Muno-IgY: Aiding the Workload of two Pleiotropic Cytokines

Pleiotropic *– "having more than one effect"*

We are working through the results of a double-blind placebo controlled study that was conducted at the University of North Texas, Kinesiology lab, under the guidance of Dr. Brian McFarlin. Not all of the results have been completed and tabulated, however we decided to share our current findings. The focus of the study is our Muno-IgY powder looking at the following key outcomes:

1) To establish an effective dose amount for this high purity IgY extract;
2) To monitor changes in immune response to dose;
3) To establish whether or not the IgY protein molecule passes through the lumen of the gut.

Four separate doses were used in the study: 14.5mg, 29mg, 43.5mg and placebo. A dose response was detectable at every level, but there was variability at the dose of 14.5mg. "**To guard against this potential source of variability, it is reasonable to recommend a dose of at least 29mg per day for all future experiments."**[i] It must also be pointed out that the study was based on a group of healthy individuals at rest, and future studies will continue to look at dose responses for individuals under stress. A recommended dose of 29mg is a significant outcome for the company as the closest any other IgY based product currently on the market offers is a daily dose starting at 4,500mg. Our results see a 98% improvement in industry standards, allowing users to take one capsule per day, compared to table spoons of powdered whole egg IgY.

While measuring results in resting biomarkers of immune health, two notable changes in specific cytokines were tabulated. Both IFN-gamma and GM-CSF had statistically significant changes. These cytokines play a role in mediating the activation of type-1 immune system cells (i.e. natural killer cells, granulocytes, and monocytes) as well as the trafficking of these type-1 cells into and out of peripheral tissue compartments. The study concludes that **"It is reasonable to speculate that IgY may have in fact improved the type-1 immune response in the treatment group."**[ii]

How does Muno-IgY Affect the Immune System?

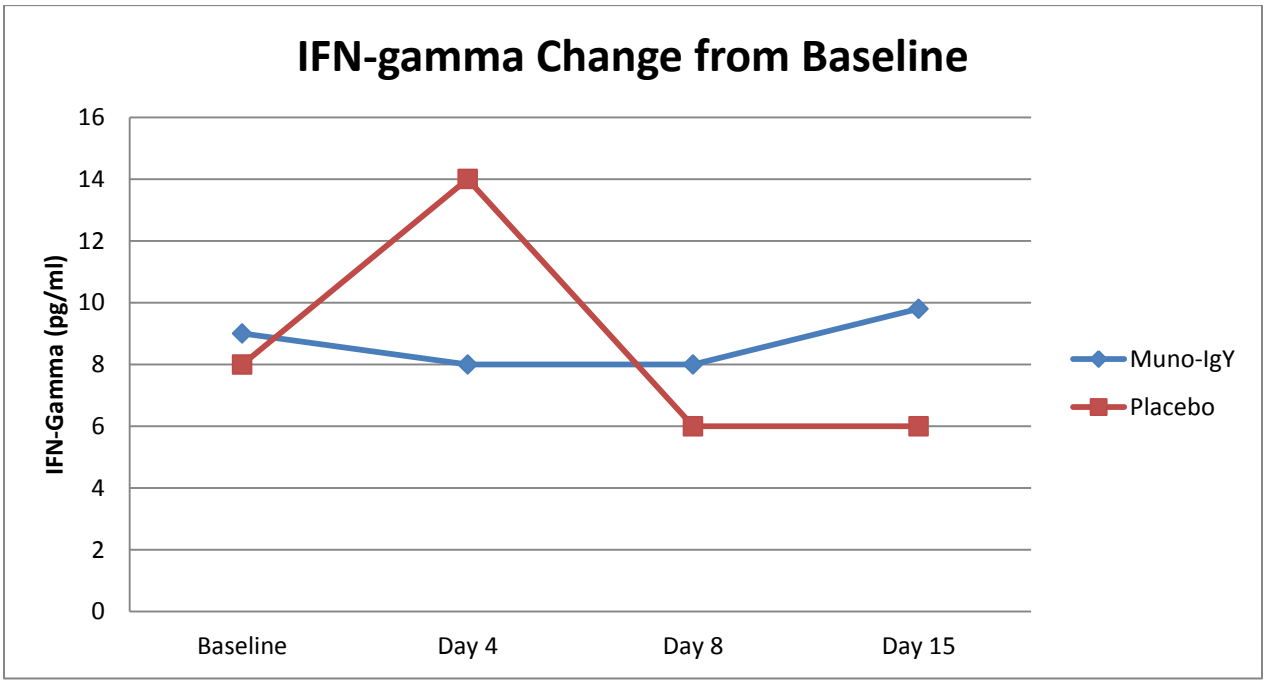
It has been said that Muno-IgY is an immune system *modulator;* it helps to restore the body to a state of immune homeostasis. We have received comments from healthy users stating that they feel nothing when taking the product, but they certainly noticed when they stopped using it for a while. Which is a perfect response if you think about a body in balance, you should feel nothing happening. On the other hand, those

