Expression of the *FoxP3* Gene in Regulatory T Cells among C3H Mice

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Introduction

The *FoxP3* (Forkhead Box P3) gene is expressed by white blood cells known as Regulatory T cells (Tregs) and is used as a discriminating marker for the detection of Tregs. Testing for the expression of this gene may provide information about the level of Tregs present in the immune system. The purpose of this project was to determine whether the *FoxP3* gene was being expressed in naïve and inoculum treated C_3H-strain mice.

Background: The Immune System and Organ Transplants

The success of an organ transplant is dependent, in part, on the body's immune system. Composed of two entities, the immune system provides both immediate defense against pathogens (the **innate** system) and long term memory of harmful invaders (the **adaptive** system). Foreign cells that are recognized by the immune system are quickly attacked. However, while the innate system is present from birth, the adaptive system must develop over time. It is this delay that has allowed infant mice to receive foreign skin grafts and heart transplants which display non-self **antigens** (protein cell markers), without eliciting an immune response. In our experiments, a C_3H infant mouse is injected with spleen or liver cells from a fetal mouse such as BALB, which is a genetically different strain. As the mouse matures, the adaptive immune system develops to tolerate the foreign cells and not attack them. Current studies have shown that infant mice injected with non-self fetal liver cells have the capacity to accept a non-self organ transplant (i.e. heart). It is thought that Tregs, which regulate the activity of other immune cells, may play a role in transplant tolerance. Investigation of differences in *FoxP3* expression in naïve (untreated), F1 spleen- and FLC-treated mice, may help to affirm this theory.

C_3H Mouse

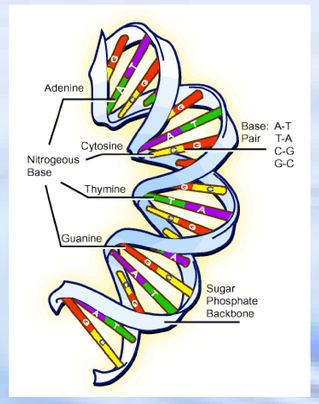
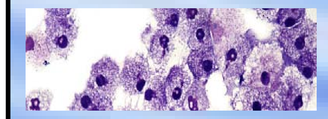


Fig.1: DNA Double Helix Structure



White Blood Cells

DNA and RNA

DNA (deoxyribonucleic acid) is the arrangement of nucleic acids into chemical instructions that allow basic components of the body, like proteins, to be made. The structure of DNA consists of a double helix, sugar-and-phosphate backbone that attaches to **base** molecules (Fig.1.), which are found in pairs. In the nucleus, DNA is made into ribonucleic acid (RNA) by the enzyme RNA polymerase, through a process called **transcription**. RNA differs slightly in chemical structure from DNA and acts as an intermediate message between DNA and protein synthesis. Gene expression, such as that of *FoxP3*, can be examined when DNA and RNA are isolated and amplified.

Methods and Materials

1) Isolating RNA

In order to detect the presence of a specific transcript, total RNA was extracted from mouse tissue. Briefly, spleen samples weighing 0.1 - 0.2 g were isolated from naïve C_3H and FLC-treated 6 week old mice. RNA was extracted with 1mL of Trizol per sample, then homogenized. The phases were then separated with chloroform and the RNA precipitated with isopropanol. RNA was finally washed with 75% EtOH, dried and resuspended in 50uL DEPC-treated water. RNA was quantified with a spectrophotometer and resuspended to a final concentration of 1ug/uL.

2) Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

Following isolation, RNA is used to create complimentary DNA (cDNA). The RNA is first treated with DNase I (1U) to remove contaminating genomic DNA. Following DNase I inactivation, the RNA was made into cDNA using the Superscript III with Platinum Taq DNA polymerase RT-PCR kit (Invitrogen). A Master Mix containing 2X Reaction mix, *FoxP3* specific primers and Superscript III RT/Taq were added to total RNA (200ng). The samples were placed into the thermocycler and the *FoxP3* cDNA was made at 45-60ºC for 10 minute intervals.

3) Polymerase Chain Reaction (PCR)

PCR allows for the amplification of a specific section of DNA (one gene, or a portion of one gene). Primers, or the corresponding components to a piece of DNA, bind to this section on the template. A Master Mix was made containing Taq DNA polymerase (Invitrogen), 10X PCR buffer, dNTP's and *FoxP3* primers. Genomic DNA (100ng) and cDNA were amplified by exposing them to three different temperatures in a thermocycler. The DNA was denatured at (94°C), uncoiling the base pairs (Fig.2a). The primers then annealed (Fig.2b) to the single stranded template DNA at 61°C. The DNA was elongated at 72ºC to form a complementary DNA strand (Fig.2c), and the process was repeated over many cycles, with a decrease in annealing temperature to 56°C in 0.5ºC increments. The same Superscript III/PlatinumTaq enzyme used in the RT-PCR process was also used to initiated the PCR reaction on the cDNA products, since the reverse transcriptase enzyme is active to perform RT-PCR between 45 and 60ºC, but becomes inactive at 94ºC, at which point the Taq enzyme is activated to complete the PCR reaction. *Actin* was used as a positive control in the PCR tests, as this gene is constantly expressed in all tissues.

