salmon, 1987) and can be found in colostrum (Harp and Moon, 1988).

Cell-mediated immunity (CMI) is a necessary component of disease control, but the cellular contribution to colostrum has traditionally been overlooked in favor of non-cellular factors such as immunoglobulins. Colostrum is rich in cells; there are more than 1×10^6 cells per ml, and it is estimated that piglets obtain 5.0–7.0×10^8 maternal cells daily (Evans et al., 1982; Magnussen, 1999). A relatively high percentage of these cells are lymphocytic (15–25%; Le Jan, 1994). Using technetium-labeled cells (Tuboly et al., 1988) or fluorescein-labeled cells (Williams, 1993), it was demonstrated that maternal colostral cells cross the neonatal intestinal epithelium and are found in the neonate's circulation and immune tissues. However, heat-killed cells and cells from a source other than the piglet's own mother do not transverse the intestinal barrier (Tuboly et al., 1988; Williams, 1993). In contrast to immune cells, colostral immunoglobulins cross the neonatal intestinal epithelium and enter the circulation independent of source, whether it be immunoglobulins from another dam or from another species (Klobasa et al., 1981); however the efficiency of cross species immunoglobulin transfer may be increased in the presence of porcine colostrum (Jensen et al., 2001; reviewed in Sanglid, 2003). The mechanism involved in selective lymphoid cell transfer remains to be clarified.

Abbreviations: PS, pre-suckling; AS, after-suckling; CMI, cell-mediated immunity; AMI, antibody-mediated immunity; *M. hyopneumoniae*, *Mycoplasma hyopneumoniae*; NK, natural killer; CFSE, carboxyfluorescein diacetate succinimidyl ester; 7AAD, 7-aminoactinomycin D; ELISA, enzyme linked immunosorbant assay; S:P ratio, sample to positive ratio; PBMC, peripheral blood mononuclear cell; CMC, colostral mononuclear cells; APC, antigen presenting cell; IgA, immunoglobulin A; IgG, immunoglobulin G; conA, concanavalin A.

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Colostral lymphocytes express activation markers (Park et al., 1992), suggesting that they are functional. The ability of human colostral cells to respond to bacterial and viral antigen stimulation (Parmely et al., 1976; Ogra and Ogra, 1978) and to produce cytokines has been observed *in vitro* (Kohl et al., 1982; Skansen-Saphir et al., 1993). However, studies on the ability of colostral cells to respond in the recipient have largely relied on mitogenic responses (Riedel-Caspari and Schmidt, 1991; Williams, 1993).

The present study was undertaken to assess CMI and antibody-mediated immunity (AMI) in sow blood, colostrum, and piglet blood to investigate differences in transfer of specific immune components and the role of those immune components, specifically colostral lymphocytes, in immune development of young pigs. The results show that colostral lymphocytes are selectively transferred into piglet blood and that these colostral lymphocytes are capable of influencing the innate and adaptive immune response of neonatal pigs. These results have important implications for sow herd management and piglet health.

2. Materials and methods

2.1. Animals

Animals (English Belle; GAP Genetics, Winnipeg, MB, Canada) were housed at a commercial farm and treated in accordance with the University's Institutional Animal Care and Use Committee regulations. Sows were fed commercial corn–soybean based diets and water was provided *ad libitum*. Randomly chosen sows (12 of a group of 24) were vaccinated with a commercially available, federally approved *Mycoplasma hyopneumoniae* (*M. hyopneumoniae*; Respisure, Pfizer Animal Health, Kalamazoo, MI, USA) vaccine. Vaccination was performed at 5 and 3 weeks prior to farrowing according to the vaccine manufacturer's instructions.

All farrowings were monitored and piglets were placed into plastic tubs immediately after birth to prohibit suckling. Piglets were returned to their dams immediately following blood collection. Cross-fostering was not practiced. Sows and their litters were kept in individual crates; farrowing rooms were maintained at 18 °C. Heat lamps provided additional heat to newborn piglets for 72 h and subsequent extra heat was provided by heat-pads (Osborne, NE, USA).

2.2. Sample collection

Blood was collected from sows prior to farrowing and from their piglets pre-suckling (PS) and 24 h after (AS) colostrum ingestion. Blood for the PS piglet sample was collected within 1 h of birth. All blood samples were collected from the jugular vein into sterile EDTA vacutainer tubes (Becton Dickinson, Rutherford, NJ, USA). Colostrum was collected manually into sterile 50 ml conical tubes from all functional teats for a total volume of 25–30 ml. Teats were scrubbed with alcohol and gloves were worn to minimise contamination. Colostrum was obtained within 1 h of farrowing.