who are under stress (from the immune system compromised to the pro-athlete) always come back with the most specific comments, making reference to lower inflammation, more mobility, faster recovery times and more endurance.

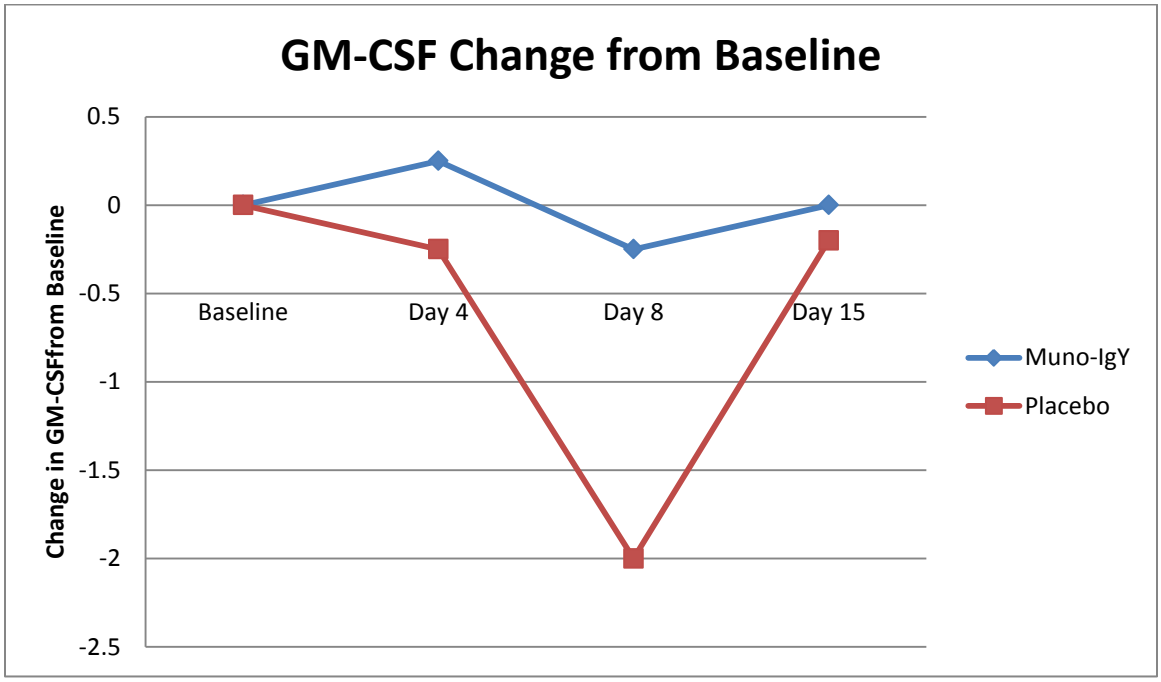
During the UNT study, the two largest movements happened in the placebo group, with significant change in two cytokines, IFN-gamma and GM-CSF. For the Muno-IgY groups, their levels stayed relatively normal. It was the extreme movement from the placebo group that caught our attention. Researcher Brian McFarlin assumes that all test subjects came into contact with the same 'negative stimulus', but only the placebo group had negative reactions.

Statistical Data from the 15-Day Trial

We witnessed from the two cytokines, in both cases, that the Muno-IgY groups had little inflection from their baseline starting points, while the placebo group had significant movement, either positive or negative. If you are trying to get from point 'A' to point 'B', the shortest distance is a straight line. And this, in short, is what the Muno-IgY has achieved. It has allowed the test subjects to get to the end of this study without any exertion placed on their immune systems. While the placebo group had to call their primary immune functions into order to overcome whatever stimulus had occurred during the trial. Our research team assumes that all participants came into contact with the same stimulus during the trial.



When the immune system is not put into action, it remains on alert and ready for duty. Once it is called into action, it has limited reserves, based on the general health of the subject, which include age, fitness level and overall health conditions. The more that Muno-IgY can do to take the work load off the body's immune system, the greater your chances are to remain in balance.



Interpreting the Data I

GM-CSF cytokine

While measuring the cytokine GM-CSF, there was a significant drop in values on day 8 of the placebo group, measuring a 10-fold difference to Muno-IgY group in serum levels (P=0.034; ES=0.31).[iii] While an additional 50 subjects is recommended to explore these changes in greater detail, it appears that the Muno-IgY treatment group was better prepared to fight off an attack on the immune system.

The significance of this difference could be substantial as GM-CSF is a primary cytokine for both the innate and adaptive immune system. The difference between the two values underscores the Muno-IgY group's ability to traffic more of innate immune response. To better understand the GM-CSF cytokine, here is a brief description of its role within the immune system. It is considered a pleiotropic cytokine, meaning it is responsible for many downstream events, an ultimate multi-tasker.

It is responsible for:

- Embryonic placenta development
- Immune response, both innate and adaptive
- Positive regulation of cell proliferation and gene expression
- Myeloid dendritic cell differentiation (very important for M.S.)
- positive regulation of DNA replication (anti-cancer)
- cellular response to lipopolysaccharide (left unregulated leads septic shock)
- Macrophage activation

- Positive regulation of DNA replication
- Positive regulation of white blood cell generation

GM-CSF as a white blood cell growth factor stimulates stem cells to produce Granulocytes, and Monocytes. Granulocytes include neutrophils, eosinophils and basophils and are an essential part of the innate immune system.

- Neutrophils are the first-responders to the site of inflammation. They are anti-microbial and attract macrophages to the infected area.
- Eosinophils are responsible for combating multicellular parasites and they control mechanisms associated with allergy and asthma.
- Basophil granulocytes are the least common, and are very important to react to allergy symptoms. They contain the vasodilator histamine, which promotes blood flow to tissues.

The UNT study contained a Complete Blood Count. Venous whole blood was used to determine a complete blood count (CBC) on a hematology analyzer (Mindray BC-3200) to determine total white blood cell (WBC), red blood cell (RBC), granulocyte, monocyte, lymphocyte, and platelet concentration. From the detailed report, I can quote UNT results as "examination of the numerical changes did not reveal any obvious effect of IgY supplementation on routine blood chemistries."[iv]

The fact that there was a ten-fold difference between the Muno-IgY group and the placebo group means that the Muno-IgY group body was better prepared for an immune response. The GM-CSF cytokine is first in line to build a type-1 immune response, building monocytes that mature into macrophages and dendritic cells.

Macrophages and dendritic cells are critical for fighting infection. They are both key to the innate and adaptive immune system. Macrophages are either going to produce killer cells, or repair cells involved in tissue repair (think athletes). They attract foreign substances, infectious microbes and cancer cells. As part of the immune response, they are critical in the development of inflammation. After they kill a foreign pathogen, they identify the molecule with an antigen which then becomes part of the adaptive immune system. Therefore, this is helping your body build more antibodies to targeted pathogens, building your immune system to better withstand future invasions.

For muscle regeneration, macrophage enters the muscles during periods of increased use, a critical process to lowering inflammation. Remember, the body has limited immune system resources and since most pathogens enter the gut, a stressed immune system is putting defenses toward a primary area of attack, leaving an athlete with sore and tired muscles. With the appearance of Muno-IgY restoring the gut side of the equation, the immune system can turn its attention to secondary issues such as muscle repair. This will help to explain why our athletes are experiencing less inflammation after extreme exercise. Macrophages promote muscle regeneration between the second and fourth day, after exertion (hence, Muno-IgY is a recovery protein, but with those other properties, using it all the time has much bigger ramifications for your immune system, including avoiding illness.)

Macrophages are essential for wound healing to begin. The spleen contains half of the body's monocytes in reserve, ready to be deployed to injured tissue. The monocytes from the bloodstream enter the area through blood vessel walls. Once there, the monocytes mature into macrophages. The macrophages' main role is to

phagocytize bacteria and damaged tissue and they also debride damaged tissue by releasing proteases. This process is critical in wound healing, and helping the body grow new blood vessels from pre-existing vessels - this is referred to as Angiogenesis. And finally, as if that was not enough, the macrophages are critical for the development of granulation tissue. This is the new tissue that is seen growing over an open wound. So the GM-CSF not only helps close the wound, it fights off the invading pathogens trying to get in.

