Immune modulation by Sterol 117™.

Gitte S. Jensen, PhD[1].

Abstract

Two bioassays targeted at key aspects of the human innate immune defense were used to examine effects of Sterol 117™ on the function of select subsets of human immune cells. The polar (water-soluble) and non-polar (water-insoluble) components of Sterol 1117™ were tested in parallel. The data showed that Sterol 117™ activated human natural killer cells in vitro, and contains compounds that support phagocytosis as an important part of anti-bacterial immune defense.

Keywords: Immune modulation, Natural Killer cells, Phagocytes, anti-viral, anti-bacterial.

1. Introduction

The project involved a nutritional product, Sterol 117™, containing a blend of antioxidants, essential fatty acids, plant sterols, and other anti-inflammatory compounds, and which is marketed as support of immune function and recovery from fatigue syndromes. The ingredients include multiple aqueous and non-aqueous sources of antioxidants with various other properties, including immune activating and anti-inflammatory properties.

The initial testing conducted at NIS Labs, showed that the product contains antioxidants capable of protecting live cells from free radical damage when the cells were exposed to oxidative stress. An additional pilot test provided data that suggested support of anti-viral immune defense mechanisms, since both the aqueous and ethanol extracts induced activation of Natural Killer (NK) cells.

This report presents data that further documents NK cell activation by Sterol 117™ in more detail, regarding this important aspect of anti-viral immune defense.

The report also documents the effect on a key aspect of our anti-bacterial immune defense mechanisms, phagocytosis.

[1]*Dr. Gitte S. Jensen, PhD (Immunology), Research Director, NIS Labs, 1437 Esplanade, Klamath Falls Oregon 97601.*

2. Materials and methods

2.1. Preparation of Sterol 117 ™ for in vitro bioassay work.

Sterol 117™ (Celt Corp, Calgary Canada) was received as an encapsulated dry powder. Immediately prior to testing in biological assays, aqueous and ethanol extracts were prepared from the powder in the following manner: Five hundred milligrams powder was added to either 5mL phosphate-buffered saline (PBS) or 5mL ethanol. Further dilutions were prepared in PBS at physiological pH.

2.2. Chemicals and reagents.

The following buffers and reagents were obtained from Sigma-Aldrich (St. Louis, MO): phosphate-buffered saline (PBS), RPMI-1640 culture medium, Histopaque 1077, and Histopaque 1119. Dichloroflurescein diacetate (DCF-DA) was obtained from Molecular Probes (Eugene, OR), a subdivision of Invitrogen (Carlsbad, CA).

2.3. Purification of white blood cells

After obtaining informed consent as approved by the Sky Lakes Medical Center Institutional Review Board (Klamath Falls, OR), peripheral venous blood from healthy volunteers was drawn into sodium heparin and layered onto a double-gradient of Histopaque 1119 and 1077. The vials were centrifuged at 2400 rpm for 25 minutes. The peripheral blood mononuclear cell (PBMC) and polymorphonuclear (PMN) fractions were harvested and washed twice in PBS without calcium or magnesium at 2400 rpm for 10 minutes. The cells were used immediately for testing in the NK activation and phagocytosis bioassays.

2.4. Evaluation of activation status of human NK cells in vitro.

Freshly purified human PBMC were used for these assays. The cells were plated in micro-well plates. Negative control samples in quadruplicate were left untreated. Positive control samples were treated with Interleukin-2 (IL-2). Two sets of samples were treated with serial dilutions of test product. One set was only treated with test product to evaluate the direct effect of the test product on NK cell activation. The second set was also treated with IL-2, to see whether the test product affects the response of NK cells to the known NK cell activator IL-2.

After 18 hours of culture, cells were stained with fluorescent markers for the T cell antigen CD3, the antigen CD56, and the activation molecule CD69. The expression level of CD69 on the surface of CD3-negative, CD56-positive NK cells was analyzed as a measure of mean fluorescence intensity (MFI). The analysis allows us to detect if compounds in a test product directly activates NK cells *in vitro*. It also provides indication of whether a test product enhances or otherwise affects the response of NK cells to IL-2. This would be an important indication of whether the product may support an already ongoing anti-viral immune response.