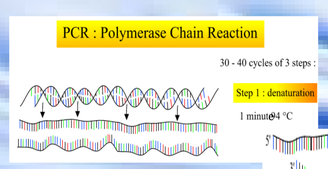


Fig.2a: Double-stranded DNA is denatured into separate strands

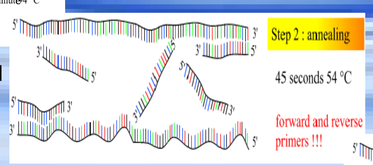


Fig.2b: Primers join to corresponding sections of the DNA

PCR Tube

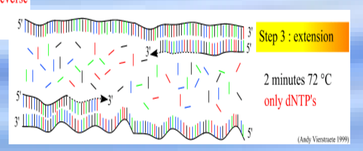


Fig.2c: The DNA elongates, producing copies of the original DNA strand

4) Electrophoresis

Following PCR, 10X DNA stop buffer was added and the samples loaded onto a 1% agarose gel. A 1kb-plus DNA ladder was used to measure the approximate size of the PCR product. Ethidium bromide was added to the gel, which intercalates the double helix allowing the DNA to be seen under ultraviolet light. Because DNA is negatively charged, exposing it to electrical current (electrophoresis) will cause it to run through the gel towards a positive charge, leaving behind visible bands. When placed under a UV light, a photograph can be taken to expose these bands, confirming a positive or negative result for DNA in the PCR samples.

Results

FoxP3 Expression in Naïve and Treated Spleen Tissue

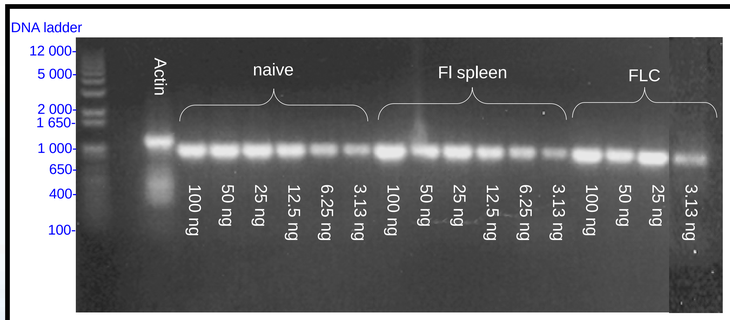


Figure 3: PCR performed on various concentrations of genomic DNA isolated from the spleen of a naïve, F1 spleen treated or FLC treated C_3H mouse.

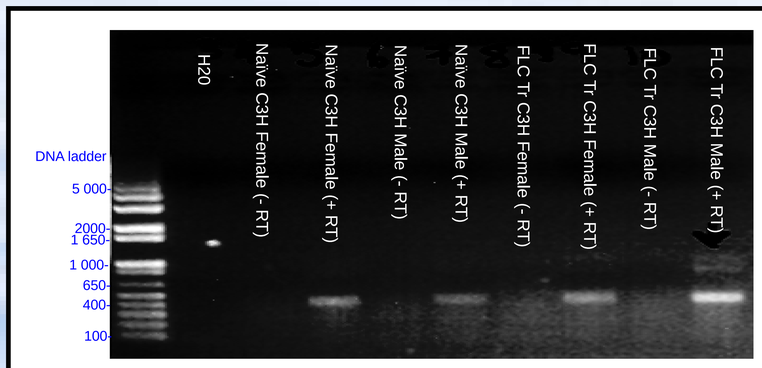


Figure 4: RT-PCR performed on 100ng/uL total RNA isolated from male and female naïve and FLC treated C_3H mice.

Results & Conclusion

The results of the PCR on genomic DNA and the RT-PCR on RNA/cDNA indicated that the *FoxP3* gene is expressed among the spleen tissue samples of both naïve and treated mice. Increasing the genomic DNA concentration created a dose-dependent relationship with the PCR product. A preliminary observation from the RT-PCR suggests that the male C_3H mouse treated with BALB fetal liver cells may have a greater expression of the *FoxP3* transcript in spleen tissue than the other samples tested. In this case, it is possible that FLC-treated mice may display higher amounts of Tregs than naïve mice. To investigate this possibility more closely, quantitative RT-PCR should be performed to accurately determine the amount of *FoxP3* transcript present in each sample.

Acknowledgements

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