



2008 Research Grant Program Winning Abstract

Role of SOCS in Treg Function

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Although it is clear that regulatory T cells (Tregs) play a critical role in the prevention of autoimmune disease, and conversely, in the inability of the immune system to eliminate certain cancers; there are not any clinically approved methods to exploit their use as therapeutic agents. Suppressors of Cytokine Signaling are a family of intracellular proteins with the ability to minimize immune responses by inhibiting cytokine signaling. Although cytokines play an essential role in the selection and survival of Tregs, the role of SOCS-1 in these processes has not been examined. We propose to examine the role of SOCS-1 in the development, survival, and peripheral function of Tregs by using a mouse model system lacking the expression of the SOCS-1 gene (SOCS-1 KO), and peptide mimetics of SOCS-1 that have shown to be restorative of SOCS-1 function. Of significance, SOCS-1 KO and mice lacking Tregs both die of a similar autoimmune disease three weeks after birth, suggesting that the two regulatory pathways may be interrelated. The objectives of this application are: 1) to characterize the thymic development and peripheral survival of Tregs in SOCS-1 KO mice, 2) to assess the capacity of Tregs from SOCS-1 KO mice to regulate immune responses, and 3) measure the capacity of T cells from SOCS-1 KO mice to become induced regulatory T cells (iTregs). Our long term goal is to utilize therapeutic strategies that incorporate the manipulation of Tregs in the treatment of autoimmune diseases and some cancers. The rationale is that once a clear role of SOCS-1 in the development and function of Tregs is established, manipulation of the development and/or function of Tregs can be accomplished through the use of SOCS-1 agonist or inhibitory peptides. In addition to potentially rescuing SOCS-1 mice from death, the studies present a novel approach to the treatment of a number of autoimmune diseases and some cancers.

Specific aim 1: Characterize the thymic development and peripheral survival of regulatory T cells (Tregs) in SOCS-1 gene deficient (KO) mice. The working hypothesis is that since SOCS-1 KO mice have abnormal T cell thymic development and die within 21 days after birth, Treg development and/or survival differs in SOCS-1 KO mice compared to normal mice. Using BD antibodies conjugated to fluorochromes for flow cytometry, we will compare the thymic and peripheral profiles of Tregs in SOCS-1 KO mice to healthy, SOCS-1 sufficient mice. Given that SOCS-1 deficient mice succumb to an inflammatory cytokine type disease, we will analyze the inflammation profile using a BD Cytometric bead array.

Specific aim 2: Assess the capacity of Tregs present in SOCS-1 KO mice to regulate immune responses. Our working hypothesis is that Tregs from SOCS-1 KO mice will have reduced capacities to inhibit T cell proliferation and cytokine production. We will compare the abilities of Tregs from SOCS-1 KO mice and healthy SOCS-1 sufficient mice to regulate immune responses. Tregs will be isolated by magnetic activation cell sorting (MACS) and flow cytometry using BD antibodies. The capacity of the SOCS-1 sufficient and deficient Tregs to inhibit the proliferative capacity activated T cells and cytokine production will be assessed by co-incubating enriched T cell population (BD



enrichment kits) with Tregs on mouse BD biocoat T cell activation plates. After 48 hours of culture, supernatant will be removed and replaced with fresh media so that cytokine production can be measured by BD ELISA kits. Moreover, we will adoptively transfer SOCS-1 sufficient Tregs, isolated using a combination of flow cytometry and magnetic activation cell sorting (MACS) (using BD antibodies), into SOCS-1 KO mice in order to rescue the mice from perinatal lethality.

Specific aim 3: Measure the capacity T cells from SOCS-1 KO mice become induced regulatory T cells (iTregs). Our working hypothesis is that T cells from SOCS-1 KO mice will be refractory to conversion into iTregs. We will compare the ability of TGF- β to convert T cells from SOCS-1 KO and healthy SOCS-1 sufficient mice into iTregs. We will also observe the effect of SOCS-1 mimetic peptide in the iTreg induction process. In similar fashion to Aims 1 and 2, the Treg conversion rate will be analyzed by Flow using BD reagents.

This project is innovative because although it is clearly evident that Tregs and SOCS proteins play a significant role in the prevention of autoimmune disease, how the two regulatory systems interrelate is currently unknown. In particular, Foxp3⁺ Tregs in the peri-lethal SOCS-1 deficient mice have not been characterized. Moreover, the role of SOCS proteins in the ability of Tregs to regulate and/or suppress immune responses has not been closely examined. We anticipate that we will achieve the following expected outcomes: 1) Foxp3⁺ Tregs will be characterized for the first time in the SOCS-1 deficient mouse model of autoimmunity. 2) Insight will be gained into the role of SOCS-1 in the development of natural and induced Tregs. 3) Considerable knowledge will be gained as to the role of SOCS-1 in the mediation of Treg suppressor function.

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