



TGF β /TGF β R3 Signaling Pathways in Autoimmune Disease

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Abstract

This research project focuses on the effect that the TGF β /TGF β R3 immune cell signaling pathway has on antibody production in mice immunized with a T cell-independent antigen. Sera were collected from two groups of mice immunized with a trinitrophenyl (TNP) antigen: a transgenic group lacking the TGF β /TGF β R3 signaling pathway and a littermate control group with an intact signaling pathway. Sera were collected 0-42 days following immunization. The indirect enzyme-linked immunosorbent assay (ELISA) was utilized to detect different subclasses of anti-TNP antibodies (IgM, IgG1, IgG2b, IgG2c, and IgG3). This project contributes to investigating whether variations in the TGF β /TGF β R3 cell signaling pathway affect antibody production associated with certain types of autoimmune disease and the potential for therapies targeting this pathway as treatment for such diseases.

Keywords: Autoimmune Disease, Cell Signaling, Immunoglobulin, Transgenic

Introduction

Autoimmune diseases arise when cells of the immune system attack and destroy components of the self. Characterizing signaling pathways among cells of the immune system that are commonly implicated in autoimmune diseases may clarify how autoimmune diseases arise and in identifying potential treatments. This research project explores the unique area of cell signaling that involves a signal protein known as transforming growth factor beta (TGF β) and a receptor that detects it called transforming growth factor beta receptor 3 (TGF β R3). TGF β has emerged in recent years as an ideal target for autoimmune therapies, as it is believed that a loss of this signal protein's ability to bind to its receptors leads to many of the symptoms associated with select autoimmune diseases (Aleman-Muench 2012). Routinely in immunological research, mice that have been transgenically modified to exhibit aberrations in cell signaling pathways are used as "models" that may be manipulated to gain a better understanding of these types of pathways. For this project, transgenic mice that do not possess TGF β R3 on specific immune cells (designated as dLCK CRE mice) and littermate control mice with an intact TGF β /TGF β R3 signaling pathway (designated as CRE NEG mice) were used. These mice were immunized with a trinitrophenyl (TNP) antigen to evaluate T cell-independent antibody production. Anti-TNP antibodies were detected via an Indirect Enzyme-Linked Immunosorbent Assay (ELISA). An ELISA is a rapid test used for detecting and quantifying antibodies or antigens against viruses, bacteria, and other foreign materials (ELISA technology 2013). Five categories of anti-TNP antibodies (IgM, IgG1, IgG2b, IgG2c, IgG3) produced during the primary and secondary immune response were evaluated in this study. Immunoglobulins can be divided into five different subclasses (IgG, IgM, IgA, IgD, IgE). These subclasses can be detected by sequence studies or more commonly by the directed use of antibodies. IgM antibodies are the structurally largest antibody. They are found in blood and lymph fluid and are the first type of antibody produced in response to an infection; the primary response antibody. They also cause other immune system cells to destroy foreign substances. IgM antibodies are about 5% to 10% of all the antibodies in the body (Schroeder and Cavacini 2010) and are frequently used to diagnose acute exposure to an immunogen or pathogen. IgG antibodies are found in all body fluids. They are the smallest, but most abundant antibody (75% to 80%) of all the antibodies in the body (Schroeder and Cavacini 2010). In mice, IgG immunoglobulins are divided into subcategories based on structural and effector differences: IgG1, IgG2, IgG3, and IgG4. IgG2 is further subgrouped into IgG2a, IgG2b, and IgG2c (Schroeder and Cavacini 2010). IgG antibodies are very important in fighting bacterial and viral infections. IgG is the most versatile immunoglobulin because it can carry out all the functions of immunoglobulin molecules (Schroeder and Cavacini. 2010). Respectively, each of these immunoglobulins act as primary contributors in the immune response. IgG antibodies contribute directly to a secondary immune response including neutralization of toxins and viruses. IgM antibodies more commonly dominate the primary response. Overall, the aim was to determine if the TGF β /TGF β R3 cell signaling pathway impacts the production of the different immunoglobulins that were examined.

Materials and Methods

Two groups of mice were used for this study: transgenically modified mice with no TGF β R3 on the surfaces of their T cells that lacked complete TGF β /TGF β R3 signaling (designated dLCK CRE mice), and littermate control mice with TGF β R3 on the surfaces of their T cells and an intact TGF β /TGF β R3 signaling pathway (designated as CRE NEG mice). Prior to the initiation of this project, three dLCK CRE mice and four CRE NEG mice were immunized with a trinitrophenyl (TNP) antigen to evaluate antibody production and primary and secondary immune response profile. Mice were immunized on Day 0 and Day 21 of a 42-day time course. Over the span of 6 weeks, sera were collected from the mice post-immunization. Sera were stored at -80°C until they were utilized for this study.

