# Will Aging Affect The Lymphocytes?


## ABSTRACT:

In this research project, I tried to answer the question whether age affects the human white blood cells, called lymphocytes. Lymphocytes are cells that play a very important role in the body's immune defense against bacteria and viruses. To analyze lymphocytes, many laboratories use flow cytometry. For this project, I performed four-color flow cytometry testing on two blood samples, one from a child and one from an adult. From this experiment, I learned that two important groups of white blood cells, namely B and T lymphocytes, are present in higher numbers in the blood of the child as compared to the adult blood. These results indicate that aging affects human lymphocytes. This may explain why children and adults respond differently to infections.


## Introduction:

The white blood cells are the body's "guardians" against germs that cause disease. They play an important role in the body's immune response against viruses and bacteria. Lymphocytes represent one important group of white blood cells. These cells present on their membrane a high number of different proteins that are called cell surface markers. The most common surface markers for lymphocytes are:

- CD45, a protein for all lymphocytes;

- CD3, CD4 and CD8, surface markers for T lymphocytes;

- CD19 & CD20, surface markers for B-lymphocytes;

- CD16 and CD56, markers for natural killer cells.

T lymphocytes are immune cells that circulate in the blood and deal with viruses. They can kill cells that are infected with a virus, and can also help other white blood cells, such as B-lymphocytes, in fighting against pathogens. B-lymphocytes also circulate in the blood and have the ability to produce antibodies. Antibodies are proteins that stop bacteria from multiplying inside the body. The human body needs healthy lymphocytes that are able to efficiently eliminate dangerous viruses or bacteria.

One powerful technique that is used in laboratories to analyze human white blood cells is Flow Cytometry. Flow cytometry is a technique that evaluates the characteristics of blood cells using one or more lasers. In contrast to a regular microscope, that allows one to observe at maximum a few hundred cells on a slide, the flow cytometer allows one to analyze tens of thousands of cells in a matter of seconds. This is possible because a solution of cells is being pushed by the instrument's pumps through the instrument's analysis chamber, where is analyzed using laser beams. The laser signals that result after the laser beams interacted with the cells are captured by the photo-multipliers, and processed by a fast computer. Thus, fluidics, optics and electronics work together to provide complex information about human or animal cells.

## Material and Methods:

**1) Antibodies.** Fluorescent antibodies that react with cell surface proteins expressed by lymphocytes were used for cell staining. Ex: Anti-CD3, CD4, CD8, etc.

**2) Human Blood Sample.** Two blood samples obtained from a healthy adult (53year-old) and from a healthy 13 month-old child were analyzed in this study.

**3) Staining Procedure.** Two tubes containing 50 microliters of blood were stained for each donor.

1. Add 10 microliters of fluorescent antibodies to 50 microliters of blood
2. Incubate sample at room temperature for 15 minutes
3. Destroy the red blood cells by adding FACSLyse solution
4. Incubate for another 8 minutes
5. Spin the samples in the centrifuge
6. Take out the solution and keep the pellet (cells)
7. Add the wash buffer and vortex
8. Spin the samples in the centrifuge
9. Add the fixing solution and the cells are ready to be run in the flow cytometer.

**4) Flow Cytometry (refer to diagram 1 on last page).** First, the sample is taken by a buffer solution called sheath fluid through the laser beam. Some of the laser energy is absorbed by the cells, and then emitted as fluorescence signals. These signals are

captured by several photo-multipliers, and further send to a powerful computer. Upon processing of these signals, the computer provides the researcher with information regarding the number, the size, and the appearance of the cells, as well as the presence of certain cellular proteins on the cells surface. The sheath fluid then takes the cells to a waste container.

**5) Data Analysis.**

1. Run samples using the 4-color analysis program.
2. Gate the white blood cells in three regions: one for lymphocytes, one for monocytes, and one for granulocytes.
3. Set up the quadrant markers to separate the positive and negative cells (cells that have certain proteins are positive, and cells that do not express that protein are negative).
4. Compare the percent of cells that express a certain cell marker in different blood samples.
5. Compare the fluorescence intensity of a certain cell marker in different blood samples.