2.5. Evaluation of phagocytic activity.

Freshly purified peripheral blood PMN cells were pretreated with test products for 3 minutes, and then introduced to fluorescent micro-particles mimicking

bacteria. The cells were allowed to ingest particles for 2 minutes, after which free micro-particles were removed by centrifugation. The fluorescence intensity of phagocyte cells was then evaluated by flow cytometry.

The treatment with test product may result in more phagocytes deciding to engage in phagocytosis, and may also lead to a faster or stronger rate of phagocytosis, resulting in higher numbers of fluorescent micro-particles per cell. The data analysis examined both aspects of the phagocytic activity of the cells.

3. Results

Effect on activation of NK cells: CD69 expression levels

Treatment of PBMC, which is the white blood cell fraction containing the NK cell subset, resulted in increased expression of CD69 on the surface of the NK cells.

Both S117 PBS and S117 EtOH increased the expression of CD69 on the cell surface of CD3- CD56+ NK cells.

This effect was dose-dependent for both product preparations with S117 EtOH performing better than S117 PBS.

For S117 EtOH the three highest concentrations of product, and for S117 PBS the two highest concentrations, produced statistically significant increases in CD69 expression above baseline ($P<0.05$).

When the effects of S117 PBS and S117 EtOH on NK cell activation were assayed in the presence of the known NK cell activator IL-2, both products showed synergy with IL-2 and increased CD69 expression to levels higher than those resulting from IL-2 treatment alone.

This effect was very pronounced with the S117 EtOH product where the 0.25 g/L and 0.063 g/L doses resulted in a doubling of CD69 expression on NK cells ($P<0.02$).

