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The Effect of Natural Compounds on Lymphocyte and Osteoblast Function

Donielle Ford

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THESIS

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THESIS

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By

Donielle Ford, B.S.

Texas Southern University

2009

Approved By:

Alamelu Sundaresan

Chairperson, Thesis Committee

Guy H. Mabry

Dean, The Graduate School

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Professor Alamelu Sundaresan, Advisor

Arthritis is the leading cause of disability in the United States, and although cost-effective interventions are available to reduce the burden of arthritis, they are currently underused. The goal of this project is to study and test natural compounds on their selective ability to improve lymphocyte function in lymphocyte function deficit model such as microgravity. A model such as this would provide a test bed to test different compounds on diseases involving osteoblast and immune dysfunction and identify biomakers in inflammation. The hypothesis of this project is that the effects of natural compounds on lymphocytes and osteoblast in modeled microgravity will increase cell growth and regulation in inflammatory diseases. To test this hypothesis cells will be cultured using a rotating-wall culture system that will model some aspects of microgravity (mg). The preliminary testing and optimization for this project will be

performed using isoflavones and nucleotides. Scanning electron micrographs (SEM) of treated and untreated lymphocytes will also be performed. Gene arrays will be used for gene expression. Results show that in lymphocytes with no nucleotide treatment cells appear stressed and pinched looking. Cell size is reduced and there appears to be erosion of cell structure. In contrast in nucleotide treated lymphocytes, cells were rounded and healthier looking. There was a significant increase in isoflavones treated osteoblast growth compared to cells. In conclusion, since the lymphocyte is the primordial cell affected in inflammatory diseases such as arthritis, these were investigated for inflammation related gene expression. Inflammatory and oxidative stress inducers were significantly up-regulated in model microgravity. In human lymphocytes fold change above '1' are considered significant since these are normal cells and not transformed. This corroborates results seen in other laboratories both in analog microgravity and spaceflight.

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LIST OF ABBREVIATIONS

g	gravity
mg	microgravity
mmg	modeled microgravity
SEM	Scanning Electron Micrograph
cDNA	Complementary DNA
cRNA	Complementary RNA
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
EDTA	Ethylenediaminetetraacetic acid
MES	2-(N-Morpholino) ethanesulfonic acid
NASA	National Aeronautics and Space Administration
µl	Micromoler
µm	Micrometer
PBMC	Peripheral Blood Mononuclear Cell
RA	Rheumatoid Arthritis
OA	Osteoarthritis
PCR	Polymerase Chain Reaction
JSC	Johnson Space Center

VITA

April 27, 1981.....Born- Monroe, Louisiana
Hometown- Grand Prairie, Texas

2005 ..... B.S., Texas Southern University
Houston, Texas

2005-Present .....Teaching Assistant
Department of Biology
Texas Southern University

2006-Present .....Graduate Research Assistant
Department of Biology
Texas Southern University

Major Field.....Biology

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CHAPTER 1

INTRODUCTION

Diseases Involving Osteoblast and Immune Dysfunction

Arthritis is the leading cause of disability in the United States, and although cost-effective interventions are available to reduce the burden of arthritis, they are currently underused. Besides the physical toll, arthritis costs the country nearly $65 billion annually. Forms of arthritis can be classified by how they disrupt the normal functioning of the joints. In most people, at the hinge where bones meet, there is cartilage at the end of the bones. A gel called synovial fluid is found between the bones which cushions movement. The synovial fluid is made up of proteoglycans, which can bend and release water molecules to absorb mechanical strain. The proteoglycans are composed of protein and glycosaminoglycans (GAGs), polysulfated molecules produced by cells called chondrocytes.

Arthritis means joint inflammation. Inflammation is a natural part of the body's response to injury and infection, and is a very complex process that produces swelling, pain, warmth and redness. Inflammation is not only a response to injury, but it may perpetuate injury as well. "Inflammation can include infiltration of inflammatory cells (monocytes), synovial hyperplasia, bone erosion and new bone formation, narrowing of

the joint space and ankylosis of the joint". (Han, J., 2007) Significant problems arise when inflammation is persistent, intense, or recurrent, or spreads to other areas of the body. When joints become arthritic, swelling will cause stiffness, rigidity and tissue damage. Pain, which is the body's signal that something is wrong, occurs as the joint is moved to the brink of its own limits. As mobility decreases, the muscles surrounding the joints are moved to the brink of its own limits.