**Data and Results:**

In the Flow Cytometry lab at Columbia University, I have learned to use a flow cytometer called FACSCalibur, produced by the Becton Dickinson Company. The research project that I undertook in this lab was to find whether age affects some characteristics of human lymphocytes. For this, I analyzed two blood samples, one from a

child and one from an adult. The blood samples were stained with fluorescent antibodies that bound to different lymphocyte subsets, namely CD4 or CD8 positive T cells, B cell or Natural Killer (NK) cell. Cocktails of 4 different antibodies carrying different fluorescent tags were used, as follows: Tube 1. CD3/CD8/CD45/CD4

Tube 2. CD3/CD16+56/CD45/CD19

Using a flow cytometer, able to measure four colors of fluorescence, I have determined the number of different lymphocyte group found in the child's and adult's blood samples. As indicated in Figure 1 & 2 and the Lymphocyte Subsets in Human Blood table below, the absolute number of lymphocytes (cells per microliter) contained in the child's blood was about 3 times higher than in the adult's blood. Also, the numbers of CD4 and CD8 positive T cells were about 4 and 2 times, respectively, higher in the child's blood compared to the adult's blood. The number of B-lymphocytes was also 4-fold higher among the child's lymphocytes. The only lymphocyte subset that did not change was the NK cells.

## ***Lymphocyte Subsets in Human Blood***

| | ADULT | CHILD |
|---|---|---|
| | ABSOLUTE COUNT (CELLS/MICROLITER) | ABSOLUTE COUNT (CELLS/MICROLITER) |
| T Lymphs (CD3+) | 1197 | 3605 |
| T Suppressor Lymphs (CD3+CD8+) | 511 | 1102 |
| T Helper Lymphs (CD3+CD4+) | 551 | 2237 |
| Lymphocyte (CD45+) | 1759 | 4924 |
| NK Lymphs (CD16+56+) | 352 | 371 |
| B Lymphs (CD19+) | 219 | 867 |

**Figure 1:**

**Adult (53 year-old)**

**Figure 2:**

**Child (13 month-old)**

Conclusion:

From the experiment I present here, I found that the number of the T and B-lymphocytes was higher in the blood sample obtained from the child as compared to the one obtained from the adult. Thus, I can conclude that age affects lymphocytes, in that children have higher numbers of T and B-lymphocytes as compared to adults. This may explain why children and adults respond differently to infections. It is known that children develop high fever more often than adults in response to a virus or bacteria. The finding that the adult has fewer lymphocytes than the child also suggests that, with age, human lymphocytes undergo changes that make them mature and able to efficiently respond to pathogens, even in lower numbers.

**Bibliography:**

1) Lewis DE Harriman GR. Cells and tissures of the immune system. In: Rich RR, Fleisher TA, kotzin BI, shearer WT, Schroeder HW Jr. editors. Clinical immunology: principles and practice. 2nd ed. London: Mosby; 2001. P.2.1-2.21.

2) Nicholson JKA, Hubbard M, Jones BM. Use of CD45 fluorescence and side-scatter characteristics for gating lymphocytes when using the whole blood lysis procedure and flow cytometry. *Cytometry*. 1996; 26:16-21.

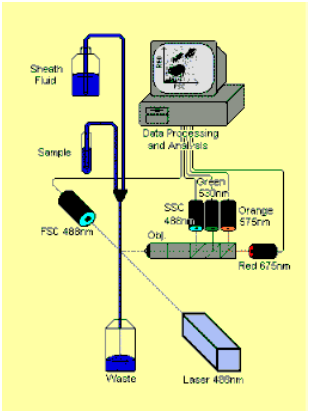
3) Nicholson JKA, Jones BM, Hubbard M, CD4 T-lymphocyte determinations on whole blood specimens using a single-tube three-color assay. *Cytometry.* 1993; 14:685-689.

4) Stein DS, Korvick JA, Vermund SH. CD4+ lymphocyte cell enumeration for prediction of clinical course of human immunodeficiency virus disease: a review. J Infect Dis 1992; 165:352-63.

5) Yanase Y, Tango T, Okumura K, Tada T, Kawasaki T. Lymphocyte subsets identified by monoclonal antibodies in healthy children. Pediatr Res 1986; 29:1147-51.

***Diagrams:***

1) Flow Cytometer:




2) Centrifuge:

3) Vortex: