THE ROLE OF REGULATORY T CELLS IN THE DEVELOPMENT OF B-CELL TOLERANCE AFTER ABO-INCOMPATIBLE HEART TRANSPLANTION

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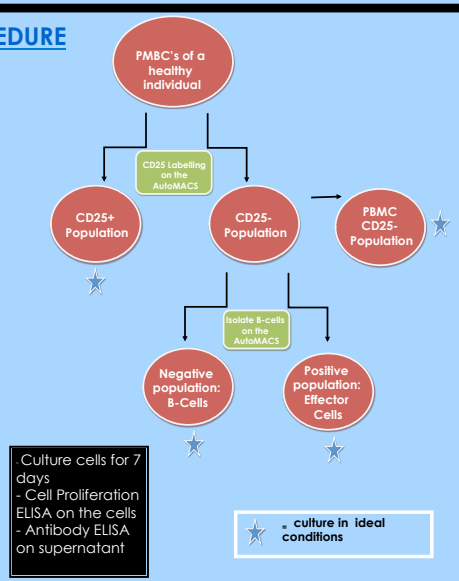
INTRODUCTION

In adult heart transplantation, blood-group incompatibility between donors and recipients is a major barrier. ABO-incompatible heart transplantation in infants, however, leads to the development of tolerance to donor blood-group antigens. The mechanisms that mediate the development of B-cell tolerance to blood-group antigens are not known.

We hypothesize that regulatory CD4+CD25highFoxp3+ (Treg) cells are capable of inhibiting the proliferation and function of blood-group A/B-antigen-specific B cells by selectively killing these cells.

The aim of this study is to investigate the effects of Treg cells on A/B-antigen-specific B cells.

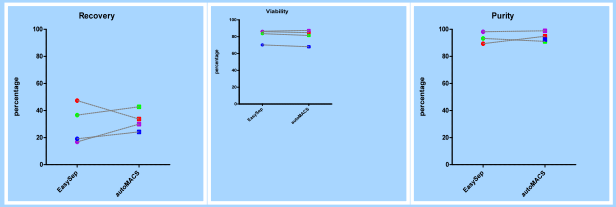
PROCEDURE



This study is currently being conducted by in vitro stimulation of B cells in the presence and absence of Tregs. The cells are isolated from peripheral blood samples from healthy individuals. We use flow cytometry to characterize the cells and ELISAs to determine the cell proliferation and production of total IgM and A/B-specific antibodies. In order to define the optimal protocol for the analysis, we initially: 1) compared two different isolation techniques for B-cells [i.e., magnetically-activated cell sorter (AutoMACS) and immunomagnet cell separator (EasySep)], 2) optimized the ELISA protocol for detecting blood-group antibodies in culture supernatant and 3) tested various culture conditions for B cells and T cells (round bottom vs. flat bottom; CpG vs. CpG + Il-2 vs. CpG + Il-2 + Il-4; with or without serum [human or bovine]).

FIGURES & RESULTS

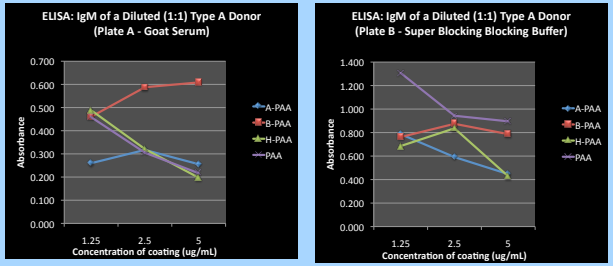
ISOLATIONS:



Figures 1- 3: Comparing the recovery, viability and purity of B-cells using AutoMACS and EasySep for Isolation

Four samples of PBMCs were divided into two groups each: one group underwent separation via a magnetically activated cell sorter, AutoMACS, while the other group underwent separation using an immunomagnet cell separator, EasySep. The populations were then analyzed for recovery, viability and purity. No differences were detected between EasySep and AutoMACS. The AutoMACS will be used in our study due to the convenience it offers.

ELISA:



Figures 4-5: Optimizing ELISA for Antibody Production

This experiment was conducted with blood type-A plasma and blood type-B plasma (data for type-B is not shown). The blocking buffer with Goat Serum drastically reduced the background which enabled the accurate analysis of the binding of the antibodies to B antigens. The Super Blocking Blocking Buffer, on the other hand, does not reduce the background. Also, this experiment demonstrated that the ideal coating concentration for the coating antibody is 5.0 ug/mL.

SUMMARY OF RESULTS:

Isolation Techniques – Comparing the AutoMACS and EasySep, there were no differences in recovery, purity, viability and function of the B cells. *ELISA* - Blocking with goat serum dramatically reduced the background. Other changes which enabled us to more accurately determine the presence of antibodies included increasing the number of washing steps and using a solution with 5% goat serum as a diluent. Also, a difference in using frozen and fresh supernatant was noted with the latter allowing for detection of higher antibody levels (increased sensitivity). *Culture Conditions* - For isolated B cells, the highest proliferation was detected after culturing in 96-well flat bottom plates in AIM-V medium supplemented with CpG, Interleukin (IL)-2 and IL-4. These conditions also yielded the highest levels of antibody production.

FIGURES & RESULTS continued

CULTURE CONDITIONS:

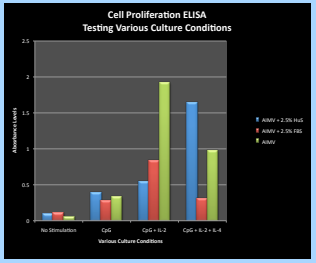


Figure 6: Testing Various Culture Conditions

This data is representative of 5 experiments. PBMCs were cultured in the particular conditions for 7 days. A cell proliferation ELISA was conducted to measure the growth.

AIM-V supplemented with Interleukin(IL)-2 and IL-4 or just IL-2 proved to be the ideal conditions for proliferation.

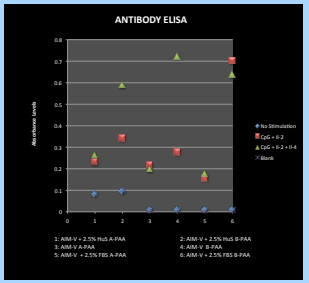


Figure 7: Antibody ELISA of Supernatant from Various Culture Conditions

An antibody ELISA was conducted on supernatant from the experiments testing various culture conditions. This particular experiment was conducted on PBMCs from a blood type-A sample. AIM-V supplemented with Il-2 and IL-4 also had the hight levels of antibody production

SUMMARY & FUTURE DIRECTIONS

For our Treg and B-cell study, we will isolate B cells and Treg cells by autoMACS and culture the cells using the following culture conditions: 96-well flat bottom plates in AIM-V medium supplemented with CpG, Interleukin (IL)-2 and IL-4. In addition, we will use goat serum as a component in the blocking and diluent solutions for the ELISA. The ELISA will be carried out with fresh supernatant from the *in vitro* stimulation of B cells in the presence and absence of Tregs.

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