Objective of Proposed Study

The goal of this project is to study and test natural compounds for their selective ability to improve lymphocyte function in a lymphocyte function deficit model such as microgravity. This model would provide the basis to test the effects of different compounds on arthritis and lupus *in vitro* and to identify biomarkers involved in inflammation.

CHAPTER 2

HISTORY AND RELATED LITERATURE

The forms of arthritis can be classified by how they disrupt the normal functioning of the joints. In most people, at the hinge where bones meet, there is cartilage at the end of the bones. A gel called synovial fluid is found between the bones, cushioning movement. The synovial fluid is made up of proteoglycans, which can bend and release water molecules to absorb mechanical strain. The proteoglycans are composed of protein and glycosaminoglycans (GAGs). GAGs are polysulfated molecules produced by cells called chondrocytes.

"Arthritis sufferers include men and women, children and adults. Approximately 350 million people worldwide have arthritis. Nearly 40 million people in the United States are affected by arthritis, including over a quarter million children. More than half of those with arthritis are under 65 years of age. Nearly 60% of Americans with arthritis are women". (medicinenet.com//arthritis) By the year 2020, the number of people with arthritis is expected to reach 60 million.

Osteoarthritis

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normally produce flexible cartilage, are replaced by osteoblasts, which produce hard bone. Bone replaces the cartilage and the synovial fluid is diminished. Cartilage is a protein substance that serves as a "cushion" between the bones of the joints. "Osteoarthritis is also known as degeneration arthritis. Among the over 100 different types of arthritis conditions, OA is the most common". (medicinenet.com) OA affects to some degree nearly 80 percent of people over 50. It commonly affects the fingers, knees, hips ankles and spine. Its common symptoms are joint pain, stiffness or swelling. "Pain in OA is localized and use-related, occurring during movement or weight bearing". (Adwanikar, H., 2007)

"Primary OA is mostly related to aging. With aging, the water content of the cartilage increases, and the protein makeup of cartilage degenerates. Eventually, cartilage begins to degenerate by flaking or forming tiny crevasses. In advanced cases, there is a total loss of cartilage cushion between the bones of the joints". (medicinenet.com) Repetitive use of the worn joints over the years can irritate and inflame the cartilage, causing joint pain and swelling. Loss of the cartilage cushion causes friction between the bones, leading to pain and limitations to joint mobility. Inflammation of the cartilage can also stimulate new bone outgrowths to form around the joints.

Rheumatoid Arthritis

Following OA in prevalence is rheumatoid arthritis (RA), a lifelong autoimmune disease that begins as early as a person's late 20s. "RA is characterized by chronic inflammation and destruction of the synovial joints leading to progressive joint damage and disability". (Alfredsson, L., 2007) It can lead to an inflammatory poly –arthritis.

Autoimmune diseases are illnesses that occur when body tissues are mistakenly attacked by its' own immune system. RA is three times more likely to occur in women as in men. "RA, along with other categories of inflammatory arthritis, is characterized by prominent synovial tissue". (Benito, M.,2004) In RA, the immune system mistakenly causes deterioration of the joint, limited movement and even deformity. RA can also cause inflammation in other body tissues and organs. "RA is characterized by an extensive dysregulation in skeletal homeostasis recognized as 1) focal articular bone erosion, 2) luxta-articular osteopenia, 3) systemic osteoporosis and fractures, as is well documented in both cross-sectional and prospective studies". (Dimunno, O., Delle Sedia, A. 2008) While RA is a chronic illness, meaning it can last for years, patients may experience long periods without symptoms. Typically, however, RA is a progressive illness that has the potential to cause joint destruction and functional disability.

Fibromyalgia

Other forms of arthritis include fibromyalgia, an inflammatory syndrome of the muscles and joints; lupus, a systemic disease that can cause inflammation of any organ or tissue; and gout, a metabolic joint disease in which crystals of uric acid are deposited in the joints, causing inflammation.

Lupus

Lupus is an autoimmune disorder that can affect various parts of the body, including the skin, joints, heart, lungs, blood, kidneys and brain. Normally the body's immune system makes proteins called antibodies to protect the body against viruses, bacteria, and other foreign materials. These foreign materials are called antigens. In an autoimmune disorder like lupus, the immune system cannot tell the difference between

foreign substances and its own cells and tissues. The immune system then makes antibodies directed against itself. These antibodies cause inflammation, pain and damage in various parts of the body. Inflammation is considered the primary feature of lupus. Inflammation is characterized by pain, heat, redness, swelling and loss of function, either on the outside of the body or both.