In addition to the macrophages, those monocytes also turn into dendtric cells which are antigen-presenting cells that are key messengers between the innate and the adaptive immune systems. These cells are in direct contact with the outside world, found in the inner lining of the nose, lungs, stomach skin and intestines. They are designed to interact with T cells and B cells to initiate and shape the adaptive immune response.

Remembering that the body has limited resources, using Muno-IgY seems to allow for greater distribution of immune system responsibilities for an overworked (stressed) pleiotropic cytokine.

Interpreting the Data II

IFN-gamma cytokine

The second cytokine that had a notable response was IFN-gamma, which demonstrated that Muno-IgY subjects had 41% lower levels of IFN-gamma than placebo subjects at day 4.[v] Looking at the chart again, you will see that essentially the Muno-IgY subjects IFN-gamma levels remained steady and true, while the spike occurred in the Placebo group. Holding onto the body's IFN-gamma reserves, the Muno-IgY group was better prepared for a further immune system event.

IFN-gamma is a class II Interferon. These cytokines are responsible for inhibiting viral infections and in stimulating the entire immune system to fight disease, in response to pathogens. They are called interferons because they "interfere" with the ability of a virus or bacteria to replicate itself within the body. Interferon-gamma is special and classified as a type II interferon for its unique ability to regulate the overall immune system. **As a pleiotropic cytokine it is involved in the regulation of nearly all phases of immune and inflammatory responses, including the activation, growth and differentiation of T-cells, B-cells, macrophages, NK cells and other types such as endothelial cells and fibroblasts. It enhances the cytotoxic activity of T-cells, macrophages and natural killer cells and thus has antiproliferative effects. It also increases the production of antibodies in response to antigens administered simultaneously with alpha-interferon, possible by enhancing the antigen-presenting function of macrophages.**

The net effect of the long list of activities associated with this cytokine is to promote inflammatory reactions dependant on macrophages, while inhibiting IgE-dependent reactions. Knockout mice in which IFN-g or the IFN-g receptor genes have been disrupted show several immunologic defects.

Clearly, this cytokine, like GM-CSF has a priority list of responsibilities and a limit to its overall effectiveness based on the stresses being placed on the body. Muno-IgY can go a long way to taking the load off this system, and returning the body to neutral.

How does Muno-IgY Benefit Athletes?

Remember, limited resources with a clear set of priorities, at the end of which would be the immune system's ability to help an athlete recover. It is just not as important as rebalancing the gut as a primary issue. With Muno-IgY able to remove stress from the immune system's workload, greater attention can be placed on secondary functions. Let me just jump back to the angiogenesis, the building of those new blood vessels. Kicking that process off, also cascades into gene activation and a set of diverse biological responses, including cell differentiation, proliferation, and matrix dissolution, which initiates a process of mitogenic activity critical for the growth of endothelial cells, fibroblasts, and smooth muscle cells. All of this of course starts to explain why our athletes recover so quickly, but also why Nik Lewis, our CFL receiver who broke his leg last August, healed in half the time expected. This of course will have to be further examined with additional research, but the anecdotal evidence we gather in the field with Vector450 users is important to our ongoing research. Angiogenesis is generally associated with aerobic exercise and endurance exercise. Angiogenesis causes changes that allow for greater nutrient delivery over a long period of time. Capillaries are designed to provide maximum nutrient delivery efficiency, so an increase in the number of capillaries allows the network to deliver more nutrients in the same amount of time. It also allows for greater oxygen exchange in the network. So the more Muno-IgY is used over a continuous time period, the athlete is able to train for an extended period of time. Initially we had the idea that Muno-IgY was allowing for an increase in red blood cells, which would explain why their endurance was improving. We have seen no change in serum blood levels to suggest this is happening. But now it seems that the increase in endurance could be due to more blood vessels delivering more nutrients and oxygen to their muscles. Every athlete's dream comes true if we demonstrate this in follow-up work. This theory should become part of the athlete recovery study planned for summer 2014.