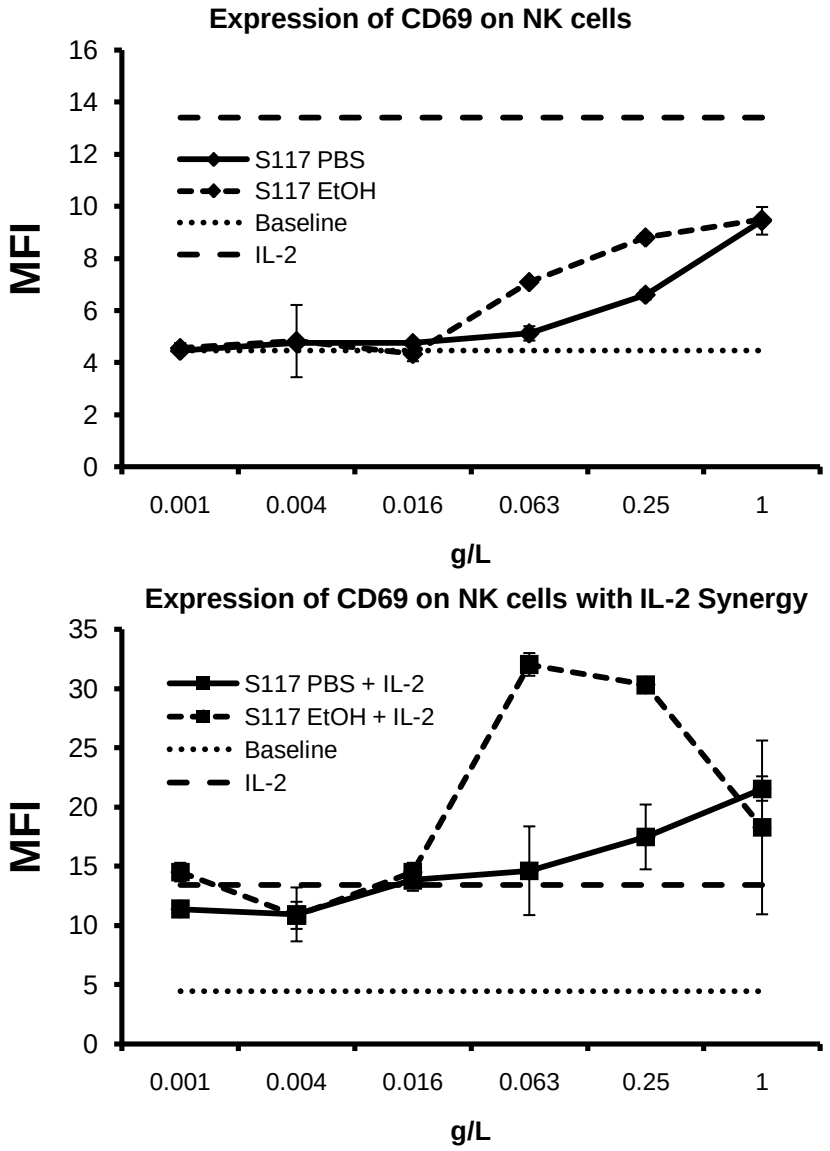

Figure 1. A) Sterol 117™ directly activates human NK cells in vitro. Dose dependent activation of NK cells by Sterol 117™ was seen both when using aqueous (PBS) versus non-aqueous (EtOH) extraction. B) Synergistic enhancement of NK cell activation in the presence of IL-2. The dotted line indicates the baseline level of CD69 expression on the NK cells. The broadly dashed horizontal line indicates the level of CD69 expression achieved when NK cells were activated by the known stimulus IL-2.

In addition to evaluating the mean fluorescence intensity (MFI) of the CD69 activation marker, analysis was also performed to evaluate if the relative number of CD69-positive NK cells changed with treatment by test product.

The highest dose of S117 PBS also produced a statistically significant

increase in the percent of NK cells expressing CD69 that was greater than result when NK cells were treated with IL-2 alone ($P<0.05$).

