ABSTRACT:

Antigen Infused Colostrum/Whey Extract, a soluble extract dialyzed from the lacteal secretion of antigen infused dairy cows, is an orally absorbable immunomodulator that enhances leukocyte anti-infective and cyto-toxic activities. There was no evidence for detrimental inflammatory cytokine production. Furthermore, there was an increase in the activation of circulating APC macrophages within a range of normalizing immune function. This study provides double blind evidence that the cytokines, peptide neurohormones and other informational molecules in Antigen Infused Colostrum/Whey Extract modulate and normalize immune function. Further, Antigen Infused Colostrum/Whey Extract demonstrates its effectiveness as a Biological Immune Response Modulator for increasing the protective functions of the immune system.

1. INTRODUCTION:

Exogenous cytokine and neuropeptide regulation of the immune system has been the subject of much research over the last decade. It is well known that various peptides influence lymphocyte and macrophage activity by either suppressing or enhancing certain functions. Some of these pathways are understood and defined while many are not. Antigen Infused Colostrum/Whey Extract has been in veterinary and human clinical use on a very limited basis, until recently, for over 40 years. Some of the observed effects have been an increase in NK cell activity with an associated increase in the production of cyto-toxic reactive oxygen intermediates. The purpose of this double blind clinical study was to more clearly elucidate the immune pathways that the material is affecting and thereby enable more decisive clinical application.

2. HISTORY OF ANTIGEN INFUSED COLOSTRUM/WHEY EXTRACT:

During the past six (6) years, as a result of expanded distribution, thousands of people have used and continue to use Antigen Infused Colostrum/Whey Extract as a dietary supplement. Many of these people have reported experiencing health benefits that they attribute to the material. Clinical studies on patient populations suffering from degenerative and chronic disease have provided consistent and dramatic documentation of increases in Natural Killer cell activity. This empirical evidence and clinical study data suggest and demonstrate an immune modulation effect caused by the material in combination with other nutrients, as well as the safety of the extract. This is the first double blind clinical study using only Antigen Infused Colostrum/Whey Extract with a healthy population.

Antigen Infused Colostrum/Whey Extract can only be produced under the most unique circumstances. All of the key factors came together in 1995 and the material became available for humans.

In the late 1940’s and early 1950’s university professors and biochemists began to explore the observable benefits of bovine lacteal secretions. By the early 1970’s, bovine colostrum was being touted as a replacement strategy for antibiotics. However, the apparent effectiveness and simplicity of manufacturing antibiotics led to the exclusion of further consideration of lacteal secretion extracts and perhaps other options.
Though many were aware in the 1970’s, all of us have recently become sensitive to the fact that antibiotics present serious side effects and consequences not fully acknowledged in the 1970’s. We now have organisms that live on antibiotics and diseases that we cannot control. Antibiotics are immune suppressive, turning-off the only defense system that we have to protect our health. In fact, many pharmaceutical products are immune suppressive.

The laboratory tests needed to study the effects of Antigen Infused Colostrum/Whey Extract became available in the mid 1990’s. Many thousands of people have benefited from this technology with millions of servings safely consumed.

Unlike other commercial colostrum or whey products, Antigen Infused Colostrum/Whey Extract is an engineered extract that requires very controlled conditions in order to obtain the desired product.

Several key factors must be in place for the manufacturing of Antigen Infused Colostrum/Whey Extract to commence:

1. A privately managed herd of organically raised dairy cows.
2. A managed breeding program to optimize and select for specific immune responses.
3. An effective strategy for antigen stimulation. Only a process known as infusion has been demonstrated consistently and reliably effective in nearly 50 years of study (U.S. Patent #4,402,938).
4. The ability to identify, recover and concentrate sufficient quantities of the immunological factors necessary to cause all of the observed changes to take place.

3. SAFETY:

To date, over 1 million servings of Antigen Infused Colostrum/Whey Extract have been taken without any observed or reported adverse or allergic reactions. Since the Antigen Infused Colostrum/Whey Extract functions as an immune modulator, a small percentage of people that receive it experience what is commonly referred to as a healing “crisis” as their immune system begins to reactivate. A healing “crisis” can consist of a few hours to a few days of experiencing flu-like symptoms. The healing crisis is a positive effect and signifies immune reactivation and the immune response to a latent infection that the body has been harboring. The healing crisis does not constitute an adverse reaction, as immune activation is the expected result of taking Antigen Infused Colostrum/Whey Extract.