The Lupus Foundation of America (LFA) estimates between one and a half and two million Americans have a form of lupus, but the actual number may be higher. More than 90 percent of people with lupus are women. Symptoms and diagnosis occur most often when women are in child-bearing years, between the ages of 15 and 45. In the United States, lupus is more common in African Americans, Latinos, Asians, and Native Americans than in Caucasians.

Lupus in some can be mild or severe and sometimes fatal. Some of the more serious complications which involve major organ systems are inflammation of the kidneys, which can affect the body's ability to filter waste from blood. This can be so damaging that dialysis or kidney transplant may be needed. Lupus can lead to an increase in blood pressure in the lungs (pulmonary hypertension); and inflammation of the heart muscles (mycocarditis), which can lead to congestive heart failure.

Treatments

"New approaches in the treatment of OA including new drug developments are hindered by the lack of objective and measurable standards for disease progression by which such treatment s can be evaluated". (Abe, M., 2004) However, primary to the treatment of all forms of arthritis is basic nutrition and exercise. A diet that is high in fiber, moderate in protein, and includes large amounts of fresh produce and cold-water

fish is the basic plan. Foods that increase acidity in the body should be avoided; these include alcohol, caffeine, saturated fat and acidic produce, such as tomatoes and eggplant. An increased level of acid in the body collects in connective tissues, promoting inflammation and pain.

Conventional treatment of arthritis may rely on non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen to relieve swelling, in addition to painkillers such as aspirin to relieve pain. A newer class of drugs called COX-2 inhibitors focuses on inhibiting a single metabolic enzyme associated with inflammation and pain. In the body, there is an enzyme known as cyclooxygenase (COX) that transforms fat into prostaglandins. NSAIDs act to block the activity of COX in the body; however, and they carry the side effect of gastrointestinal (GI) distress in some patients.

It was discovered in recent years that COX is actually two enzymes. COX-1 acts to make prostaglandins that protect the kidneys and GI tract; COX-2 makes prostaglandins that increase inflammation and pain. This led to the evolution of COX-2 inhibiting drugs, which aim to stop the inflammation without inhibiting the positive actions of COX-1. While the effects of drugs have been shown to be effective, the long-term effects of drug supplementation to suppress enzyme activity are unknown. "We know that COX-2 plays an important role in the body's ability to repair damaged tissues and in maintaining proper blood flow through the kidneys" wrote Earl Mindell, Ph.D., in *Arthritis: What You Need to Know* (Avery Publishing, 2000). "The result of suppressing this enzyme long-term remains to be seen."

Medical journals and drug companies have been open to new roads for preventing and treating these inflammatory conditions. A review in the *British Journal of Nutrition*

concluded, "There is a growing scientific rationale for the use of dietary supplements as adjuncts in the treatment of inflammatory disorders such as rheumatoid arthritis and osteoarthritis." Researchers in the nutrition field have been busy exploring different compounds that may work to prevent inflammation, rebuild joints and mediate pain.

Repairing Joints

Nutritional compounds that prevent inflammation, rebuild worn joints areas, or simply prevent further degeneration, also assist in pain management. However, many compounds go to the heart of the matter to directly impact the joint area. Less well-known, but increasingly found on the market, is green-lipped mussel. While early studies were contradictory, current research has been positive regarding its anti-inflammatory abilities. Clinical studies have demonstrated very anti-inflammatory activity in patients with osteoarthritis, rheumatoid arthritis, asthma and other inflammatory conditions. The primary compound studied has been a lipid-rich extract, prepared from New Zealand green-lipped mussel powder. According to researchers in Scotland, the stabilized lipid fraction appeared to reduce both pain and inflammation without having adverse side effects on prostaglandin and immune systems.

Newer research is being conducted on the active lipid ingredient extracted from the lip of the mussel. Sarken Nutrition, for example has studied its GlycoSea product for its anti-inflammatory abilities. "Our studies have shown that almost 80 percent of people taking this extract find relief from inflammatory condition." said Sarken's Rick Money. Two research studies are still pending publication, both examining the effects of the GlycoSea extract on animals with joint diseases. The studies found that this product relieved symptoms, showing a clinically significant beneficial effect on the signs of

degenerative joint disease. Sarken attributes the product's ability to treat RA and OA to the combination of anti-inflammatory activity and chondroprotective compounds such as GAGs within the extract.

