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805 Immune components of colostrum and milk. K. Stelwagen*, T. T. Wheeler, and E. A. Carpenter, *AgResearch, Ruakura Research Centre*, *Hamilton, New Zealand*.

Colostrum and milk provide for a complete diet for the neonate. In ruminants it is also the sole source of initial immunity for the offspring. However, milk also plays an important role in host defence. The level of immunoglobulins is particularly high in colostrum, with IgG being the predominant immunoglobulin class in ruminant milk, compared to IgA in human milk. Immunoglobulin transport into milk is only partially understood. In addition to immunoglobulins, both colostrum and milk contain viable cells, including neutrophils and macrophages, which secrete a range of immune-related molecules into milk. These include cytokines and some antimicrobial proteins and peptides such as lactoferrin, defensins and cathelicidins. Mammary epithelial cells themselves also contribute to host defence through secretion of a range of innate immune effector molecules, such as lactoferrin, RNAses, lysozyme, cathelicidins and defensins. A detailed understanding of these proteins and peptides offers great potential to 'add value' to milk. This is demonstrated by the wide ranging commercial applications of milk-derived lactoferrin (e.g. as an ingredient in food, toothpaste, cosmetics and fish food). Knowledge of the immune function of milk, in particular how the gland responds to pathogens, can also be used to boost the levels of immune factors in milk through farm management practices and vaccination protocols. The latter approach is currently being used to maximise yields of bovine milk-derived IgA directed at specific antigens for therapeutic and prophylactic use. Increasingly sophisticated proteomics technologies are being applied to identify and characterise the function of the minor components of milk. An overview of immune factors in colostrum and milk as well as the results of research aimed at realising this untapped value in milk will be presented.

Key Words: Milk, Innate Immunity, Mammary Gland

806 Mammary immunology and protection of the neonate. H. Salmon*, *IASP, Lymphocyte et Immunité des Muquesuses*, *Nouzilly, France*.

Colostrum and milk, secretions of mammary gland (MG) are the two components of the post-natal delivery of maternal immunity to the neonate. In monogastrics, sIgA is the predominant colostrum or milk Ig depending upon to the degree of prenatal Ig transfer, whereas in ruminants IgG1 predominate. In ungulate such as swine and ruminant absence of prenatal Ig transfer is compensated for by IgG enriched colostrum. These immunoglobulins enter neonate circulation and provide the newborn with the maternal serum antibodies that arose from antigenic stimulation of the mother's systemic immune system and sustain the systemic protection of the neonate against invasive pathogens. In contrast, the passive mucosal protection of neonatal mammals is dependent on the continuous supply until weaning of maternally dimeric IgA (Monogastric) and IgG1 (Ruminants), the so-called lactogenic immunity.
Based on multistep model of lymphocyte migration between compartments we analyzed the spatio-temporal relationships between adhesion molecules and chemokines in the gut and/or nasal mucosa and MG. In sows and mice, localization of $\alpha 4\beta 7$ T-cells follows MadCAM-1 development on capillaries. In contrast, $\beta 7$/c-IgA B plasmablasts increased in mid- and late lactation when MadCAM-1 density declined; this result implicates additional factors; one of these has been identified as chemokine CCL28 (MEC) interacting with CCR10 receptor onto sIgA B cells. As the same pattern of adhesion molecules and chemokines was observed in small gut and nasal mucosa, that indicates the existence of a cellular link between the upper respiratory tract and MG in addition to the entero-mammary link. By comparison, absence of MadCAM-1 in ruminant MG is in agreement with the absence of a link with the intestinal immune system and explains the low levels of IgA in bovine mammary secretions. Further, in ruminants and in mice VCAM-1 is not present on capillaries in lactation but only on larger blood vessels.
In conclusion, knowledge of these humoral and cellular factors of mucosa-mammary links may pave the way of optimal route of vaccination to protect the mammary gland itself and to protect the neonate via its secretion.

807 Characterisation of the bovine RNAse gene family: Evidence for rapid evolution and acquisition of an innate immune function in the mammary gland. T. T. Wheeler*[1], N. Maqbool[2], A. Molenaar[1], P. Harris[1], and M. Callaghan[1], [1]*AgResearch*, *Hamilton, Waikato, New Zealand*, [2]*AgResearch*, *Mosgiel, New Zealand*.

