METHOD DEVELOPMENT FOR THE ANALYSIS OF PBMC-MEDIATED KILLING OF K562 CELLS BY BOVINE COLOSTRUM*

Technical White Paper

Anna Andersen, Dr. Paula L. Vieira-Brock, Dr. Brent Vaughan, Dr. David Vollmer 4Life Research, Sandy, Utah

OBJECTIVE

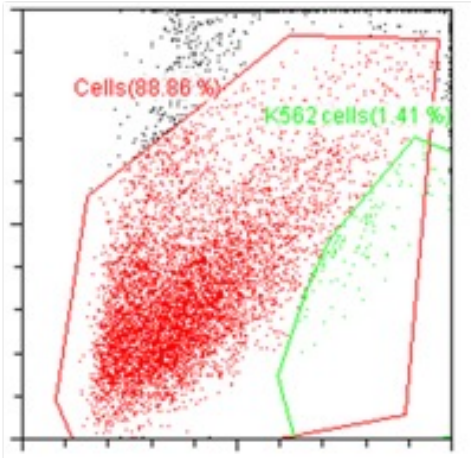
The purpose of this study was to develop a method to use peripheral blood mononuclear cells (PBMCs) to test the immune system activity of compounds, most notably 4Life Transfer Factor®.*

BACKGROUND

Assessing immune system activity in laboratory experiments using PBMCs has many challenges and limitations. Some of these limitations are common for these types of methods, such as variability in immune system responses from different blood donors or even from the same donor at different times. Other limitations are sample-related, such as natural variability between groups of 4Life Transfer Factor. The method used in this study is called flow cytometry.*

Flow cytometry is an effective method for evaluating the activity of compounds on many immune system cell types, including Natural Killer (NK) cells. If this current set of studies worked, flow cytometry would be a viable future screening method for natural products.

The value of such experiments is to not only test and verify the activity of 4Life Transfer Factor, but to also explore the immune system activity of other natural compounds. This knowledge can assist 4Life in the development of new, exciting products to support the immune system.*



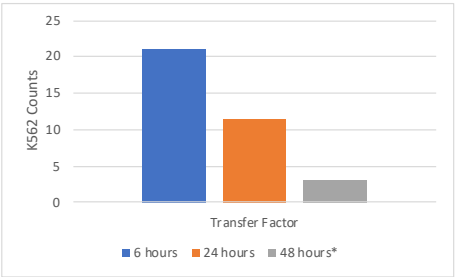
Representative flow cytometry dot plot showing NK cells (red dot) and K562 cells (green dot).*

METHODS

The immune system activity of a bovine colostrum blend was evaluated by its ability to enhance PBMC-mediated killing of cells known as K562s using flow cytometry. PBMCs from multiple donors were investigated. Different doses and different batches of 4Life Transfer Factor were assessed, and various incubation times were explored. Different solvent systems were tested to attempt to improve the solubility of the natural products.*

RESULTS AND DISCUSSION

Several parameters were evaluated to develop a robust method for testing the immune system activity of 4Life Transfer Factor. An incubation time of 48 hours with PBMCs, K562s, and 4Life Transfer Factor resulted in an optimal immune system response, compared to earlier timepoints. Evaluating several common biological solvent systems revealed that phosphate buffer solution (PBS) improved the solubility of 4Life Transfer Factor. This, in turn, improved the immune system response, presumably because the solubilized peptides and proteins from 4Life Transfer Factor were better able to interact with immune system cells. This premise was also suggested in lack of immune system response in the less soluble solvents.*



Using the optimized method parameters, concentrations up to 1.0 mg/mL of 4Life Transfer Factor were evaluated. The most effective response across 48 hours was shown in the 1.0 mg solution of 4Life Transfer Factor. Different blood donors and different batches of 4Life Transfer Factor were tested to explore 4Life Transfer Factor's ability to activate immune system cells. All donors and all batches of 4Life Transfer Factor demonstrated robust immune system activity.*

CONCLUSIONS

A new method using flow cytometry to determine the immune system activity of 4Life Transfer Factor was developed. A number of different parameters were altered and tested. An optimum concentration, incubation time, and solvent were identified. Testing across different donors with several batches of 4Life Transfer Factor demonstrated a robust immune system response. These findings open up additional research opportunities to better understand the immune system effects of 4Life Transfer Factor.*