



## 2011 Research Grant Program Winning Abstract

### Neonatal Immunity and CD36<sup>hi</sup> Monocytes

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Umbilical cord blood (CB) has been used as a source of hematopoietic stem cells (HSC) in the treatment of patients for the last 30 years. The success of CB in transplantation is partially due to the low rate of rejection by the host immune system and the low incidence of graft-versus-host disease (GvHD). It is generally accepted that this low rate of rejection is due in part to the immaturity of the infant immune system, which is highly immunosuppressive. For infants, this immunosuppressive state makes them susceptible to numerous infectious diseases and limits their immune responses to protective and life-saving vaccines. At the same time, this state of immunosuppression is life saving because it prevents the infant from being in a constant state of inflammation due to the onslaught of thousands of environmental microorganisms to which the infant is exposed immediately after birth. The immunosuppressive state also abrogates reactivity to non-inherited maternal antigens such as milk proteins.

While numerous studies demonstrate the weak immune response of infants, relatively little is known about the mechanism by which this response is regulated, including how the immunosuppressive state is maintained, and the mechanisms by which children outgrow this suppression and become immune competent. We have performed preliminary studies to obtain insight regarding the immunoregulatory mechanism of the infant immune system. A striking difference between CB and blood from adults is the ratio of monocytes to lymphocytes. Whereas most mononuclear cells in adult peripheral blood are CD3<sup>+</sup> lymphocytes, most mononuclear cells in CB are CD14<sup>+</sup> monocytes. Our preliminary study identified two populations of monocytes (CD14<sup>+</sup>) in CB, CD36<sup>hi</sup> and CD36<sup>low</sup>, with the majority of these being CD36<sup>hi</sup>. By culturing CD36<sup>+</sup> monocytes with naïve T cells, we found that the CD36<sup>hi</sup> monocytes specifically drive development of Foxp3<sup>+</sup> regulatory T (Treg) cells in a transforming growth factor beta (TGF-beta) dependent manner. These Foxp3<sup>+</sup> Tregs remain Foxp3<sup>+</sup> over 8 weeks in culture and have potent immunosuppressive functions. These data lead us to hypothesize that the immunosuppressive state of cord blood is due in part to the predominance of immunosuppressive CD36<sup>hi</sup> monocytes, which promote expansion of Treg cells.

Foxp3<sup>+</sup> regulatory T cells play significant roles in maintenance of immunological tolerance against self and non-self antigens. Ex vivo expanded Foxp3<sup>+</sup> Tregs enabled antigen-specific immune modulation against transplanted antigens in animal models. While the method for induction of Foxp3<sup>+</sup> Tregs is well established for murine T cells, de novo induction of stable human Foxp3<sup>+</sup> Tregs remains unsuccessful. Our system using CB CD36<sup>hi</sup> monocytes provides a unique opportunity to study the mechanism of human Foxp3<sup>+</sup> Treg generation. Though retinoic acid and aryl hydrocarbon receptor (AHR) ligands have been shown to enhance the induction of Foxp3 expression, our data showed that these factors do not play significant roles in Treg induction by CD36<sup>hi</sup> monocytes. The data indicate that CD36<sup>hi</sup> monocytes induce Foxp3<sup>+</sup> Tregs in a mechanism discrete from previously reported stimulation.



We hypothesize that CD36<sup>hi</sup> monocytes provide a thus far unidentified “second” signal to induce Foxp3<sup>+</sup> Tregs. This second signal may be mediated by (i) an unidentified molecule produced by CD36<sup>hi</sup> cells, and/or by (ii) TGF-beta/T-cell antigen receptor (TCR) stimulation by CD36<sup>hi</sup> cells in a manner distinctive from the soluble form of TGF-beta stimulation. These two possibilities are not mutually exclusive. A significant fraction of CD36<sup>hi</sup> monocytes, but not CD36<sup>low</sup> monocytes, expresses surface latency associated peptide (LAP), which forms a complex with active TGF-beta and tethers the complex to extracellular matrix by binding to latent TGF-beta binding protein (LTBP) or Glycoprotein A repetitions predominant (GARP). Direct contact is required for Foxp3<sup>+</sup> Treg induction by CD36<sup>hi</sup> monocytes. Based on these data, we hypothesize that CD36<sup>hi</sup> cells provide stimulation via membrane-bound TGF-beta to naïve T cells and provoke a signaling process distinct from that of soluble TGF-beta to establish stable expression of Foxp3.

Aim 1. Determine the molecular mechanism of membrane localization of the TGF-beta/LAP complex by CD36<sup>hi</sup> monocytes

Aim 1.1 To determine the isoform of LTBP or GARP expressed by CD36<sup>hi</sup> cells and decipher their roles in membrane LAP tethering

Aim 1.2 To determine if CD36 plays a functional role in activation of TGF-beta and induction of Foxp3<sup>+</sup> iTregs by CD36<sup>hi</sup> monocytes

Aim 2. Determine the TGF-beta induced signaling process following CD36<sup>hi</sup> monocyte stimulation

Aim 2.1 To determine the spatial and temporal regulation of TGF-beta receptor localization during the activation of T cells by CD36<sup>hi</sup> monocytes

Aim 2.2 To test if CD36<sup>hi</sup> cell induced-TGF-beta receptor activation provokes crosstalk with the TCR, distinctive from soluble TGF-beta-induced activation

To complete this study, reagents and equipment from BD Biosciences will play essential roles. We plan to use a variety of antibodies against human surface molecules such as GARP, CD36, and LAP. To analyze the signaling process, we will utilize the BD Phosflow™ system, while BD™ Cytometric Bead Array will be used to quantify multiple cytokines. Cell sorting will be performed using the BD FACSAria™ system, and the data analysis will be carried out using the BD FACSAria or 2-photon/confocal microscopes. Naturally, the study will utilize tissue culture reagents and tools such as tissue culture plates, pipettes, and media additives.

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