4. ELIGIBILITY CRITERIA and STUDY DESIGN:

4.1 Eligibility Criteria

The following eligibility criteria were applied to human subjects for this double-blind study. They were between the ages of 21 and 65. They were not be suffering from any known severe or chronic diseases, were not experiencing any unusual high stress circumstances, not taking any pharmaceutical medications and could not be pregnant.

For agreeing to participate in this clinical study, each participant received a six months supply of a currently manufactured and distributed product that includes Antigen Infused Colostrum/Whey Extract.
4.2 Study Design

Twenty (20) subjects were included in the study and each of them had a series of laboratory blood tests prior to starting the study (Appendix 1). Ten (10) subjects received 200 mg of Antigen Infused Colostrum/Whey Extract three times a day for fifteen (15) days. The other ten (10) subjects received a capsule of the placebo (calcium carbonate) three times a day for fifteen (15) days.

Neither the subjects nor the principle investigator knew which of the subjects were taking the placebo or the Antigen Infused Colostrum/Whey Extract. After the 15 days, the laboratory studies were repeated for comparison evaluation. The blood test evaluations included a variety of immunological tests designed to measure the structure and functional capacity of the immune system.

The actual patient population studied was as follows:

Four (4) men and six (6) women in the group that received Antigen Infused Colostrum/Whey Extract.

Six (6) men and four (4) women in the control group that received the placebo.

Ages ranged from 32 to 61.

5. MATERIALS and METHODS:

5.1 Materials

Antigen Infused Colostrum/Whey Extract is a specially purified extract of the dialyzable (filterable) fraction of bovine lacteal secretions. These peptides are derived from the pasteurized lacteal secretion of a standing herd of organically raised cows that are immunologically stimulated in a prescribed manner while they are pregnant. The lacteal secretion is processed to preserve the immuno-regulatory agents. The material is then lyophilized and mixed with flowing agents, encapsulated and bottled.

5.2 Natural Killer (NK) Cell Function Assay

Natural Killer (NK) Cells are an important line of cytolytic defense against newly arising malignant cells and cells infected with viruses. They are a distinct group of circulating mononuclear lymphocytes with no immunological memory and are independent of the adaptive immune system. Natural Killer Cells typically constitute 5 to 16 percent of the total lymphocyte population. They mediate non-MHC restricted cytolytic activity against a number of tumor cell lines, particularly against virus-infected or virus-transformed cells without prior sensitization. They are distinguishable from T or B-lymphocytes by their surface phenotype and cytokine profile.

Currently, the standard test for NK cell activity, function, is a cyto-toxicity assay using Cr51 labeled targets. K562 cells (2X10^6) in CM are labeled with 350 uCi radioactive sodium chromate (Cr51) for 1 hr at 37 degrees Centigrade and 5% CO2. The labeled cells were washed twice in PBS, resuspended and adjusted to 1X10^5 cells/ml CM. Subsequently, duplicates of 1X10^4 target cells in 100 ul were added to the effector cells in 96-well U-bottomed plates yielding E:T ratios of 50:1-1.5:1. The plates were centrifuged at 200Xg for 1 minute and incubated for 4 hr at 37 degrees Centigrade in the presence of 5% CO2. Spontaneous release was measured from the wells containing the target cells and medium and maximum release from wells containing target cells and 0.1% Triton X-100. After incubation, the plates were centrifuged at 200Xg for 5 min, 40- wl supernatant was harvested from each well into wells of
MicroBeta Plus standard plates and 15ul of super scintillation cocktail were added. Emitted radioactivity was measured in a 1450 Micro Beta Plus scintillation counter. Percentage specific release of Cr51 was calculated as follows:

$$100 \times \frac{\text{cpm(test)} - \text{cpm(spontaneous)}}{\text{cpm(max)} - \text{cpm(spontaneous)}}$$

Individuals with low levels of NK cell function have an associated increase in susceptibility to many different viral infections. Furthermore, low NK cell function is related to solid tissue malignancies, autoimmune diseases, chronic fatigue immune dysfunction syndrome and post-radiation or chemotherapy of cancer patients.

