

## **Intestinal Regulation of Neonatal Development of B Lymphocyte Subsets**

The period immediate after birth is the most challenging period for the developing immune system. This is particularly true in domestic animals, which develop in utero in the absence of exogenous antigen and are immediately bombarded with a diverse array of environmental microflora upon birth. During this early period, very few circulating B cells are present and the majority of neonatal immunity is obtained passively from the mother via the milk and colostrums. Immediately post-birth, there is an explosion in the production of new B cells which eventually rise from a minimal 5-10% of peripheral blood lymphocytes at birth to a stable level of roughly 50% of PBLs at 6 months of age. Recently, it has become clear that colostrums contains not only maternal antibody, but also cells and soluble factors which may stimulate B cell production. Furthermore, a unique subset of non-recirculating B cells develops beginning at 6-8 weeks of age, to reach levels of roughly 50% of peripheral blood B cells. Coincidentally, the appearance of this subset develops alongside the development of reactivity to a number of intestinal microflora. This research project is aimed at investigating two distinct questions: (a) the role of colostrums, and specifically soluble CD14 and macrophages, at promoting the development and release of ileal Peyer's patch B cells in the neonatal period. (b) the role of intestinal microflora in the development of B cell subsets and immune competence. Identification of the unique stages of B cell development have relied and continue to rely upon 2, 3, and 4-colour phenotypic analysis to define functional and developmental subsets. The experiments to define the function and development of these subsets will necessarily rely upon the use of fluorescent tracking dyes (PKH, CellTracker dyes, 5,6-Carboxyfluorescein succinimidyl ester) in conjunction with multicolour flow cytometry. Typically, mature B cell subsets can be defined based upon the combined expression of surface immunoglobulin, CD21, and either CD11b or CD11c. The addition of either a red or green fluorescent tracking label to monitor the phenotypic changes and the physiological growth of the B cell pools will require 4-colour cytometry to obtain statistically meaningful results. These data will allow a greater understanding of the unique development of the ruminant immune system, and enhance the utility and design of neonatal agricultural vaccines.