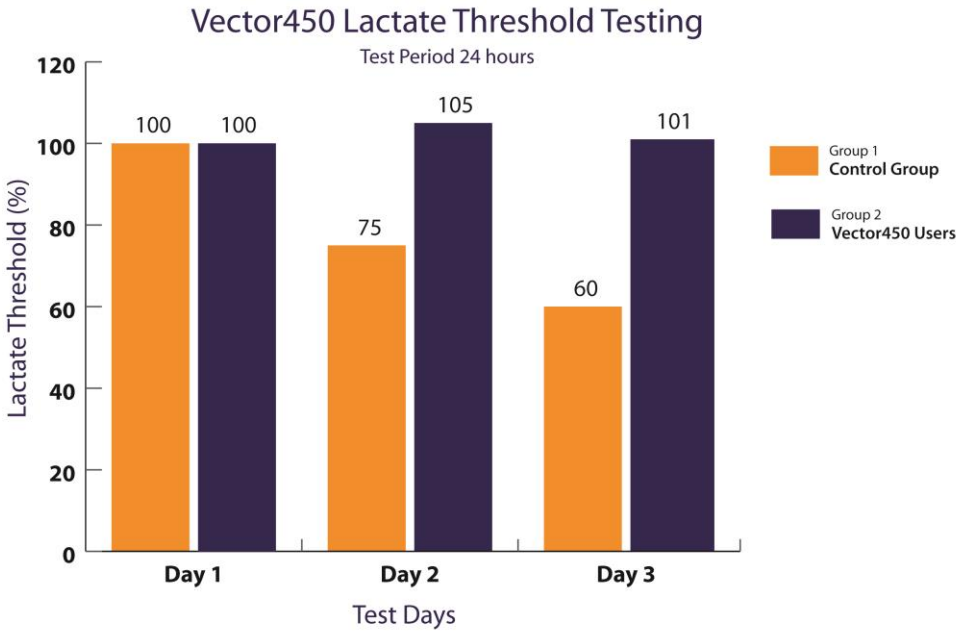
Early Indications that Muno-IgY is Effective in Athletes

Overtraining is a process of excessive exercise training in high-performance athletes that may lead to overtraining syndrome. Although high-performance athletes are generally not clinically immune deficient, there is evidence that several immune parameters are suppressed during prolonged periods of intense exercise training. These include decreases in neutrophil function, serum and salivary immunoglobulin concentrations and natural killer cell number and possibly cytotoxic activity in peripheral blood.

It is best identified by a combination of markers, such as decreases in urinary norepinephrine output, maximal heart rate and blood lactate levels, impaired sport performance and work output at 110% of individual anaerobic threshold, and daily self-analysis by the athlete (i.e. high fatigue and stress ratings).

No work, to our knowledge, has been done on urinary output while using IgY, but there is clinical work using an IgY recovery protein which indicated a 3% decrease in Submaximal heart rate after 10 days supplementation. While this was a short study, it also used a whole-egg IgY that was 98% less pure than Muno-IgY.

Blood lactate levels is a more interesting test which we have performed on 4 subjects, each demonstrating the ability to exceed their lactate thresholds while using Vector450 in back-to-back testing. Normal results always indicate a lower lactate threshold the 2nd day, once the athlete has pushed through their threshold on the first day of testing. This is a test where you can not fool the machine, and the results we see in the lab are similar to the field reports from our professional athletes who train for Iron Man competitions.



Currently there are over forty professional athletes who have been using Vector450 to supplement their immune systems and they continue to provide feedback, which we share on the Vector450 website. Their comments not only focus on their ability to avoid illness, or to rebound very quickly at the first sign of getting sick, they also talk about their recovery times. The next focus will be to combine the information from the dosing study into the higher stress model of an athlete recovery study.

Beyond Pro-Athletes

While Vector450 had been created to market toward the athlete market, initially to keep them healthy from the stresses of over training, the strategy was to demonstrate that if we could keep this group of individuals healthy then the product could also work on immune systems suffering from other stress loads such as seasonal allergies, cold and flu season and possibly auto-immune issues that result in inflammation.

Similar to the discovery that Vector450 was helping athletes recover quicker and deal with inflammation, a group of individuals with known Lupus and Rheumatoid Arthritis had become consistent users of the product. As they suffer from constantly challenged immune systems, the initial aim was the same as the athletes, to keep them healthy and avoid simple colds and flus. The surprises came when they reported lower inflammation issues, less fatigue and muscle soreness. We then decided to assemble an open label study for Systemic Lupus Erythematosus (SLE) suffers and provided them with enough products to get through a 90-day trial. To date, 15 subjects have entered the study with a reported 8 having completed all three study questionnaires.

