Grant-in-Aid of Research, Artistry and Scholarship

Using Stat5b to Suppress Autoimmune Disease
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Abstract

A normal immune system defends an organism by ensuring a proportionate response to pathogens. A weakened immune system results in increased susceptibility to infections, whereas overly aggressive immune responses can cause autoimmune diseases. Many factors influence the normal set-point of the immune system. Importantly, white blood cells called regulatory T cells act to dampen unwanted autoimmune responses. Lack of regulatory T cells causes autoimmune disease in mice and humans. Conversely, increasing the number or function of regulatory T cells can be used to treat autoimmune diseases. Recent research indicates that augmenting the function of a protein called Stat5b leads to increased numbers of regulatory T cells in mice. This proposal seeks to determine if the presence of constitutively active Stat5b can suppress the development of autoimmunity in a well-characterized model of rheumatoid arthritis. If successful, this approach would spur the development of new therapies for patients with autoimmune diseases.
1. Category Justification

This is a Category 1 application. Dr. Binstadt is an Assistant Professor in the Department of Pediatrics and has been at the University of Minnesota since April 2007. Dr. Binstadt does have Departmental start-up funds and external funding. This proposal describes a new, currently unfunded collaborative project requiring additional resources.

2. Present Status of Knowledge

Rheumatoid arthritis is a common autoimmune disorder that, untreated, causes progressive destruction of joints leading to permanent disability. Effective treatments for rheumatoid arthritis and similar autoimmune diseases work by suppressing the immune system. Immunosuppressive medications are associated with significant side effects, however, and currently no cure exists for rheumatoid arthritis or any other autoimmune disease.

T lymphocytes, a type of white blood cell, are the primary immune cell responsible for initiating and perpetuating autoimmune diseases. Recent investigations of autoimmune diseases have focused attention on two particular subtypes of T lymphocytes, termed regulatory T cells (Treg) and Th17 cells. These two subsets of T lymphocytes have opposing functions. Namely, Treg cells suppress autoimmune responses, whereas Th17 cells promote autoimmune diseases. Importantly, the development of Treg and Th17 cells is reciprocally regulated: conditions that stimulate the development of Treg cells impair Th17 cell development and vice versa (1). Healthy immune systems are characterized by an appropriate balance between Treg and Th17 cells.

The balance between Treg and Th17 cells is controlled by cytokines, soluble messenger proteins of the immune system. Cytokines function by binding to specific receptors on the surface of cells, resulting in the generation of signals within the cell that in turn cause changes in the transcription of key genes via activation of transcription factors. The resulting cytokine-induced changes in gene expression alter the development and/or function of the cell. Much is known regarding the cytokines that control Treg versus Th17 cell development. The current proposal focuses on Stat5b, a transcription factor activated by these key cytokine receptors. Stat5b is intimately and critically involved in controlling the delicate balance between Treg and Th17 cell development (Figure 1) (2-5).

![Figure 1: Stat5 maintains the balance between regulatory T cells and autoimmune Th17 cells. Absence of Stat5 impairs the development of Treg cells, leading to Th17 cell-mediated autoimmunity. In contrast, constitutively active Stat5b-CA (dashed box) promotes the development of Treg cells and may prevent autoimmunity, the hypothesis explored in this proposal.](image-url)
Evidence for the role of Stat5b in controlling Treg and Th17 cell development comes from both mice and humans. Mice genetically lacking Stat5b and its relative, Stat5a, lack Treg cells, have increased numbers of Th17 cells, and develop autoimmune disease (2, 4, 5). Mutations of the Stat5b gene in humans have also been described, and are similarly associated with impaired Treg cell development and “hyperactivation” of immune cells, leading to inflammation of the skin and lungs (6).

These observations suggested that augmenting the function of Stat5b might, conversely, increase Treg cell development, impair Th17 cell development, and prevent autoimmunity. Indeed, transgenic mice whose lymphocytes express a constitutively active variant of Stat5b termed “Stat5b-CA” have greatly increased numbers of Treg cells (3). Whether this Stat5b-CA-induced skew toward Treg development can actually prevent autoimmune disease remains unknown and is the focus of this proposal.

Our laboratory studies a mouse model of rheumatoid arthritis. This arthritic mouse model is called K/BxN. Arthritis in K/BxN mice shares several features with rheumatoid arthritis in human patients, and the K/BxN model has therefore been useful in dissecting the myriad inflammatory pathways responsible for the development of autoimmune arthritis. The K/BxN mouse model involves a transgene-encoded T cell receptor termed “KRN”. T cells expressing the KRN T cell receptor recognize a ubiquitously-expressed self-antigen presented by a particular major histocompatibility complex class II molecule (7, 8). The immunologic details of this reaction are not important; what is important is that the T cells activated by this autoimmune interaction cause arthritis.

