ImmunoTools *special* Award 2013

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Immunophenotyping of milk somatic cells in murine and in bovine species (immune cells and epithelial cells)

Objectives

1. Progress in the characterization of stem and progenitor cell populations in the bovine and murine (used as experimental model) mammary gland thought biochemical, immune and functional approaches
2. Evaluation of the quantitative and qualitative differences of these cell populations in relation to age of the animal and its physiological state during pregnancy and lactation by analyzing somatic cells in milk
3. Find a relationship between the state of "wear" of the mammary gland according to the level of aging and longevity in the production with a correlation between specific population of somatic cells (progenitors) in lactation.

Background:

The complicated and extensive modifications to which the mammary gland in mammalians and more specifically in bovine, cyclically undergoes can be attributed to adult stem cells. Since the amount of milk that a cow is able to produce at any time is closely related to the number of epithelial cells secreting, the more the mother will be able to maintain a high number of these cells, higher will be milk production. In lactation curve of dairy cow, after an initial increase due to an increase in the secretory capacity of the epithelial cells, this is kept constant and the slow decline of productivity is due to a slow and steady reduction in the number of epithelial cells. We have collected information about the amount of these cells by collecting samples of mammary tissues, however these data represent only a single moment in the bovine productive life without providing information on variations associated with specific conditions (quiescence, pregnancy, early vs late lactation). Very recently it has been reported as milk is an alternative source for progenitor cells.

My research group was able to functionally demonstrate the existence of a population of adult stem cells in ruminants and proposed a method based on flow cytometry to isolate different subpopulations of progenitors [Martignani et al, 2010, Prpar et al., 2012]. We have now also unpublished data that show how luminal (ALDH1) progenitor cells and 49f-positive cells observed by flow cytometry in somatic cells isolated from the milk of a primiparous Friesian cows. We are interested to understand the physiological role of this cell population in milk both in murine and in bovine species, possibly with a better immune characterization.

Methodology section related to FACS analysis:

Cells recovered from milk will also be analyzed by CFC assay in order to better characterize progenitor content and an aliquot of cells will be subjected to cytospin and subsequent immunocytochemistry to assess the expression of several epithelial cell markers both of the luminal

lineage (CK18, MUC1) and the myoepithelial lineage (CD49f; Musashi 1, CK14, p63, αSMA, Notch 3 receptor, nuclear receptor subfamily 5 group A member 2, nucleoporin 153 and fibronectin type III domain containing 3B). The last three markers have been very recently examined and we consider them very interesting as potential markers for bovine mammary stem cells. We found that NR5A2 and NUP153-positive nuclei were more abundant in prepubertal than lactating mammary glands and their distributions were consistent with expectations for a MaSC marker. FNDC3B was localized mainly in the nucleus prepubertally and in the cytoplasm during lactation. Furthermore, preliminary results showed colocalization of the novel markers with label retaining mammary stem cells, whereas Msi1 staining was distributed in a fashion consistent with mammary stem cell localization.

Immunophenotyping both in bovine and murine milk somatic cells will focused on markers like EpCAM, MUC1, CD24, CD49f are able to separate adult stem cells, myoepithelial progenitors and luminal progenitors (cytokeratin18 and cytokeratin 14). We also are going to carry out immunophenotyping for leukocytes present in milk according to stage of lactation and age. We are interested to test monoclonal antibodies that crossreact works in bovine, we just set up protocol for CD4 but we are interested also for CD3, CD8, CD11, CD14. A great effort has been done in these years to validate monoclonal antibodies, not only for leukocytes, that usually work in human and murine species for bovine. A great change would be to have the opportunity to test other Abs for this purpose.