Herbal compounds also function as anti-inflammatory agents. The gum resin of *Boswellia serrata*, a tree that grows abundantly in India, contains a combination of boswellic acids that have emerged as effective NSAIDs without the GI side effects. Studies have indicated that the anti-inflammatory action of boswellia is inhibiting 5-lipoxyganase, the enzyme responsible for production of inflammatory leukotrienes. It also decreases the level of tissue-destructive enzymes.

Natural Compounds

Natural compounds have shown to be beneficial because they are not regulated by the FDA and there are no known side effects. There are many products on the market featuring boswellia, primarily, in the Ayurvedic lines (since it was traditionally used in Ayurvedic medicine). Studies using natural compounds to alleviate arthritis are few and far between. These need to be undertaken with more zeal in conformance with the call from NIH regarding natural compounds and their importance to the NIH critical path. Since the side effects are nil to none this is important in the context of the patient's quality of life and reduction of health care costs.

Research in several areas of healthcare has shown that consumption of isoflavones may play a role in lowering the risk for disease. They can fight disease on several fronts. Soy isoflavones help in the preservation of the bone substance and fight off osteoporosis. This is the reason why people in China and Japan rarely have osteoporosis, despite their low consumption of dairy products, whereas in Europe and

North America the contrary happens. Unlike estrogen, which helps prevent the destruction of bone, evidence suggests that isoflavones may also assist in creating new bone. Other studies are not entirely consistent, but evidence suggests that genistein and other soy isoflavones can help prevent osteoporosis. Nucleotides have also been proven to have many beneficial effects in overcoming immune suppression and inflammation in both "in vitro" and "in vivo" models of microgravity in human lymphocytes. (Sundaresan 2006) Both isoflavones and nucleotides will be used in this study. Nucleotides (Otsuka pharmaceuticals, Japan), will be used to treat lymphocytes prior to Scanning Electron Micrography and isoflavones (Amino Up, Japan), will be used in the osteoblast experiments. If the results show promise, the nutritional compound palate will be expanded.

CHAPTER 3

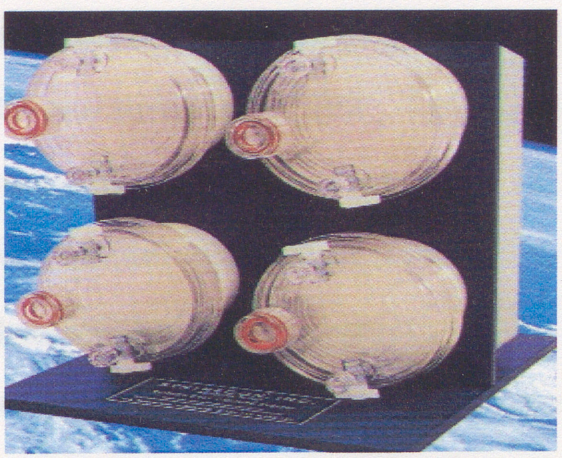
DESIGN OF STUDY

Modeled Microgravity and Control Cell Cultures

This model was developed using human lymphocytes (Gulf Coast Bank, La Concha, Houston, Texas) and human osteoblast cells (Figure 1). The vessel was developed by NASA. This rotating wall vessel was used to optimize conditions for culturing cells in a preliminary function-deficit model. (Sundaresan et al, 2002; Sundaresan et al, 2004; Sundaresan et al, 2005; Sundaresan et al, 2006). The vessel stimulates microgravity.

FIGURE 1

Rotating Wall Vessel Culture System



To model some aspects of microgravity (MG), a specialized rotating-wall vessel culture system developed at the NASA-Johnson Space Center and commercially available from Synthecon, Inc. (Friendswood, TX) was used. This very low shear culture system randomizes gravitational vectors and approximates the microgravity environment by sustaining cells in continuous free fall (Figure 1). This culture system was successfully used previously for the analysis of the effects of microgravity on peripheral blood mononuclear cell locomotion in parallel studies on Earth and during space flight missions STS-54 and STS-56. Control cells were cultured under stationary conditions in plastic tissue culture flasks. All cultures were maintained at 37°C in an atmosphere of 95% air and 5% CO_2.with rotation set to 17 rpm.