5.3 Neutrophil Phagocytosis (Pi)

The term macrophage is used to define a ubiquitous population of large mononuclear cells that have the ability to engulf and destroy particulate matter that it deems to be foreign or debris. Macrophages are the tissue equivalent of monocytes and are involved at all stages of the immune response. They act as rapid, first line protection, which can respond before T cell-mediated amplification has taken place. Activated macrophages not only play a key role in host defense against intracellular parasitic bacteria, pathogenic protozoa, fungi and helminthes but also against tumors, especially metastasizing cancers.

When monocytes enter the tissues and become macrophages they undergo several changes. The cells enlarge and increase the amount of intracellular lysozymes allowing greater phagocytosis. In the tissues, macrophages live for months or years and may be motile.

The flow cytometric assay of Phagocytic Index measures neutrophil-associated fluorescence after incubation of leukocytes with fluorescent microspheres at both 4 degrees Centigrade and 37 degrees Centigrade. The index value represents the quotient of reactivity at 4 degrees Centigrade (adherence only). A deceased Phagocytic Index is indicative of impaired phagocyte function, which is associated with repeated bacterial infections and anergy.

5.4 Tumor Necrosis Factor (TNF)

Tumor necrosis factors are pleiotropic cytokines that are considered primary modifiers of inflammatory and immune reactions and are produced in response to tumors, injury or infection. Tumor necrosis factor-a (cachectin) and tumor necrosis factor-B (lymphotoxin) are two closely related proteins that share sequence homology of 34% in their amino acid sequence. Both mediators act on their target cells via the same receptors and therefore show many similar, but not identical, biological effects. Many different cells are shown to produce TNF including CD4+ T-cells, smooth muscle cells, polymorphonuclear neutrophils, astrocytes, etc.

Due to the occurrence of TNF receptors on nearly all cells, TNF shows a wide variety of biological actions. It has cytolytic and cytostatic effects on tumor cells and shows chemotactic activity on neutrophils. TNF enhances the proliferation of T-cells after stimulation with IL-2. In the absence of IL-2, TNF induces the proliferation and differentiation of B cells.

TNF levels were analyzed with the Immulite® assay. Immulite® is a solid-phase, two-site chemiluminescent immunometric assay. The solid phase, a polystyrene bead enclosed within an Immulite test unit, is coated with a monoclonal antibody specific for TNF. While the patient sample and alkaline phosphatase-conjugated polyclonal anti-TNF antibody are incubated for 60 minutes at 37 degrees Centigrade in the test unit with intermittent agitation, TNF in the sample is bound to form an antibody sandwich complex. Unbound conjugate is then removed by a centrifugal wash, after which substrate is
added and the test unit is incubated for a further 10 minutes.

The chemiluminescent substrate, a phosphate ester of adamantly dioxetane, undergoes hydrolysis in the presence of alkaline phosphatase to yield an unstable intermediate. The continuous production of this intermediate results in the sustained emission of light, thus improving precision by providing a window for multiple readings. The bound complex - and thus also the photon output, as measured by the illuminometer - is proportional to the concentration of the TNF in the sample.

6. RESULTS

An effective immune system response involves all aspects and components of the immune system from macrophage activation to T-8 cell suppression. Stimulation affects specific immune reactions but is lacking in expression of proper immune modulation. Immunomodulatory reactions cause activity in the immune system that can be monitored, but not predicted, until the final states that are reflected in the activity of natural killer cells, macrophages and TNF (tumor necrosis factor). These three immune system markers largely indicate the state of modulation that is occurring in the milieu of the actions and reactions that cascade from immune system response to immune system homeostasis.

In monitoring the immune system “behind” the NK, TNF and PI markers we see an array of changes that reflect the processes of modulation. B cell activity will increase and decrease in predictable patterns and the production of antibodies varies accordingly. T lymphocytes are activated at regular intervals and the production of cytokines is appropriately responsive effecting related changes in immune function.