The role of Treg cells in the K/BxN mouse model of arthritis has been explored. A transcription factor called Foxp3 is essential for the development of Treg cells. Mutations in the gene encoding Foxp3 result in autoimmune disease in mice and humans. When this mutant mouse Foxp3 gene (termed scurfy) was introduced into K/BxN mice, the mice developed arthritis faster and in more joints (9). The conclusion is that Treg cells normally act to restrain arthritis in K/BxN mice, and impairment of Treg cell development results in more severe arthritis.

Our clinical goal, however, is to discover means to prevent or cure arthritis. We have two key tools that might, in combination, provide a new means for doing so. As described above, (1) mice expressing the constitutively active Stat5b-CA transgene have increased number of Treg cells and (2) arthritis in K/BxN mice is restrained by Treg cells. We seek to test the hypothesis that introduction of the Stat5b-CA transgene into K/BxN arthritic mice will impede or prevent the development of autoimmune arthritis by augmenting the development of Treg cells. This finding would serve as a proof of principle, opening new avenues for manipulating the balance between Treg and Th17 cells to provide more effective treatment for a wide variety of autoimmune diseases.
3. Plan of Work

Hypothesis:
The Stat5b-CA transgene will increase the number of Treg cells and decrease the number of Th17 cells in K/BxN mice, thereby resulting in less severe arthritis.

This hypothesis will be tested via the following inter-related Specific Aims:
1. Determine how the Stat5b-CA transgene influences autoantibody production and the development of arthritis in K/BxN mice.
2. Investigate the effect of the Stat5b-CA transgene on Treg cells in K/BxN mice.
3. Investigate the effect of the Stat5b-CA transgene on Th17 cells in K/BxN mice.

Materials and Methods:
Achieving the above Aims principally involves breeding the Stat5b-CA transgene into the K/BxN mouse arthritis model. The Stat5b-CA transgenic mice were developed by Dr. Michael Farrar in our Department of Laboratory Medicine and Pathology. This Stat5b-CA transgene is under the control of a promoter that results in expression of the gene product in lymphocytes. Our laboratory currently utilizes the K/BxN model. Thus, all necessary mouse lines are readily available. The two-generation breeding scheme is depicted in Figure 2.

Figure 2: Breeding scheme to generate K/BxN Stat5b-CA transgenic mice. A two-generation breeding scheme is depicted. As described in the text, the resulting progeny include arthritic control K/BxN mice, experimental K/BxN Stat5b-CA transgenic mice (red), along with non-autoimmune (KRN-negative) BxN mice with or without the Stat5b-CA transgene. These four groups of mice are expected to be generated in normal Mendelian ratios.

Our laboratory has abundant experience with similar breeding schemes, and thus we expect no difficulties in generating mice of the critical genotypes. The KRN and Stat5b-CA transgenes are easily detected via polymerase chain reaction (PCR)-based genotyping, permitting rapid identification of the genotype of each mouse. Importantly, the breeding scheme also generates important control mice, namely standard arthritic K/BxN mice (without the Stat5b-CA transgene), as well as non-arthritic controls (BxN mice lacking the autoimmune KRN transgene, both with and without the Stat5b-CA transgene). Thus, all of the second generation progeny of the breeding scheme will be included in our analysis.
Specific approaches:

Aim 1

a) Arthritis in K/BxN mice is first noticeable around 3-4 weeks of age. Arthritis is measured by a standardized, qualitative scoring system (0-3 for each paw, maximum score = 12) once weekly, along with a quantitative measurement of ankle thickness using calipers. Scores and measurements are obtained twice weekly from 3 weeks of age until 8 weeks of age. The development of arthritis will be compared in arthritic control K/BxN mice versus K/BxN mice bearing the Stat5b-CA transgene. Student’s T-test will be used to compare differences between these two groups at each time point, with a goal of 4-5 mice/group. Similar statistical analyses and numbers of mice will be used in all other portions of this proposal.

b) The development of arthritis in K/BxN mice is accompanied by the production of autoantibodies recognizing the key self-antigen (anti-GPI antibodies). These antibodies are present in the serum of mice, and are detectable by an enzyme-linked immunosorbent assay (ELISA) used routinely in our laboratory. Serum will be collected from mice once weekly between the ages of 3-8 weeks. We will compare the level (titer) of anti-GPI autoantibodies in arthritic control K/BxN mice versus K/BxN mice bearing the Stat5b-CA transgene. It should be noted that the same mice will be used for parts a and b of this Aim.

