
# Comparison of the Cytotoxic Activity of Transfer Factor Extracted from Colostrum, Egg Yolk and Human Leukocytes

**David L Vollmer**[1*]
**Ramon Simon-Lopez**[2]

[1]*Sandy, PhD, 4Life Research, USA*
[2]*Zug, MD. Consultant, Switzerland*

** Author for correspondence: Zug, MD. Consultant, Switzerland. E-mail: ramonsimonlope@gmail.com*

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**Abstract**

**Introduction:** One of the ways to evidence the immunological activity of any substance is to mix the candidate substance to a mix of Peripheral Blood Mononuclear Cell (PBMC), and a K-652 cell line. Transfer Factors (TF) are very small polypeptides (sequence of amino acids) that are the messengers of the immune system. It has been suggested that they play a very im-portant role in the immune system, including the increase of the natural killer cell function.

In this study the purpose was to compare the Immunological activity of Interleukin-2 (IL-2), Transfer Factor extracted from Colostrum, Transfer Factor extracted egg yolk and Human dialyzable leukocyte extracts (Blood-based TF).

**Methods:** We compared the Immunological activity killing K652 cells of 5 different substances:

- PBMC+K562+IL-2
- PBMC+K562 (control)
- PBMC+K562+Colostrum-based TF
- PBMC+K562+Egg-based TF
- PBMC+K562+Blood-based TF, including these components in a cell culture.

**Results:** It was found an equivalent function of Colostrum based TF (45.23% of killing K652 cells compared to baseline and similar for IL-2 (44.81% of killing K652 cells) and then a lower activity of Egg based Tf (35.18% of killing K652 cells) and Blood based TF (37.80% of killing K652 cells).

**Discussion:** Sherwood Lawrence discovers Transfer Factors in 1949 and demonstrated their role. In the activation, regulation, and training of the immune system. Originally the TF were obtained from Human dialyzable leukocyte extracts and in 1998 it was developed a method to ob-tain TF from Bovine Colostrum and egg yolk. It was not clear which source was the best activating NK cells and killing K-652 cells. In this study it is suggested that the Bovine ex-tracted TF are the most effective and equivalent to IL-2.

**Keywords: immune system • transfer factors • cell function • egg yolk • human leukocyte extracts**

## Introduction

The immune system plays a very important for human and animal health. Two of the recent Nobel Prizes in medicine have been given to the development and research on the immune system: 2011 for discovery of the dendritic cell and its role in adaptive immunity and or their discoveries concerning the activation of innate immunity and 2018 for the for the discovery of cancer therapy by inhibition of negative immune regulation (i. e., check-point inhibitors).

Transfer Factors (TF) are small bioactive polypeptides that are the messengers of the immune system. It has been suggested that TF play an important role in the immune system, including the increase of the natural killer cell function that is crucial for the defence against cancer and against viral infections. In addition,

it has been demonstrated by Dr Sherwood Lawrence that TF can transfer passive immunity to the recipient, providing information about more than 100,000 antigens. Neonate protection from different infectious disease may also occur owing to the high content of TF in the colostrum. Finally, another important property of TF has been described, that is the capacity to help the patient immune system to regulate down to normal the immune system in cases of Allergies and Autoimmune disorders [1-5].

One of the ways to test for immunological activity is to mix the candidate substance with Peripheral Blood Mononuclear Cell (PBMC), and K-562 cell line [6-8]. In this study, the purpose was to compare the Immunological activity of Interleukin-2 (IL-2), Transfer Factor extracted from colostrum, egg yolk and human leukocyte extracts [9].

## Methodology

We assessed the immune-boosting potential of a TF as a colostrum-based ingredient (UltraFactor, 4Life Research), egg yolk-based ingredient (OvoFactor, 4Life Research), and a human dialyzable leukocyte extracted (Transferon, National Polytechnic Institute), including these components individually in a cell culture, by examining its ability to enhance PBMC-mediated, K562 cell killing. Interleukin-2 (IL-2) served as a positive control. In addition, we used a negative control with PBMCs alone (see Summary Material and Methods) [10].

Frozen PBMCs (composed basically of lymphocytes and monocytes) **(FIGURES 1 and 2)** were thawed, washed, and counted, then diluted in RPMI medium with 10% Heat-Inactivated Fetal Bovine Serum (HI FBS). They were plated at 96,000 cells per 90 uL and allowed to "recover" for 16 hours. CD56 expression in PBMCs was assessed, showing an average of around 12.6% CD56-positive cells during initial testing [11].

The compounds mentioned before, were solubilized to 10 mg/mL in various solutions, added to PBMCs for 24 hours at 1000 µg/mL, followed by the introduction of K562 cells (4000 cells per 90 uL) for incubation, up to 48 hours. Samples included were:

- PBMCs+K562 cells+IL-2 (20 ng/mL)
- PBMCs + K562 cells,
- PBMC + K562+Colostrum-based TF
- PBMC+K562 + Blood-based TF

All tests were run in triplicate. After incubation, cells were resuspended in PBS with Dioctadecyloxacarbocyanine perchlorate (DiO), and K562 cell killing was assessed using flow cytometry **(FIGURES 3)**.

**Materials**

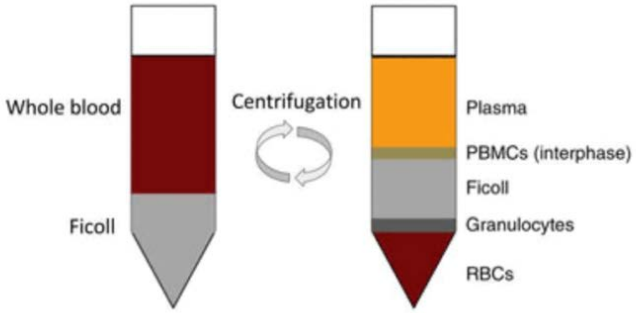
- PBMC+K562+IL-2 (20 ng/mL)
- PBMC+K562 (control)
- PBMC+K562+Colostrum-based TF
- PBMC+K562+Egg-based TF
- PBMC+K562+Blood-based TF

**Flow cytometer**


**FIGURE 1. Flow Cytometer used in this study.**

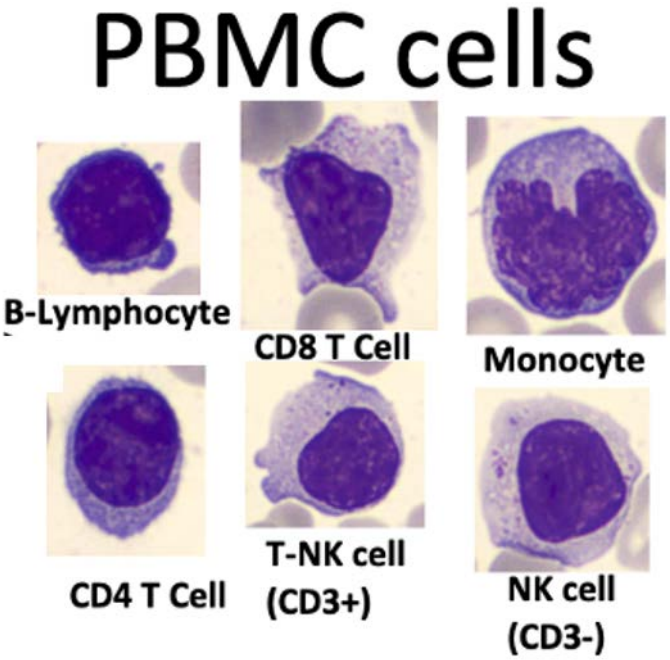
**Obtention of PBMC's**

**FIGURE 2. Graph on how to obtain PBMC.**




**Summary of composition of PBMC's**

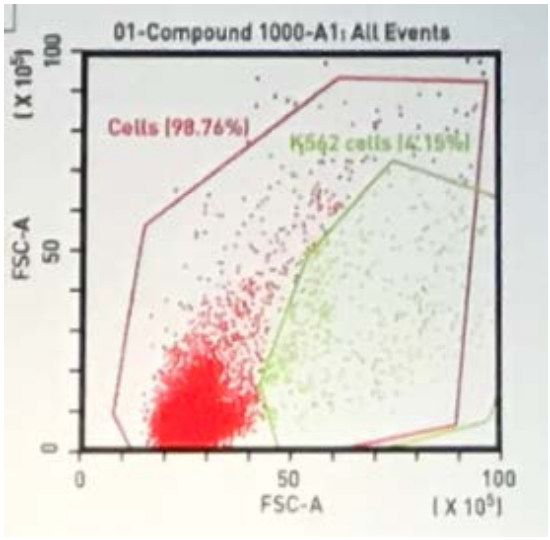
**FIGURE 3. Main components of PBMC cells.**




## Results & Statistical Analysis

It was found an equivalent immune function of colostrum-based TF compared to baseline control and IL-2, with a lower activity of egg based TF and blood based TF [12] **(FIGURES 4-6 and TABLE 1).**