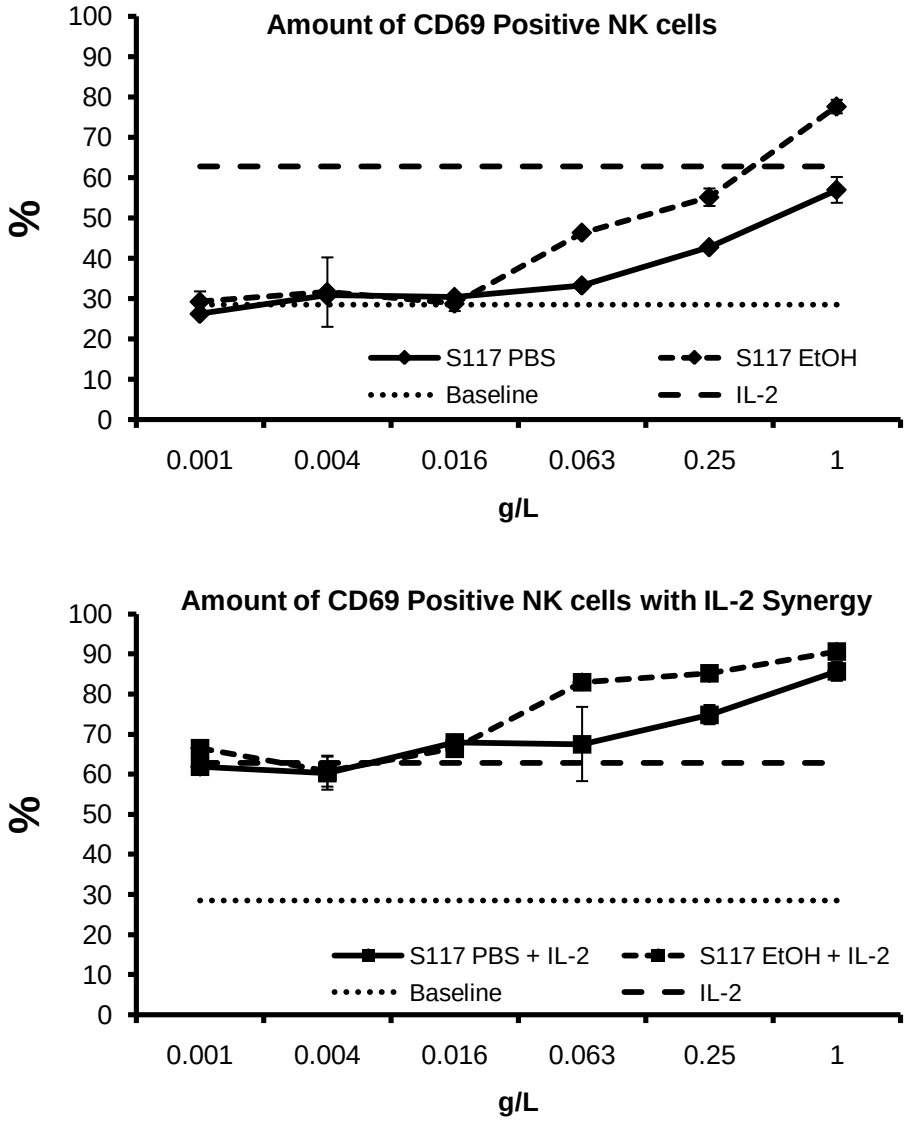

Figure 2. Sterol 117™ increases the numbers of NK cells that express the CD69 marker, both in a direct manner (top graph) and in synergy with IL-2 (bottom graph). A dose dependent increase was seen both when using aqueous (PBS) versus non-aqueous (EtOH) extraction.

Effect on activation of Natural Killer T (NKT) cells

In most normal healthy human donors, the proportion of a rare CD3+ CD56+ cell subset called NKT cells is almost non-detectable. During analysis, we found that two out of the three donors

had detectable levels of NKT cells. This allowed us to gather pilot data on how the test product may affect the activation status of this type of cell, known to conduct rapid immune modulating effects including cytokine secretion.

Expression of CD69 on NKT cells was increased by treatment of cells with S117 PBS and S117 EtOH.

In the presence of IL-2, S117 EtOH nearly quadrupled the expression level of CD69 on NKT cells when assayed at the 0.25 g/L concentration. This increase was much higher than the effect of IL-2 alone and was highly statistically significant ($P<0.003$).

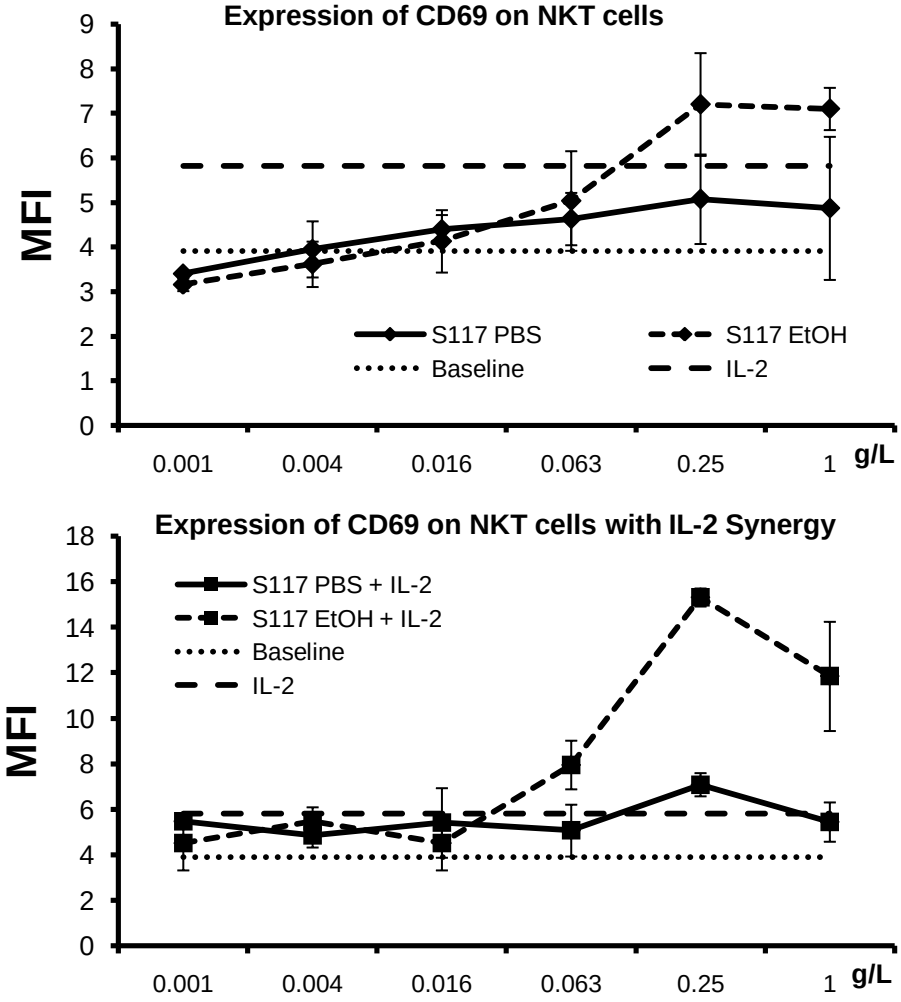

Figure 3. A) Sterol 117™ directly activates human NKT cells in vitro. Dose dependent activation of NKT cells by Sterol 117™ was seen both when using aqueous (PBS) versus ethanol (EtOH) extraction. B) Synergistic enhancement of NTK cell activation in the presence of IL-2 was seen for the EtOH extract.

