STRONG CHILDREN'S RESEARCH CENTER

Summer 2016 Research Scholar

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ABSTRACT

Title: ***Role of CD31 in Naïve Neonatal T Cell Activation***

Background: CD31 (PECAM-1) is a platelet endothelial cell adhesion molecule that is cross-linked in the presence of antigen presenting cells, resulting in the phosphorylation of an immunoreceptor tyrosine inhibitory motif (ITIM), and downstream inhibition of T cell activation.[1] CD31 has been shown to inhibit activation-induced cell death (apoptosis) and promote proliferation.[2,3] Our research indicates that infants born at <29 weeks gestational age and with <60% CD31+CD4+ T cell frequencies at time of hospital discharge are 3.5-fold more likely to develop Persistent Respiratory Disease (PRD, manuscript in progress). T cell CD31 expression strongly correlates with gestational age at birth.[4] These finding may be important because preterm neonates (born at less than <37 weeks gestation) are more susceptible than full term neonates to intracellular pathogen infection,[5] suggesting a deficiency in T cell function. Around 50% of preterm infants born at <1500g (Very Low Birth Weight, VLBW) are diagnosed with pulmonary morbidity at one year of life.[6] VLBW is also associated with T cell activation.[7] Based on the observation that pulmonary morbidity is correlated with decreased CD31 expression in preterm neonates, **we hypothesize that CD31 plays a central role in the regulation of naïve neonatal T cells, and loss increases potential for immunopathology and suboptimal protection from infection.**

Objective: The purpose of this study is to determine the effect of CD31 activity on proliferation (expression of Ki67), apoptosis (CC3), and differentiation (CD45RO) in naïve and TCR-activated neonatal T cells. Additionally, the study will determine if this effect is due to direct engagement of a CD31 cross-linking antibody and if CD31's activity may be modulated with enhancers and inhibitors. Finally, the study will determine differences in TCR signal strength by comparing calcium flux between CD31+ and CD31- naïve neonatal T cells.

Results: Cellular markers of T cell activation were measured by flow cytometry in umbilical cord blood mononuclear cells from full term infants and peripheral blood mononuclear cells from healthy adult donor controls. We measured an increase in Ki67 expression and a decrease in CC3 expression in activated neonatal T cells in the presence of a CD31 cross-linking antibody. Activated neonatal T cells were also less prone to differentiation during proliferation when compared to healthy donor controls. Changes in Ki67 and CC3 expression were not observed when activated neonatal T cells were treated with autologous CD31 supernatant containing secreted mediators from a previous experiment. However, activated neonatal T cells exhibited increased proliferation and decreased apoptosis in the direct presence of a CD31 cross-linking antibody. Variable findings in regard to dose-response changes in Ki67, CC3, and CD45RO expression in CD4+ and CD8+ cells were observed in the presence of metalloprotease inhibitor and CD31 Inhibitor. Results also indicated that naïve neonatal and adult CD31+ T cells exhibit decreased calcium flux compared to CD31- cells.

Conclusion: Our results support the hypothesis that direct engagement of CD31 modulates naïve neonatal CD4+ T cell signaling as evidenced by increased proliferation, decreased apoptosis, and decreased calcium flux in activated neonatal T cells. Our results also

demonstrate that fewer activated neonatal T cells differentiate during proliferation compared to activated adult T cells. These findings suggest that the inhibitory signal of CD31 modulates activation threshold in naïve neonatal CD4+ T cells. In addition, CD31 on neonatal T cells may be important in proliferation and prevention of activation-induced cell death downstream of TCR engagement in the context of infection. Understanding the role of CD31 during neonatal development may provide important insight into mechanisms underlying their susceptibility to infection and subsequent respiratory morbidity.

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