Figure 1 summarizes the steps of the indirect ELISA procedure employed for this project. To assess anti-TNP antibody presence in sera, the capture antigen trinitrophenyl-bovine serum albumin (High-Affinity= TNP (4), TNP (21) for normal) was diluted in a carbonate-bicarbonate coating buffer (pH 9.6) to 10 μ g/ml. The wells of a 96 well EIA/RIA clear flat bottom polystyrene high bind microplate (Corning; Corning NY) were coated with the antigen by pipetting 100 μ l into each well. The plate was then incubated overnight at 4°C. The plate was then washed three times with 0.05% Tween 20 diluted in phosphate buffered saline (PBS-T), allowing 30 s-2 min between washes. A squirt bottle was used to fill wells. Once the wells were filled, the plate was turned over the sink and flicked to decant the wash solution in one motion. Then the overturned plate was blotted on a paper towel to wick away remaining moisture. To block wells to prevent nonspecific antibody binding, 200 μ l of a 5% bovine serum albumin (BSA) solution (in 1x PBS) was added to each well. The plate was covered with an adhesive plastic and incubated at room temp for 2 hrs or overnight at 4°C. Following the incubation, the plate was washed as previously described.

Serum samples were diluted in 2% BSA (in 1x PBS). For IgM detection during the primary response (Day 0-21), serum samples were diluted 1: 4000. For IgG detection during the primary response, serum samples were diluted 1:1000. For IgG detection during the secondary response (Day 28-42), serum samples were diluted 1:8000. Diluted samples were added in triplicate at 100 μ l/well. The plate was then incubated for 2hrs at room temp or overnight at 4°C. Following the previously-described plate washing procedure, 100 μ l of a 1:8000 dilution of the desired Horseradish peroxidase-labeled goat anti-mouse IgM, IgG1, IgG2b, IgG2c, or IgG3 antibody (Sigma-Aldrich; St. Louis, MO) was added to the wells for specific antibody class detection. Following a 1 h incubation at room temperature, the plate was again washed three times with 0.05% PBS-T and 100 μ l of tetramethylbenzidine (TMB) ELISA substrate (Biolegend; San Diego, CA) was added to each well. The plate was stored in a dark place and the reaction was checked periodically for color change. Following color change, 50 μ l of a 2N H₂SO₄ stop solution was added to the wells. Finally, the absorbance values for the color changes in the sample wells were assessed at 450 nm using a Synergy HTX Multi-Mode Plate Reader (BioTek; Winooski, VT).

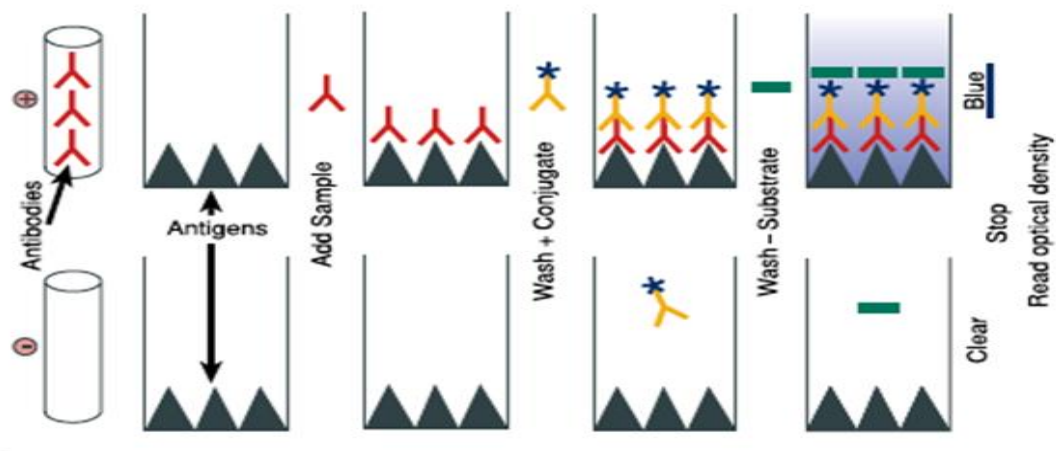
Steps in an Indirect ELISA

FIGURE 1. Depicts the methods used in an Indirect Enzyme Linked Immunosorbent Assay (ELISA technology 2013).