Phagocytic activity.

When measuring the percent of PMN cells that were actively engaged in phagocytic activity, it was seen that treatment with S117 EtOH at the two highest doses tested, showed an effect on increasing this percent. Treatment of cells with S117 EtOH also increased the number of particles ingested per phagocytic PMN cell (indicated by an increase in fluorescence intensity).

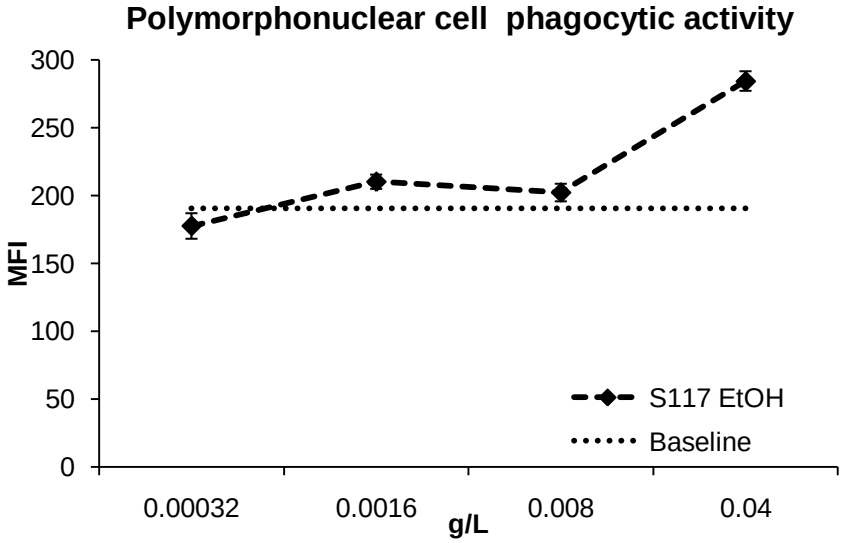

Figure 4. The treatment of human phagocytes with the EtOH extract of Sterol 117™ resulted in an enhanced function of these cells in terms of the anti-bacterial behavior of engulfing foreign particles.

4. Discussion

Our body's primary defense mechanisms towards cancers and viral diseases involve a group of cells called NK cells. These cells travel in our blood stream in a state of rest, but can be immediately recruited into tissues by chemical signals and activated through various mechanisms to a) kill cancer cells, b) divide and make more NK cells, and c) secrete substances that attract other cells into the site.

The data showed that Sterol 117™ contained both water-soluble and water-insoluble antioxidants capable of providing a significant increase in the activation marker CD69 on both NK cells and NKT cells. Expression of the CD69 marker on NK cells has been equated to an increased cytotoxic capacity, in terms of the capacity of the NK cells to kill transformed target cells [1].

Phagocytosis of microbial particles is an important part of the innate (immediate) immune response. It is a rapid process, and the effect of test products on enhancing this cellular function is often almost immediate

Sterol 117™ contains compounds that are extractable by ethanol, i.e. non-polar compounds, that induce an enhanced phagocytic capacity in vitro. This may suggest that ingestion of Sterol 117™ supports phagocytes in the gut mucosal lining, and possibly be supportive of anti-bacterial defense mechanisms at the gut interface.

These findings taken together suggest that Sterol 117™ contains compounds that are capable of supporting two distinct function of the human innate immune defense.

References

1. Clausen J, Vergeiner B, Enk M, Petzer AL, Gastl G, Gunsilius E. Functional significance of the activation-associated receptors CD25 and CD69 on human NK-cells and NK-like T-cells. Immunobiology. 2003;207(2):85-93.