The mammalian RNAse A family of genes comprises a cluster of 13 genes on human chromosome 14 that share significant similarity with RNAse A (RNAse1). Several members of the family are secreted from circulating immune cells and some mucosal epithelia. RNAse5, which is secreted by intestinal cells, has been reported to have bactericidal activity. RNAse5 is also secreted from mammary epithelial cells and is present in milk. The aim of this study was to address the hypothesis that members of the RNAse family have undergone rapid evolution (typical for many innate immune effector proteins) and play a role in the host defence function of bovine milk.
Analysis of the assembled bovine genomic sequence revealed significant expansion of the RNAse locus in cattle, with 22 orthologous or paralogous RNAse genes being identified within the cluster. As with the human RNAses, the protein coding regions of the bovine RNAse genes were contained within a single exon. Orthologues for 12 of the 13 human RNAse genes were found, the exception being RNAse3. An additional 10 genes appeared to be found only in cattle. Two of these encode a significantly shortened protein, and thus appear to be pseudogenes. Phylogenetic and substitution analyses suggest that the bovine RNAse locus is under evolutionary pressure, consistent with rapid evolution.
Northern, quantitative PCR, in-situ hybridisation and western analyses of RNAse4 and RNAse5 revealed these genes are expressed most abundantly in the mammary gland, liver and small intestine. RNAse5 was purified from milk and tested for antimicrobial activity. Microbicidal activity was obtained against Candida albicans, with a 50% kill rate being obtained at a concentration of 31ug/ml. This level of activity was typical among four independent purifications). However, no growth

suppression activity was observed in RPMI medium. The results are consistent with the idea that some members of the RNAse family have a role in host defence against pathogens in the mammary gland and/ or digestive tract.

Key Words: Lactation, Mammary, RNAse

808 Neonatal protection by an innate immune system of human milk consisting of oligosaccharides and glycans. D. S. Newburg*, *Massachusetts General Hospital and Harvard Medical School*, *Boston, MA*.

Infants not breastfed have a higher incidence of severe diarrhea and respiratory diseases than those breastfed. In the past, this had been attributed primarily to human milk secretory antibodies. However, the oligosaccharides are major components of human milk, and milk is also rich in other glycans, including glycoproteins, mucins, glycosaminoglycans and glycolipids. These milk glycans, especially the oligosaccharides, are comprised of thousands of components. The milk factor that promotes gut colonization by Bifidobacterium bifidum was found to be a glycan, and such prebiotic characteristics may contribute to protection against infectious agents. However, the ability of human milk glycans to protect the neonate seems primarily to be due to their inhibition of pathogen binding to their host cell target ligands. Many such examples include specific fucosylated oligosaccharides and glycans that inhibit specific pathogens. Most human milk oligosaccharides are fucosylated, and their production depends on fucosyltransferase enzymes; mutations in these fucosyltransferase genes are common, and underlie the various Lewis blood types in humans. Variable expression of specific fucosylated oligosaccharides in milk, also a function of these genes (and maternal Lewis blood type), is significantly associated with the risk of infectious disease in breastfed infants. Human milk also contains major quantities and large numbers of sialylated oligosaccharides, many of which are also present in bovine colostrum. These could similarly inhibit several common viral pathogens. Moreover, human milk oligosaccharides strongly attenuate inflammatory processes in the intestinal mucosa. These data support the hypothesis that oligosaccharides and other glycans are the major constituents of an innate immune system of human milk whereby the mother protects her infant from enteric and other pathogens through breastfeeding. These protective glycans may prove useful as a basis for the development of novel prophylactic and therapeutic agents that inhibit disease by mucosal pathogens in many species.

809 Immune signaling during mammary development and involution. C. J. Watson*, *University of Cambridge*, *Cambridge, UK*.