Results

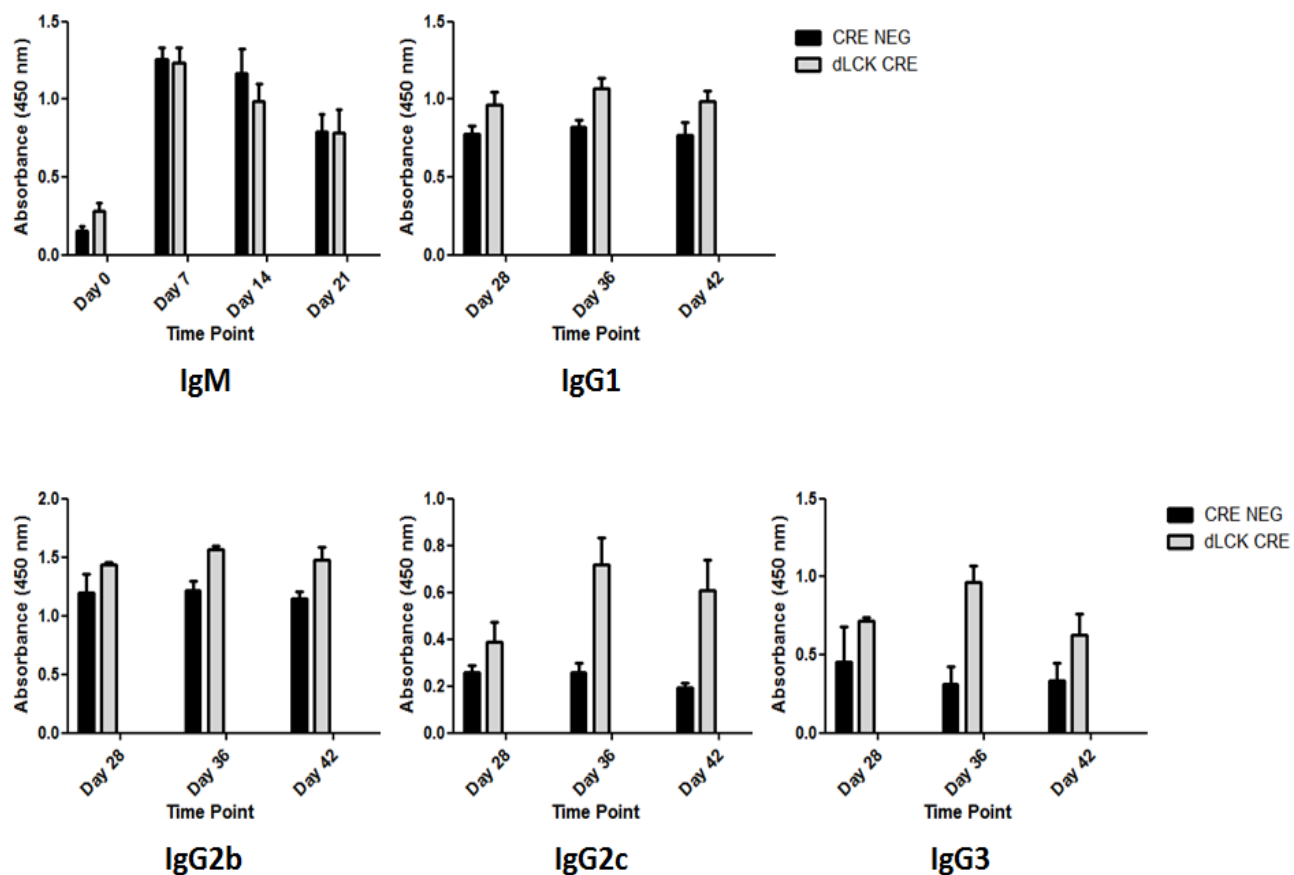


FIGURE 2. Graphs depicting the detectable amounts of five different categories of anti-TNP antibodies over an extended period. Error bars depict the standard deviations of the data. Data were collected from 4 littermate control (CRE NEG) mice and 3 transgenic (dLCK CRE) mice. All samples were run in triplicate.

Discussion

Based on the data collected during this project, the indirect ELISA indicates that an increase in select anti-TNP antibody production is exhibited by transgenic mice during the secondary immune response (days 28-42 post-immunization), to the TNP antigen. The secondary response is dominated by IgG production. No dramatic difference was observed during the primary immune response (days 0-21 post-immunization), when IgM production occurs. The varying levels of detection suggest that throughout the time course, the concentrations of the different subclasses of immunoglobulins (IgM, IgG1, IgG2b, IgG2c, and IgG3) varied as the immune response to the TNP antigen progressed. Elevated levels in the transgenic mice suggest that the TGF β /TGF β R3 signaling pathway plays a role in mediating T cell-independent antibody production that warrants further investigation.

Signaling pathways in autoimmune disease are just one component of research stemmed in investigating potential avenues for symptom treatment and prevention, but understanding these pathways are essential. This project provides support for ongoing investigation into the TGF β /TGF β R3 signaling pathway as a candidate treatment target. Such studies are conducted with the goal of learning more about cell signaling pathways that will potentially lead to autoimmune disease prevention, optimized treatments, and cures. Future work with more detailed models for studying components of this pathway will expand our knowledge and help in this effort.

References

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