**Figure 4. Representative flow cytometry diagram at 0 Time point; red dots = NK cells, green dots = K562 cells.**



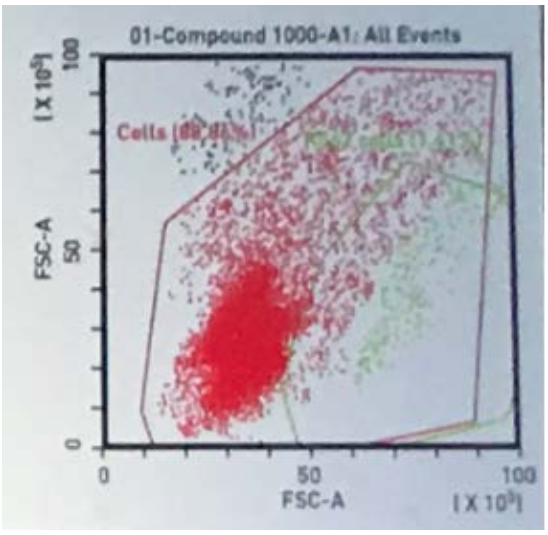



**FIGURE 5. Representative flow cytometry diagram at 48 hr Timepoint; red dots = NK cells, green dots = K562 cells.**

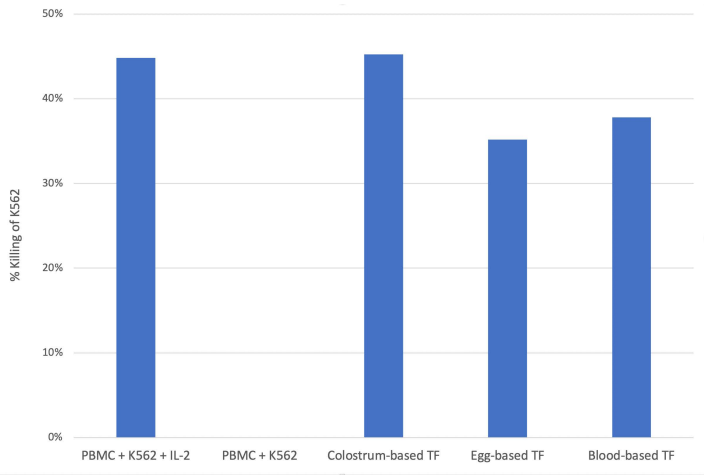



**FIGURE 6. Histogram of results.**

**TABLE 1. Table of results.**

| Sample | Live K562s Mean | Events/µL(V) % Killing |
|---|---|---|
| PBMC+K562+IL-2 | 0.1 | 44.8 |
| PBMC+K562 | 8.3 | 0 |
| Colostrum-based TF | 8.2 | 45.2 |
| Egg-based TF | 9.8 | 35.2 |
| Blood-based TF | 9.4 | 37.8 |

## Discussion

Sherwood Lawrence discovers Transfer Factors in 1949 and demonstrated their role in the activation, regulation, and training of the immune system. Originally the TF were obtained from human dialyzable leukocyte extracts, and in 1998 it was developed a method to commercially extract TF from bovine colostrum and later in 2003, from chicken egg yolk. In the absence of a reliable immune assay, it was possible until recently to determine which TF source was optimal at activating NK cells and killing K-652 cells. In this study, we present evidence that the bovine-extracted TF are the most effective, and equivalent to IL-2 [13, 14].

## Conclusion

This study evaluated the immunological activity of different Transfer Factors (TF) sources—Colostrum-based TF, Egg-based TF, and Blood-based TF-compared to Interleukin-2 (IL-2) in their ability to kill K562 cells. The results indicate that Colostrum-based TF and IL-2 exhibit similar effectiveness, with approximately 45% and 44% cell killing activity, respectively. In contrast, Egg-based TF and Blood-based TF showed lower activity, at 35% and 38% cell killing, respectively.

Sherwood Lawrence's discovery of Transfer Factors in 1949 highlighted their crucial role in immune system function. Initially, these factors were sourced from human dialyzable leukocyte extracts, but later methods developed in 1998 enabled the extraction of TF from bovine colostrum and egg yolk. Our findings suggest that TF derived from bovine colostrum is as effective as IL-2 in activating natural killer cells and inducing the killing of K562 cells. Therefore, bovine colostrum-based TF may be considered a potent alternative to IL-2 for enhancing immune responses.

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