# MAMMARY SERUM AMYLOID A3, PROGRESS TOWARD DETERMINING STRUCTURE AND FUNCTION.

A. Molenaar, P. Harris, J. Green, M. Callaghan, K. Kim, R. Wieliczko, K. Johannessen, R. Dines, R. Watson, V. Arkus, J. Dalziel, K. Singh, G. Rajan, M. Pearson, V. Farr, M. Miles, K. Oden, L. Good, R. McLaren, C. Prosser, M. Grigor, S. Davis, K. Stelwagen.
*AgResearch, Ruakura Research Centre, Hamilton, New Zealand.*
*Email*: *adrian.molenaar@agresearch.co.nz*

The serum amyloid A protein (SAA) family are one of the major reactants in the acute-phase response (Sellar 1993, Eckersall 2004). We found the mRNA encoding for the SAA protein (M-SAA) to be present in bovine mammary tissue by representational difference analysis in a comparison of RNA from normal and involuting quarters of a dairy cow udder (Molenaar, 1999). SAA3 proteins have also been detected by in situ hybridisation in a range of human tissues including; some epithelial cells of the intestine, breast and pancreas (Urieli-Shoval, 1998). It is thought that local serum amyloid production may fulfill a short term requirement (Sellar, 1993), or that the locally expressed proteins may play a role related to the site of expression and be produced under conditions that do not initiate the systemic acute phase response (Urieli-Shoval, 1998).

The mRNA was found by in situ hybridisation to be localised to restricted populations of mammary epithelial cells. *In-situ* hybridization, northern analysis and real-time PCR revealed that it was expressed at a moderate level in late pregnancy, at a low level throughout lactation, was induced during early milk stasis, and expressed at a high level during mid to late involution and inflammation/mastitis. The expression patterns of the mRNA and the location of the protein in the udder were consistent with an involvement of M-SAA in mammary remodelling during pregnancy and involution, and response to stress caused by cellular engorgement and consequent inhibition of milk protein mRNA transcription, and, as it was elevated during mastitis, during inflammation and infection. The association of the M-SAA with the vesicle or fat globule membranes was demonstrated by immunohistochemistry and this association is also consistent with a role in the removal of accumulated lipid which becomes trapped in the alveoli during milk stasis (Molenaar, 1995). An extra-mammary role has been suggested in that M-SAA, and in particular its TFLK motif, has been shown to be protective in neonates and possibly adults against gastrointestinal infections by inducing mucin production (McDonald 2003).

In order to attempt to clarify the function of the M-SAA we cloned the cDNA, expressed the mature peptide in *E. coli*, confirmed the reading frame using LCMS, developed a sensitive antibody and attempted the isolation of the recombinant and native form for functional testing. Difficulties were encountered such as poor solubility and aggregation, nevertheless these give some clues about the properties of the molecule. Various extracts have been tested in direct and indirect assays and the work is ongoing. Our experiences, the results of bioinformatic and theoretical structural studies, and future plans will be presented.

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