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Finding a probable origin for the secretion of Interleukin-4 cytokine in the immune response

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I. Introduction

Our bodies are under constant attack from foreign invaders, and we have our immune systems to thank for keeping us healthy and productive every day. One of the many important pieces to our immune response is Interleukin-4, which has many essential functions. It stimulates and signals the division of activated B cells and T cells, and help B cells turn into Plasma Cells, which will allow for more antibodies to be produced and more foreign invaders to be destroyed as a result. IL-4 also switches antibody production over to the Immunoglobin E and Immunoglobin G4 classes, to help better fight certain kinds of infections. It up regulates the production of Major Histocompatibility Complex Class II, which are mostly found on cells that present antigens to the T cells and B cells, meaning there will be more cells to present antigens for making antibodies against.

Needless to say, it is a key component of our immune response, and as such is made by quite a few different cells. It is currently known to be made by Th2 T cells, B cells, NK1.1 T cells, Basophils, and Mast cells, and it is also known that just the presence of IL-4 will stimulate further production of it. But we don't have a definite answer as to which cell starts making IL-4 first, nor do we know at what point IL-4 is first produced. My experiment hopefully will shed some light as to when it is produced and the order in which cells produce it.

II. Experiment

My experiment will be provoking an immune response in mice, harvesting them at predetermined intervals, and analyzing the harvested cells for IL-4 production. By analyzing different cells at each interval, we can establish an order in which IL-4 is hopefully produced.

To start with, I would inject a batch of mice with a solution of ovum proteins and an Aluminum Hydroxide adjunct to provoke an immune response. Over the course of 10 days, I would administer a boost of the ovum protein and Aluminum Hydroxide solution on days 3 and 7, and harvest a portion of the mice on the remaining days.

When the mice are harvested, I will collect splenocytes, lymph node cells, and periteneal cells as these places will contain the particular cells I am looking for. I will then incubate the cells with the ova in a cell culture with 5μM Monensin for 5 hours. The Monensin will block the secretion of any IL-4 made from exiting the cell and will allow us to detect the amount of IL-4 present. Next, the cell membranes would be fixed in place by adding a solution of 4% paraformaldehyde to the culture. This would prevent the cell from lysing from osmotic pressure when the next step is preformed, which is to permeabilize and stain the membrane with a Saponin detergent buffer

and antibodies. The antibodies would bind to specific molecules and IL-4 depending on the cell. The antibodies are dyed so that they could be analyzed using flow cytometry, and will fluoresce a different color depending on the molecule it was bound to (Table 1)

Cells harvested	FITC Dye	PE Dye	Alexafluor Dye
Control	IgG	IgG	IgG
Th2 T Cells	CD4	T1/ST2	IL-4
NK1.1 T Cells	CD4	NK1.1	IL-4
Basophils	FcERI	Kit	IL-4
Mast Cells	FcERI	Kit	IL-4
B Cell	B220	IgE	IL-4

Table 1: Upon harvesting, antibodies with the dyes listed with enter the fixed, permiablized membrane and bind to a specific molecule based on the cell type.

III. Discussion

After flow cytometry is done, I would analyze the data and counting the amount of IL-4 produced by each cell type. After repeating this procedure 8 times of the course of 10 days, I should be able to graph each cell's production of IL-4 as well find an origin point for production.

That would be the ideal result, having only one cell begin IL-4 production and in enough quantities to be picked up by the floe cytometer. Another possible result would be that two or more cell types begin production of IL-4 at the same time, in which case I would have to begin looking for a common element or trigger that would initiate production of IL-4.

Some possible errors this experiment could face is that the ovum solution would not produce a strong enough immune response to warrant IL-4 production, and if so a stronger infection agent would be required. Another obstacle would be that IL-4 would already be present in the background in sufficient quantity to mask the cell's production, but the possibility of this is low and most likely the result of a genetically defective mouse that run counter to the experiment's design, and would be replaced.

The known limitations would be that the time intervals may not be short enough to catch the first cell producing IL-4, and that it would not be definitive proof of the origin of IL-4.