To determine if the product was having any positive effect, we used a standardized Quality of Life Survey called the Rand 36. It is comprised of 36 items that assess eight health concepts: physical functioning, role limitations caused by physical health problems, role limitations caused by emotional problems, social functioning, emotional well-being, energy/fatigue, pain, and general health perceptions. Physical and mental health summary scores are also derived from the eight RAND-36 scales.[vi]

Of those whom have completed the survey, the following positive outcomes have been observed:

- 26.43% improvement in pain levels
- 15% improvement in ability to perform vigorous activities
- 39% improvement in the ability to perform daily activities
- 25% improvement in joint mobility

The most common comment from respondents when asked about taking Muno-IgY was in reference to energy. Here is a sample respondent:

> *This product is amazing, my muscle strength was completely changed, I was always having muscular pain in my chest, totally resolved, wasn't able to run anymore and was having great runs in record time. My energy levels were like before I became ill, an incredible difference. I have enjoyed an active summer, which hasn't happened for a couple years. I also noticed a lot less swelling in my hands and face.*

The work on our Lupus study is not yet complete, but now that we are seeing the results from UNT on two key cytokines, I went looking for other literature that focused on Lupus and IFN-gamma and GM-CSF. From the 2004 edition of Lupus journal comes a study titled **Increased frequency of GM-CSF secreting PBMC in patients with active systemic lupus erythematosus can be reduced by immunoadsporption.** [vii]

Their observation was that patients with active SLE had a significantly increased number of GM-CSF secreting peripheral blood mononuclear cells (PBMC) and a significant decrease of IFN-gamma. The strategy of the study was to use an immune system inhibitor to lower the over production of GM-CSF and IgG, which is in abundance in SLE patients. From our observations it would seem logical to add these cytokine measurements to our future studies as the Muno-IgY appears to be having the net effect that the Westphalian University researchers were looking to achieve.

Conclusion

Understanding the mechanisms of action of a dietary ingredient takes time and effort. We are fortunate that those who are using the Vector450 with Muno-IgY product have been generous in sharing their outcomes, which we bring back to our team of experts in an attempt to interpret what is going on with their immune systems. Within the UNT report, we see two pleotropic cytokines, each with a long list of duties to re-balance the human immune system. In both cases they are being called into action within the placebo group. Meanwhile, there appears to be balance within the immune systems of the Muno-IgY group. As an immune system modulator, this is the ultimate goal, to have a nutraceutical removing the action before it even starts. To feel 'nothing' is the best outcome.

Vector450 is being used by both athletes and many people with auto-immune issues. Less inflammation is one of the first comments back from both groups, even though the source of inflammation is decidedly different. What is not different however is the body's defence mechanism to deal with stressors that cause inflammation, and both GM-CSF and IFN-gamma are involved in this repair process. If Muno-IgY makes that process easier, we are on our way to developing the story of the next great Super-Nutraceutical.

[i] *To Determine an Appropriate Oral Muno-IgY Dosing Regime to Elicit Physiological Changes in Serum Cytokines; Dr. Brian McFarlin, University of North Texas, Department of Kinesiology, unpublished research report, page 9.*

[ii] *To Determine an Appropriate Oral Muno-IgY Dosing Regime to Elicit Physiological Changes in Serum Cytokines; Dr. Brian McFarlin, University of North Texas, Department of Kinesiology, unpublished research report, page 9.*

[iii] *To Determine an Appropriate Oral Muno-IgY Dosing Regime to Elicit Physiological Changes in Serum Cytokines; Dr. Brian McFarlin, University of North Texas, Department of Kinesiology, unpublished research report, page 7.*

[iv] *To Determine the Dosing Pattern for IgY along with Appropriate Biomarkers for Detection in Serum – University of North Texas, unpublished paper by Dr. Brian McFarlin, page4.*

[v] *To Determine an Appropriate Oral Muno-IgY Dosing Regime to Elicit Physiological Changes in Serum Cytokines; Dr. Brian McFarlin, University of North Texas, Department of Kinesiology, unpublished research report, page 4.*

[vi] *The Rand 36 Quality of Life Survey, www.rand.org*

[viivii] *P. Willeke, Department of Medicine B, Westphalian Wilhelms-University, Muenster, Germany, as reported in Lupus (2004) 13, 257-262.*