2.3. Lymphocyte purification

Peripheral blood mononuclear cells (PBMC) were isolated from whole blood by density centrifugation using Lymphocyte Separation Media (Cellgro, Inc., VA, USA; Bautista et al., 1999). Colostral mononuclear cells (CMC) were purified as described (Le Jan, 1994) with modifications. Colostrum was diluted 1:3 in sterile PBS and centrifuged at 800*g* for 10 min. The cell pellet was retained and the supernatant was centrifuged again at the same specifications. The cell pellets were combined and washed 3× and resuspended in PBS then centrifuged on Lymphocyte Separation Media for 35 min at room temperature at 1000*g*. The lymphocyte-enriched layer was retained and washed with PBS. Isolated PBMC and mononuclear colostral cells were resuspended in RPMI medium supplemented with 10% heat-inactivated, irradiated fetal calf serum, 2 mM L-glutamine, 100 U penicillin G per ml, and 100 μg of streptomycin per ml. The serum portions of blood and whey portions of colostrum were retained and stored at −80° C until IgG, IgA, and *M. hyopneumoniae*-specific antibody analyses were performed.

2.4. Lymphocyte phenotyping

Cells were enumerated using a hemocytometer and viability was assessed by trypan blue exclusion. Lymphocytes were phenotyped as described (Yang and Parkhouse, 1996) with modifications. Anti-pig CD8-PE, CD4-PE, and γδ-FITC antibodies (VMRD, Inc. Pullman, WA, USA) were added at a concentration of 1 μg/10^6 target cells in 200 μl of PBS. Cells were allowed to incubate for 30 min in the dark at room temperature. Samples were washed and immediately analysed via flow cytometry. A Facs Caliber flow cytometer was used and the FL-4 laser was calibrated using the manufacturer's calibration beads (Becton Dickinson Immunocytometry System, San Jose, CA, USA). Non-stained cells were used to establish a base line for phenotyping. Event acquisition was set at 10,000 within the regions encompassing PE or FITC depending on subset being analysed.

2.5. Natural killer assay

The natural killer (NK) assay was performed as described (Olin et al., 2005a). K562 cells were membrane stained with carboxyfluorescein diacetate succinimidyl ester (CFSE; 10 μM concentration; Immunochemistry Technologies, LLC, Bloomington, MN, USA). CFSE-stained K562 cells were added to PBMC or CMC at a ratio of 1:50. Samples were incubated for 4 h at 37 °C in 5% CO_2. The live/dead cell stain 7-Aminoactinomycin D (7AAD: Immunochemistry Technologies, LLC, Bloomington, MN, USA) was added and the samples were placed on ice for 15 min and analysed via flow cytometry. Non-stained effector (PBMC or CMC) and target cells (K562) were used to establish a baseline for NK activity. CFSE stained K562 cells in suspension with non-stained PBMC or CMC were used to verify the separation of effector and target cells. Water-lysed K562 cells stained with 7AAD were used to calibrate acquisition of live and dead K562 cells. Event acquisition was set for 10,000 events in a region encompassing the CFSE positive, and 7AAD positive and negative quadrants (upper and lower right quadrants only). NK activity was assessed by flow cytometry and analysed by CellQuestPro (Olin et al., 2005b).

2.6. Antigen specific blastogenesis

Mononuclear cells isolated from colostrum and blood were stained with CFSE as stated above and plated in duplicate in v-bottom 96-well-plates at a concentration of 5×10^5 cells per well. Cells were stimulated with 10 μg/ml purified *M. hyopneumoniae* antigen as described (Thacker et al., 2000); *M. hyopneumoniae* antigen was prepared as described (Bandrick et al., 2008). Nonstimulated cultures served as negative controls and concanavalin A (conA) stimulated cultures (5 μg/ml) served as positive controls. Experimental, negative, and positive controls were analysed for each animal. *M. hyopneumoniae*-specific proliferation data are described as the percentage of mononuclear cells proliferating to *M. hyopneumoniae* antigen minus the percentage of cells spontaneously proliferating in the negative control (*M. hyopneumoniae*-stimulated proliferation – nonstimulated proliferation).