Based on our main hypothesis, we expect that K/BxN mice bearing the Stat5b-CA transgene will develop lower anti-GPI autoantibody titers and less severe arthritis when compared to arthritic control K/BxN mice.

Aim 2

Regulatory T cells (Treg) are identifiable based on their expression of the transcription factor Foxp3, along with the cell surface molecules CD4 and CD25. The standard method for identifying Treg cells utilizes flow cytometry, a commonly used technique in our laboratory.

Lymphoid organs (thymus, spleen, and lymph nodes) will be harvested from K/BxN mice and K/BxN Stat5b-CA mice. The lymphoid cells will be analyzed by flow cytometry via surface staining for CD4 and CD25 and intracellular staining for Foxp3 via standard protocols. The numbers and percentages of CD4+CD25+Foxp3+ Treg cells in these organs will be compared in arthritic control K/BxN mice versus K/BxN mice bearing the Stat5b-CA transgene. We will also analyze KRN-negative BxN control mice with or without the Stat5b-CA transgene, to determine the effect of the Stat5b-CA transgene on Treg cells in non-autoimmune, non-arthritic mice on this genetic background.

Based on our main hypothesis, we expect that K/BxN mice bearing the Stat5b-CA transgene will develop an increased number and/or percentage of CD4+CD25+Foxp3+ regulatory T cells when compared to arthritic control K/BxN mice.
Aim 3

Th17 cells are identified based on their production of the cytokine interleukin-17 and cell surface expression of CD4. Similar to Aim 3, this identification is based on standard flow cytometric techniques used in our laboratory.

Peripheral lymphoid organs (spleen and lymph nodes) will be harvested from K/BxN mice and K/BxN Stat5b-CA mice. Following standard brief in vitro stimulation, the cells will be analyzed by flow cytometry via surface staining for CD4 and intracellular staining for IL-17 and another cytokine, gamma-interferon. The numbers and percentages of CD4⁺ IL-17⁺ Th17 cells will be compared in arthritic control K/BxN mice versus K/BxN mice bearing the Stat5b-CA transgene. As in Aim 2, we will also analyze KRN negative BxN control mice with or without the Stat5b-CA transgene; these mice will provide a baseline enumeration of Th17 cells in non-autoimmune mice on this genetic background.

Based on our hypothesis, we expect that K/BxN mice bearing the Stat5b-CA transgene will have lower numbers and/or percentages of autoimmune Th17 cells when compared to arthritic control K/BxN mice.

History, Timeline, Feasibility, and Future Work:
This project is a new project for Dr. Binstadt's laboratory, but is a logical extension of work ongoing in the lab.

Based on our experience utilizing the K/BxN mouse model, it is expected that we will be able to introduce the Stat5b-CA transgene within 3-4 months of initiating the breeding protocol, leaving sufficient time for careful analysis. We have experience with all elements of the methods necessary to complete the studies. We therefore anticipate no difficulties in generating the appropriate mice in sufficient numbers, or in carrying out the analysis of the mice as described above.

If our hypothesis that the Stat5b-CA transgene will decrease arthritis severity in K/BxN mice is found to be accurate, we expect that the preliminary data obtained from this project will be sufficient to apply successfully for external funding focused on harnessing the power of Stat5b to control autoimmune diseases. It is of course possible that the Stat5b-CA transgene will have unanticipated effects on the autoimmune response or the development of arthritis in K/BxN mice, which may also open new avenues for exploration.
4. Budget Justification
The budget covers the period 7/1/2009 through 1/15/2011. The total amount requested is $25,000.

Personnel: 1.2 Calendar months/year are requested to provide salary support for a research assistant (total = $10,780).

Supplies:
$6,090 is requested for animal housing and purchase
$4,060 is requested for flow cytometry supplies (antibodies and related reagents)

Other expenses:
$4,070 is requested for time on the flow cytometer.

There is no scientific overlap between this project and any ongoing or other submitted projects in the Binstadt lab.

5. Need Justification
Dr. Binstadt's current external and start-up funds are being utilized for several active projects in the laboratory. The proposed project is intended to establish a new collaborative project with Dr. Michael Farrar and to generate preliminary data for external funding in the future. To initiate this exciting collaboration and to avoid diminishing the effort dedicated toward other projects currently ongoing in the Binstadt lab, additional funding for personnel, animals, and flow cytometry supplies and time is requested for this proposal.

6. Word Count (sections 2-3): 1902, including Figure legends
References:


