Modulation of Protein-X using a blocking antibody

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Abstract

Rheumatoid arthritis is an autoimmune disease that primarily affects joints and connective tissue in the heart and blood vessels in both men and women. It is especially prevalent in the United States, where more than 1.3 million people are affected. In previous research, it has been shown that one specific protein, labeled as Protein X, plays a critical role in the development of rheumatoid arthritis and of the inflammatory response behind it. It has been shown that levels of Protein X increased in synovial fluid and the serum of RA patients. However, its role is still quite unknown. To do this, we have made a collagen-induced arthritis mouse model to study the autoimmune effects of rheumatoid arthritis. A blocking antibody has been made that targets and blocks Protein X. We aim to find out whether or not a reduction in Protein X has any correlation with a reduction in clinical score in order to better understand the role of Protein X in RA. For this purpose, we have used several different methods consisting of bacterial transformation, intraperitoneal injections, and ELISAs to perform this experiment.

Background

Anti-type II Collagen Antibody

Rheumatoid arthritis (RA) is a chronic and autoimmune inflammatory arthritis of unknown cause. One potential joint-specific autoantigen is type II collagen (CII), proposed as an autoantigen in RA in 1970. Antibodies to CII have been shown to be present repeatedly in both serum and synovial fluid of RA patients, especially towards the onset of the disease. They have been shown to peak around the time when they are associated with active inflammation. The detection of anti-collagen antibodies was performed using an ELISA using buffered normal rabbit serum as a blocking agent to diminish non-specific binding of human IgG to the plastic plates.

Table 1: Incidence of antibody against Type II collagen in sera from patients with rheumatoid arthritis

ARA criteria	No. of patients	Total no. of sera positive for Type II collagen
Probable	35	25 (71.4%)
Definite	69	26 (37.7%)
Classic	376	58 (15.4%)
Total	480	109 (22.7%)

- ELISA plates were coated with 100 ul of collagen
-Of the 480 RA sera assayed, 109 (22.7%) were found to react with native human Type II collagen
- All the sera confirmed to be positive for anti-Type II collagen antibodies and were negative

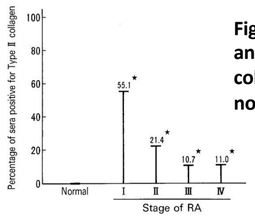


Figure 1: Incidence of antibodies against Type II collagen in sera from normal control subjects

-Of the 480 RA patients, 89 were classified as Stage I, 168 as Stage II, 150 as Stage III, and 73 as Stage IV.
-Asterisks indicate a significant difference relative to the normal sera ($p<0.001$)

Protein X

Protein X is a part of the cytokine superfamily that regulates fundamental cellular properties such as proliferation, apoptosis, and differentiation.
Specifically, Protein X is a cytokine that is secreted by various cell types in response to inflammatory compounds. It has been shown that protein X has a role in RA synovitis as an inducer of cell proliferation of fibroblast-like synoviocytes, ultimately activating interleukin-1β (IL-1β), tumor necrosis factor α, and transforming growth factor β. Past research has shown significantly higher levels of Protein-X in synovial fluid and serum of patients with RA.

TFH Cells with RA

Follicular helper T cells (TFH cells) are a unique subset of CD4+ cells that regulate the survival of B cells. It is well known that high levels of autoantibodies and abnormal GC B cell responses contribute to inflammation in the joints. Recent studies have found that TFH cells could provide help for B cells and allow for long-lived antibody responses. The frequencies and correlations of high proportions of TFH-like cells in RA patients were measured, as well as with clinical activity and auto-antibody production.

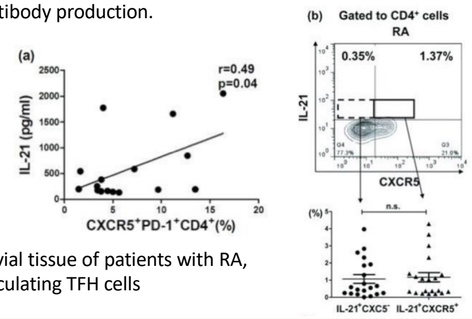


Figure 2: Positive correlation of increased T follicular helper (Tfh)-like cells with disease activity and auto-antibody production in patients with rheumatoid arthritis

-TFH cells are detected in the synovial tissue of patients with RA, accompanied by a frequency of circulating TFH cells

Objective

The primary goal of our experiment is to determine the effect of using a blocking antibody on protein X in circulation.
We hypothesize that using a blocking antibody will reduce the amount of protein X in circulation and ultimately show a reduction in clinical score.

Methods

The collagen-induced arthritis (CIA) mouse model is the most commonly studied autoimmune model of rheumatoid arthritis. The joint inflammation that develops in CIA resembles inflammation in human patients with RA. Autoimmune arthritis is induced in this model by immunization with an emulsion of complete Freud's adjuvant, followed by a booster immunization with Bovin type II collagen in Incomplete Freund's Adjuvant. The Complete Freund's adjuvant contained heat killed Mycobacterium tuberculosis while the Incomplete Freund's adjuvant did not. Around three to four weeks after the booster immunization, inflammation begins to develop in the paws of the mice. The model takes around 40 to 50 days long.

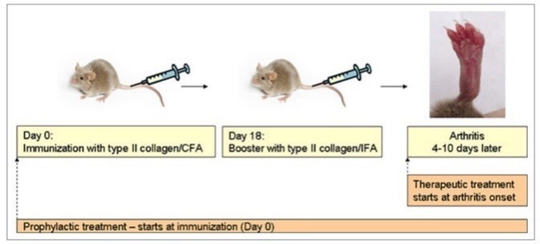
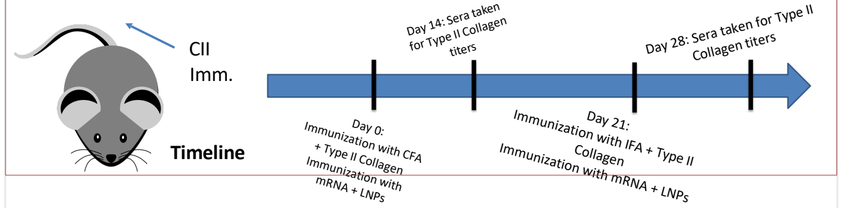


Figure 3: CIA Induction and Development

Steps:

1.) To increase the amount of blocking antibodies present, antibodies were selectively isolated from the serum of the hybridoma. Bacterial transformation was done to select the bacteria that uptook the gene producing the blocking antibody. A Maxi Prep was then prepared to isolate the plasmid DNA.
2.) In the experiment, two groups of DBA1/J female mice were used. Each group had 10 mice that were around 8 weeks old. One group received the isotype antibody while the other group received the protein X blocking antibody. Both groups were given the antibodies through intraperitoneal administration every other day starting the day before their 1st immunization and then every other day starting the day before their 2nd immunization (booster immunization) at Day 21 until euthanization at Day 49.
3.) Clinical score of the mice was measured beginning the day of the booster immunization until euthanization.
4.) After mouse euthanasia, blood was extracted from the heart and serum was isolated from the mice. Anti-collagen antibodies (IgG, IgG1, IgG2a, and IgG2b) were harvested from the serum.
5.) Ran ELISAs for the IgG antibody, IgG1, IgG2a, and IgG2b antibody at both Day 21, which was a week post boost, and at Day 49, which was the time of sacrifice (euthanization) of the mice, to check for Type II Anti-Collagen specific antibodies.



Results

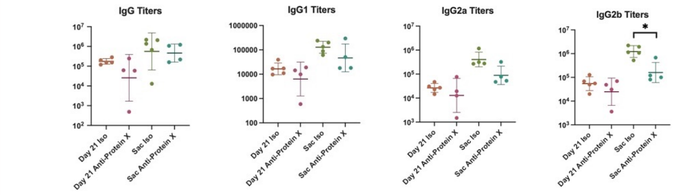


Figure 4: Titer Graphs of Antibodies (IgG, IgG1, IgG2a, IgG2b)

- The higher the titer, the greater the concentration of antibody
-Titers are higher at time of sac (sacrifice) compared to Day 21 because the mice are boosted with more collagen immediately after the Day 21 serum timepoint
-Mice with the isotype antibody have higher anti-collagen titers than mice who received the Protein X blocking antibody
-Mice with the isotype antibody have a higher clinical score compared to the mice with the Protein X blocking antibody

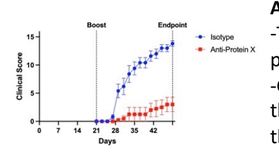


Figure 5: Graph of Clinical Scores of Mice with Isotype Antibody and Blocking Antibody

-The lower the clinical score, the less swelling that is present on the mice
-Graph shows the clinical score of mice injected with the isotype antibody to be much higher compared to the mice injected with the blocking antibody for Protein X (Anti-Protein X)

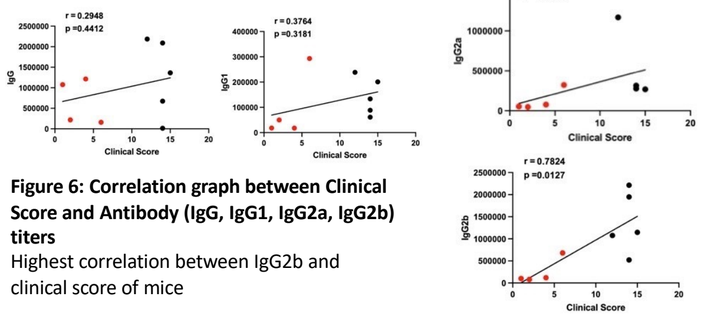


Figure 6: Correlation graph between Clinical Score and Antibody (IgG, IgG1, IgG2a, IgG2b) titers

Highest correlation between IgG2b and clinical score of mice

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