Synthetic stimulation of the immune system will often cause a change in one of the markers but will not reflect a modulation effect. Consequently, there is a limited effect on immune function. This is evident in the activation of natural killer (NK) cells. NK cell activity can be increased readily by a number of natural or nutritional substances including some vitamins and minerals. However, increasing NK cell activity alone has very limited value if immune modulation is not achieved. B cell stimulation, as initiated by substances such as transfer factor, is a good example of partial immune system activation without immunomodulation and has thus proved to be of limited clinical value.

Drugs or any substance or influence that stimulates the immune system without having a balancing effect on the immune system is severely limited in its value as compared to immune modulation. The immune system must be in a state of modulation (dynamic equilibrium) to function optimally, which makes immune modulation a critical pathway to good health.

There is now clinical double blind evidence that the cytokines, peptide neurohormones and other informational molecules in Antigen Infused Colostrum/Whey Extract modulate and normalize immune function and is thus an orally effective immune response modulator for increasing the protective functions of the immune system.

Of the ten patients in the group that received the Antigen Infused Colostrum/Whey Extract (Group A) seven had a significant increase in all three of the immune markers, NK cell, TNF and PI (macrophage) activity. Of this group, 1 person decreased TNF activity, 3 decreased in NK activity and 2 decreased in macrophage activity. Three participants had no change in the markers. These are highly significant changes reflecting a dramatic increase in immune system function and modulation within the fifteen-day period of the clinical trial. It should be noted that two people in this group were found to have significant immune system compromises, after completing the blood tests, upon entering the study.
In the control, or placebo, group (*Group B*), 5 participants had an increase in TNF, 4 had an increase in NK function and 4 had an increase in PI. Of this group there was decrease in function for 3 participants in TNF, 5 participants in NK function and 5 participants in PI. Three participants had no change in the three markers. These results reflect typical responses from a population that is not receiving specific intervention to enhance immune system function.

This chart presents the net changes in each category of measurement.
Quantitative changes are not particularly significant in viewing the activity of NK cells, TNF and macrophages in short term studies as the quantity of increases will vary from individual to individual. The three markers are in a state of flux towards increased immune function and modulation in **Group A** and in random state of change in **Group B**.

Increases and decrease in marker values can be viewed in patterns that may have some significance in monitoring immune modulation. Patterns have implications in long-term studies and are not so relevant in short term studies but this study did reveal a pattern that is pointing towards the improved function and immune modulation effects of this immune response modulator.

**Patterns Peculiar to Group A**

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In **Group A** there were two distinct patterns that were peculiar to that group. Three participants had increases of all three markers and one participant had an increase in NK activity and no change in the other two markers. In both groups there was a mixture of increases and decreases in random patterns however there were three patterns distinct for four **Group B** participants that showed a decrease in one or more of the three markers.

**Patterns Peculiar to Group B**

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Immune system modulation is not present when the marker values are below normal or out of balance with one another. When an immune system response modulator is effective there is a change in the quantities of the individual markers with all, eventually, moving into normal ranges if the immune system is not compromised in function to the point where this is not possible. The movement of TNF and PI are much less than NK cell activity as the reference ranges for those two marker are much more narrow than the NK cell activity range. However, the movement of all three markers into a high normal range is expected to occur in time with the administration of an effective immune response modulator that modulates the immune system.
7. DISCUSSION

This study investigated the immunomodulatory effect of Antigen Infused Colostrum/Whey Extract on the human immune system in vivo. Fueled by the worsening trend of severe and chronic diseases that our society is facing, many attempts are under way to find new - artificial or natural – immune response modifiers or modulators that can modulate immune function and lead to a successful counterattack against the consequences of biological entropy.

Traditionally, the battle plan has been focused upon T-cells and utilized molecular biological models in which various compounds have either increased or decreased some aspect of immune function. This paradigm utilizes analytical methods of cell and molecular biology e.g. lymphocyte sub-population response monitoring, NK function and quantitative levels, mitogen lectin proliferation, cytokine assays and the like are but merely an inadequate reflection of the flexible, complex, information processing system that the immune system represents. Some researchers have likened the data obtained by this sort of testing as analogous to the data acquired by the electroencephalograph (EEG) machine when doing research about the brain. Such data can provide valuable information as to whether the brain is dead or alive, but the qualitative functions of the brain, intelligence, the expression of emotions or the creation of a sonata can never be ascertained from such quantitative information.

