ImmunoTools *special* Award 2015

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Characterization of the earliest human immune responses in the intestine

Necrotizing enterocolitis (NEC; severe intestinal inflammation) is one of the most critical diseases in preterm infants with a mortality rate of 30%. In addition, the surviving infants are often inflicted with severe morbidities. Although NEC has been researched extensively, the pathogenesis remains unresolved and an effective therapeutic intervention is lacking.

Based on studies that show the absence of memory T (T_{mem}) cells in cord blood, it is assumed that the adaptive immune system in the neonate is naïve and favoring regulatory responses after birth. However, the infant gut is capable of mounting extensive inflammatory responses such as is seen in NEC. Interestingly, our group has shown that $CD4^+$ T cells with a memory phenotype ($CD45RO^+CCR5^+$) are present in the gut mucosa of the human fetus. Furthermore, the majority of fetal mucosal $CD4^+$ T_{mem} cells express $ROR\gamma t^+$ which is indicative of T helper (T_h) 17 cells *(Bunders et al., Blood, 2012, 120:4383-4390)*.

T helper cells play a crucial role in shaping immune responses. However, when not controlled appropriately, they contribute to the pathogenesis of inflammatory diseases. We hypothesize that in the preterm infant, $CD4^+$ T_{mem} cells with a T_h17 profile are key players in the pathogenesis of NEC as the induction of a T_h17 response in the relative absence of regulatory T (T_{reg}) cells can activate inflammatory pathways when infants are prematurely exposed to pathogens and food antigens. $CD4^+$ T_{mem} cells are induced by dendritic cells (DCs), but it is unknown how this process takes place in the fetal and infant gut. We want to elucidate which T_h subsets are present in the neonatal gut and unravel the critical steps that allow the induction of inflammatory responses. This research will provide the opportunity to devise potential strategies to prevent or treat inflammatory diseases such as NEC, for example utilizing DCs to skew T-cell responses towards regulation.

In order to identify $CD4^+$ T_{mem} cells in the neonatal gut, we have thus far used 3-color immunohistochemistry (IHC). However, to fully understand the contribution of T helper cells to the pathogenesis of NEC, a deeper understanding of their nature and functioning is required *(Farber et al., Nat. Rev. Immunol., 2014, 14:24-35)*. Therefore, we aim to identify subtypes of $CD4^+$ T cells with specific functional roles using flow cytometry which allows for the assessment of up to 18 single-cell characteristics at a time.

We will determine the phenotypical and functional traits of the $CD4^+$ T cells in unique intestinal tissues of 15 fetuses without NEC and preterm infants with NEC. We will use the hallmark antibodies for T-cells (CD3, CD4, CD8, CD45) that reveal antigen exposure (CD45RA, CD45RO), homing (CD62L, CD103, CCR7), co-stimulation (CD27, CD28), and activation (CD25, CD69, CD95, HLA-DR).

Furthermore, we intend to investigate the potential of using DCs to skew T cell responses. However, at present nothing is known regarding DC populations in the human neonatal gut. Characterization of DCs in human tissue requires the identification of $CD3^-CD19^-CD20^-CD56^-HLA\text{-}DR^+$ cells. Further differentiation into DC subsets requires CD14, CD141, CD123, CD1c, CD1a, and CD304.

Together these studies are aimed at gaining a better understanding of the induction of memory responses in the neonatal intestinal immune system which will lead to a better understanding of the underlying pathogenesis of NEC. Furthermore, by skewing these immune responses away from inflammatory responses and towards regulation by T_{regs} this may provide the opportunity to develop preventative and treatment strategies for NEC. The antibodies from ImmunoTools would greatly aid in creating the opportunity to assess this severe disease in preterm infants and additionally would help unravel the earliest human adaptive immune responses with the potential for treating other inflammatory diseases and enhancing vaccination strategies in infancy.

ImmunoTools *special* AWARD for **Renée Schreurs** includes 24 reagents

FITC - conjugated anti-human CD1a, CD3, CD19, CD20, CD45, CD45RA, CD56, HLA-DR,

PE - conjugated anti-human CD3, CD4, CD8, CD45, CD95,

PerCP - conjugated anti-human CD3, CD4, CD8, CD45, CD45RA,

APC - conjugated anti-human CD3, CD4, CD8, CD25, CD62L, CD69

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