Title: Exploring mechanisms of salt-induced T cell polarization into T helper 17 cells
Keywords: Immunology, physiology, T cells, salt
Background and Rationale: How immune cells sense and respond to their environment is a basic fundamental question that needs further exploration. Interleukin 17 (IL-17) producing T helper 17 (Th17) cells are highly regulated proinflammatory cells that are activated in infectious, autoimmune, and cardiovascular diseases (1-4). Recently, it has been shown that increased sodium chloride (NaCl) concentrations *in vivo* and *in vitro* drive Th17 polarization and maintenance via increased expression of serum and glucocorticoid regulated kinase 1 (SGK1). Downstream effects of SGK1 activation include enhanced expression of the IL-23 receptor which is important for Th17 cell maintenance and IL-17 production (1, 2). Despite this clear connection between NaCl and IL-17 production, a critical unanswered question is how T lymphocytes actually sense NaCl concentration changes. Our laboratory demonstrated that several sodium channels and transporters are expressed on T lymphocytes, including sodium-potassium-2 chloride 1 (NKCC1) transporter and sodium calcium exchangers 1 and 2 (NCX1/NCX2). Preliminary data using pharmacological blockade of NKCC1 in cultured CD4+ T cells revealed complete abrogation of the salt-induced upregulation of SGK1. **Thus, I hypothesize that the NKCC1 transporter is the molecular NaCl sensor in T cells necessary for salt-induced Th17 cell differentiation and function.** Furthermore, since intracellular sodium concentrations must remain relatively constant, **I hypothesize that a sodium/calcium exchanger (NCX1 or 2) is activated in the presence of excess intracellular sodium that normalizes sodium concentrations and leads to increased intracellular calcium concentrations, thus mediating salt effects in T cells.**

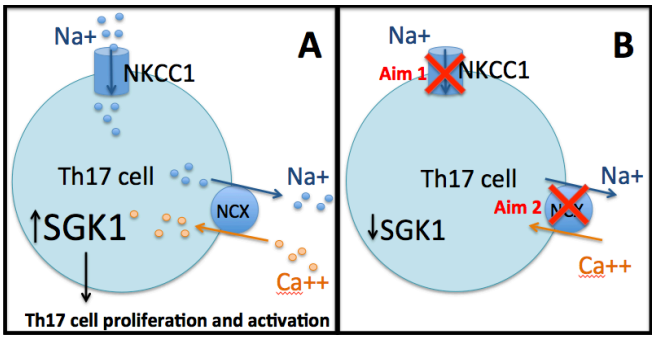

Figure 1: (A) Proposed model of Th17 cell activation by salt. (B) Sodium transporters and exchangers that will be investigated in this proposal.

Specific Aims and Research Plan

Aim 1: To determine if NKCC1 transports NaCl in T-cells, thus triggering salt-induced Th17 differentiation and function.

Aim 1a: To determine the effects of T cell NKCC1 on salt-induced SGK1 expression *in vitro*, naïve splenic CD4+ T-cells from wild type (WT) mice will be treated with NKCC1 small interfering RNA (siRNA) or control non-targeting siRNA and cultured in either normal (150 mM) or high salt (190 mM) conditions with Th17 polarizing cytokines. After 7 days of exposure, NKCC1 and SGK1 expression will be analyzed by qRT-PCR. I expect that siRNA-mediated inhibition of NKCC1 will abrogate the salt-induced increase in SGK1 in Th17 cells.

Aim 1b: To determine the effects of T cell NKCC1 on T cell function *in vivo*, NKCC1$^{+/-}$ mice will be bred to obtain homozygous NKCC1-deficient mice. T cells from these mice and B cells from WT mice will be adoptively transferred by tail vein injection into mice lacking T and B cells (RAG1$^{-/-}$ mice) to obtain mice with NKCC1 deficiency in T cells only (5). These mice will be used to determine the effect of T-cell NKCC1 during systemic increases in sodium concentrations. To sustain high sodium concentrations, which kidney autoregulation would typically normalize in WT mice, one kidney will be removed and a pellet containing a mineralocorticoid agonist, deoxycorticosterone acetate (DOCA), will be implanted followed by supplementation of the drinking water with 1% NaCl. Control mice will be subjected to a sham

surgery without the DOCA pellet. In each group, we will use flow cytometry to measure the percent of IL17A+CD4+ Th17 cells out of total CD4+ cells in the spleen and lymph nodes. I expect to observe that WT mice with DOCA-salt treatment will have a greater percent of Th17 cells as compared to sham treated mice, and that these increases will be absent in Th17 NKCC1 deficient mice, suggesting that NKCC1 is critical for Th17 pathogenicity in vivo.

Aim 2: To determine if excess intracellular sodium activates sodium/calcium exchangers and increases intracellular calcium concentrations, thus inducing Th17 polarization.

Aim 2a: To determine the effect of T cell NCX exchangers on T cell function in vitro, CD4+ T-cells will be cultured with a selective inhibitor of NCX (SEA-0400) or vehicle to reduce NCX activity. Alternatively, cells will be exposed to NCX1 and/or NCX2 siRNA or control non-targeting siRNA. Cells will be cultured in normal (150 mM) and high salt (190 mM) conditions with Th17 polarizing cytokines. After 7 days, SGK1 expression will be analyzed by qRT-PCR to determine the effects of T-cell NCX1/2 on the expression of SGK1 in both normal and high-salt conditions. I expect that SGK1 expression in normal conditions will not be affected by NCX inhibition, but that salt-induced SGK1 expression will be abrogated by NCX inhibition.

Aim 2b: I will measure calcium concentrations in Th17 cells *in vitro* before and after salt treatment using the IonOptix Calcium Imaging system, which is designed for time-lapse imaging of intracellular calcium in living cells. I predict that calcium concentrations will be higher after salt treatment. If this is the case, I will then repeat the experiment using SEA-0400 or NCX1/2 siRNA to determine if NCX inhibition blunts the salt-induced increase in calcium.

Personal Qualifications: Both my didactic coursework and research experience have prepared me to conduct immunology and physiology research using the types of experiments outlined in my proposal. In my first semester of graduate school, I am taking a genetics techniques course which will help me plan and conduct my experiments, and I have already been trained in [redacted] s lab in several of the methods that I am proposing to use, including flow cytometry, cell culture, and siRNA mediated transfections. Additionally, our lab and [redacted] cores are fully equipped with everything I will need to carry out these experiments, and I will receive excellent training from Dr. [redacted] a leading expert in the field.

Broader Impact: My graduate training and the research I will perform will be in two independent fields, immunology and physiology. I will integrate knowledge from both disciplines to research the influence of salt on Th17 cell activation. This topic can influence and connect these disparate scientific fields, which will pave the way for interdisciplinary collaboration. Understanding how environmental changes impact the immune system is also relevant to public health interest, for example, since dietary sodium content and salt mediated diseases are rising worldwide. I will leverage this general public interest in the field to engage in outreach explaining to the public how these systems interact and influence our health. This public outreach could also improve public opinion of scientific research and its benefits to society. Finally, this project is suitable for training and mentoring emerging scientists, as it is conceptually simple and largely uses basic molecular biology techniques. Funding from the NSF will allow me to attend international conferences, foster collaborations, and grant me financial support while participating in outreach efforts and mentoring students.

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