Dramatic changes in cell composition and function occur in the mammary gland during a pregnancy/ lactation/involution cycle. We have investigated the transcriptional changes associated with these biological events using microarray analysis and identified the critical genes involved using genetically modified mice. Two surprising findings arose from these studies. Firstly, our microarray data showed that post-lactational regression was associated with an acute phase/inflammatory response in addition to cell death. Conditional deletion of Stat3, or the NF-κB regulatory kinase IKKβ, resulted in a failure to induce cell death indicating that these signaling pathways are essential mediators of the involution process. Both Stat3 and NF-κB have been shown regulate acute phase gene expression in addition to apoptosis regulators. Four distinct transcriptional profiles are present in the first 4 days of involution while there are three in lactation. At the peak of lactation (day 10 in mouse) over 400 genes reach their maximum expression level before declining dramatically in the first 12 hours of involution. A reciprocal pattern was observed for over 500 genes that were specifically upregulated within the first 12 hours of forced involution. We are now investigating the role of a subset of these genes in involution. We also uncovered a role for genes normally associated with immune cell signaling in differentiation of luminal mammary epithelial cells during pregnancy. Genetic deletion of the transcription factor Stat6 resulted in delayed development during pregnancy and this phenotype was recapitulated in mammary tissue from IL-4/IL-13 doubly deficient mice. Furthermore, we showed that mammary epithelial cells secrete T cell regulatory cytokines. T helper (T_H) type 1 cytokines such as IFNγ and IL12a are secreted by undifferentiated mammary epithelial cells while T_H type 2 cytokines including IL-4 and IL-13 are secreted by differentiated cells. This unexpected finding demonstrates a role for immune cell signaling in mammary epithelial cell fate and function. Support by BBSRC and AICR is acknowledged.

810 Effect of lipopolysaccharides on plasminogen activator activity and lactoferrin mRNA expression in a bovine mammary epithelial cell line. C. Pecorini, R. Rebucci, E. Fusi, F. Galante, L. Rossi, F. Cheli, and A. Baldi*, *University of Milan*, *Milan, Italy*.

The aim of this work was to examine the urokinase-plasminogen activator (uPA) activity and the mRNA expression of bovine lactoferrin (bLf) after LPS treatment using BME-UV1 (Bovine Mammary Epithelia – University of Vermont 1) cell line as an in vitro model of bovine mammary epithelium. In a preliminary experiment, the effect of LPS on cell growth was examined. Cells were incubated with medium containing 0, 1, 10 or 20 μg/ml of LPS from Escherichia coli O111:B4 for 24 h. The effect of LPS on cell growth was evaluated by MTT test. In order to evaluate the effect of LPS exposure on uPA activity and mRNA bLf expression, cells were treated with 0, 1, 10 or 20 μg/ml of LPS from E. coli O111:B4 for 3, 6, 12 or 24 h. Media were stored at -80°C for the determination of uPA activity using a colorimetric assay and for the quantification of bLf using an ELISA kit. Cells were recovered and total RNA was extracted. The bLf mRNA expression was evaluated by RT-PCR. The experimental design included at least three replicates per treatment and all treatments were repeated twice. Results were evaluated using the GLM procedure of SAS. MTT test provided evidence that LPS treatment did not have any effect on cellular proliferation of BME-UV1 cell line. uPA activity was not affected by LPS treatments at 3 and 6 h, whereas 1 and 10 μg/ml of LPS significantly stimulated ($P<0.05$) uPA activity at 12 and 24 h. After 24 h of LPS exposure (1 and 10 μg/ml) bLf secretion was stimulated compared to the control ($P<0.05$). RT-PCR analysis confirmed that bLf is constitutively expressed by BME-UV1 cells and LPS treatments slightly modified bLF mRNA expression depending on time and concentrations. Under our experimental conditions, BME-UV1 represented an useful in vitro model to study the inflammatory response in mammary epithelial cells.

Key Words: Lipopolysaccharides, uPA, Lactoferrin

811 Pathogen-dependent variations in the innate immune response to intramammary infection. D. D. Bannerman*, *USDA-ARS, Beltsville Agricultural Research Center*, *Beltsville, MD*.

Mastitis remains one of the most prevalent diseases among dairy cows and one of the most economically costly diseases to the dairy industry. The majority of cases of mastitis result from intramammary bacterial infection, and numerous genera of bacteria are capable of inducing mastitis. Following penetration of the physical barriers of the teat canal, the innate immune system encompasses the initial and primary mechanism by which the cow defends itself against invading bacterial pathogens. It has been well-described that some pathogens are readily eliminated from the mammary gland, and the accompanying mastitis quickly resolved. In other cases, bacterial pathogens can persist in the gland resulting in chronic mastitis that may endure throughout the lifespan of the cow. During the past few years, there have been rapid gains in the knowledge surrounding the innate immune responses that are evoked in response to intramammary infection. A major finding from this area of research is that despite the highly conserved nature of the elements involved in host innate immunity, marked variation in innate immune responses to different bacterial pathogens can occur. This review summarizes evidence that the nature of the innate immune response, as well as the rapidity in which it is evoked, influences the outcome of intramammary infection. Furthermore, the utility that immune response modifiers may have in preventing and/or treating mastitis will be considered.

Key Words: Immunity, Infection, Mastitis