What’s ultimately important about the human immune system is not just the numbers describing its activity, but that aspect of the wisdom of the body that we can refer to as “immune intelligence”. The famous medical physiologist Dr. W.B. Cannon wrote a highly acclaimed book entitled The Wisdom of The Body.

He stated therein that the main task of the wisdom of the body is to improve the quality and maintain the quantity of health. This is accomplished through control processes that are even more intricate than the feedback auto-regulatory controls of homeostasis but cannot be expressed by physiochemical laws and equations.

Generally today, instead of testing how intelligent the immune system is, our current technology allows us to merely measure the potency of some of its weapons. This is a critical flaw in most of today’s research. In-vitro studies that are so commonly performed rarely have any useful clinical application. At the same time, experimental data on various uniquely created, or herbally extracted immunomodulating compounds created by different research groups, owing to their adynamic nature, are all but impossible to integrate into a comprehensive therapeutic protocol.

Previously presented clinical research papers demonstrated that the immune system is a dynamic multidimensional system that can protect us by using a variety of very different strategies. Evolutionary biochemistry has imbued our immune system with several overlapping protective tactics that can at least partially replace one another. To presuppose that administering a single biochemical substance with static immunomodulatory effects to correct an immune deficiency will work reproducibly well in most patients appears short sighted and naïve. In fact, such substances administered in this manner will generally only undermine the potential good that the agent may do in the long run if properly balanced and supported.

Antigen Infused Colostrum/Whey Extract is the first of a new type of immune-modulator for two reasons.

First, it is produced in a living mammal for other living mammals and thus has dynamic elements reflected in the wisdom of the body.

Secondly, as the studies with Icorin™ have demonstrated single antigen (or no specific antigen)
stimulation of a dairy cow does not produce a wide enough range of immunological factors to reliably produce a beneficial response in the recipient. Therefore, when making Antigen Infused Colostrum/Whey Extract such as that used in the study five (5) different antigens are used to produce a response. The resulting material is then combined and used to get not only a primary immune response but also a broader secondary effect, by "spreading", of NK-cell activation. This is most clearly seen in people with good biochemical support but a very compromised immune system.

Current statistics clearly demonstrate that the incidence of severe and chronic diseases is worsening. Furthermore, according to published studies virtually every severe and chronic disease is associated with some level of immune dysfunction at its core. This dysfunction can only be caused by one (1) or more of five (5) different factors:

1) Stress – cortisol suppresses immune function
2) Infection – EBV, CMV, HIV, etc.
3) Toxin – heavy metals, petrochemicals, etc.
4) Trauma – radiation
5) Deficiency – vitamin C, Zinc, Iodine, Arginine, Vitamin A, etc.

Each of these potential causes of immune dysfunction must be investigated and addressed in each patient. Correcting these problems does not, in itself, automatically re-establish the normal immunoregulatory pathways of the immune system. As a result of global pollution, viral exposure and our fast paced stressful society virtually everyone has some level of immune dysfunction that requires intervention to minimize their risk of illness. Based upon our current knowledge of clinical immunology, it is an imperative of our species to modulate and support our immune system.

The implications of widespread use of an immune response modulator such as that examined herein would have an enormous impact on the general health of a population such as that in the United States, where immune suppression by lifestyle is an inherent factor in the nature of the culture at this time. Improvement in immune performance as experienced by the study group that utilized Antigen Infused Colostrum/Whey Extract would result in a reduction in dependence upon prescription medications ranging from antibiotics to antidepressants and an improved ability to cope with the stresses of everyday life. The long-term benefit could be expected to manifest in reversing the rising incidence of degenerative and chronic disease in people of all ages, and especially the most vulnerable, so directly related to the stresses discussed herein.
Appendix 1

Blood tests examined for all participants:

- T & B Cell Subset
- Natural Killer Cell Function
- Eosinophilic Cationic Protein
- TNF
- Phagocytic Index
- IL-2r
- IL-6
- a-INF
### Statistical Summary

#### Group A

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