Lymphocyte Isolation

Peripheral blood mononuclear cells were isolated from buffy coats of healthy donors (Gulf Coast Regional Blood Center, Houston, TX) using a Ficoll Hypaque gradient (Ficoll-Paque, Pharmacia Biotech AB, Uppsala, Sweden) with centrifugation at 700 g for 20 min. at room temperature. Cells from the interface were washed three times with Hank's Balanced Salt Solution (HBSS) and resuspended in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS). The cells were then counted using a hemocytometer and suspended at $1x10^6$ cells per ml in 1g (T flask) and modeled microgravity. Cells were then harvested at 24 and 72 hours.

Treatment of Cell Culture with Natural Compounds

The preliminary testing and optimization for this project was performed using isoflavones and nucleotides. Cell viability and proliferation using Trypan blue exclusion was the prime end point assessed. Scanning electron micrographs (SEM) of treated and

untreated lymphocytes was also performed. This was the only method applied to the lymphocytes since SEMs using osteoblasts are very cumbersome to perform. The osteoblasts being adherent cells are difficult to handle. 5μm of isoflavones and 12μm of nucleotides were the natural agents tested.

Gene Arrays

First-strand cDNA synthesis was performed using total RNA (10 to 25mg), and a T7-(dT)24 oligomer

(5`-GGCCAGTGAATTGTAATACGACTCACTATAGGGAGGCGG-dT24-3`).

The enzyme Superscript II reverse transcriptase (GIBCO-BRL Life Technologies, Gaithersburg, MD) was used for the reaction. Second-strand synthesis converted the cDNA into a double stranded DNA template for use in an in vitro transcription reaction. The T7 promoter introduced during the first cDNA synthesis provided the necessary sequence for directing the synthesis of cRNA using bacteriophage T7 RNA polymerase. The target cRNAs were labeled with biotin during the transcription reaction. Biotin-labeled target cRNAs were fragmented to a mean size of 200 bases to facilitate their hybridization to probe sequences on a gene chip array (Affymetrix, Santa Clara, CA).

Each target cRNA sample was initially hybridized to a test array containing a set of probes representing genes commonly expressed in the majority of cells (e.g., human: actin, GAPDH, transferring receptor, transcription factor ISGF-3, 18S RNA, 28S RNA, and Alu). Test arrays were used to confirm the successful labeling of the target cRNAs and to prevent the use of degraded or non-representative cRNAs. Hybridization of gene chip (Affymetrix) arrays will be performed at 45°C for 16 hours in 0.1 M MES, pH 6.6, 1.0 M sodium chloride, 0.02 M EDTA, and 0.01% Tween 20.

Four prokaryotic genes, Bio B, Bio C, and Bio D from the E. coli biotin synthesis pathway, and the Recombinase gene from P! bacteriophage were added to the hybridization cocktail as internal controls. These control RNAs were used to normalize expression levels between experiments. Because control RNAs were added at varying copy numbers (Bio B, 1.5 pmol; Bio C, 5 pmol; Bio D, 25 pmol and cre, 100 pM), the arrays will be analyzed using the Affymetrix Gene Chip Analysis Suite 3.3 software (Affymetrix, Santa Clara, CA).

Microscopy

Both Scanning Electron Microscopy and Phase Contrast Photomicrography experiments were performed on the cells at the NASA Johnson Space Center.

SEM /ESEM Sample Preparation

Samples were fixed with 4% Gluteraldehyde for 1 hour or longer if needed and stored at 4°C where necessary. About 200-500 ul of sample was removed and rinsed with PBS by centrifugation at 2500 rpm for 5 minutes for 2 repetitions. The PBS was removed and the sample was then incubated in 0.1M Osmium tetroxide for 1 hour. Samples were centrifuged (2500 rpm for 5 minutes) and the supernatant was removed. The samples were rinsed with PBS as before. Dehydration of the samples was carried out using increasing dilutions of Ethanol (20%, 50%, 75% and 100%) for each 30 minute incubation. Samples were spun between ethanol gradients. Subsequent to dehydration, the samples were kept in 100% ethanol. Approximately 200 ul of each sample (in 100% ethanol) was then placed on a 3 μm filter and allowed to filter by gravity. The filter was removed its housing unit and placed on carbon tape. Samples were dried by critical point

drying or by using 2 drops of HMDS and air dried (repeated twice). Samples were then coated with gold palladium and the images were viewed on ESEM.

CHAPTER 4

RESULTS

To analyze the differences and deficiencies caused by modeled microgravity with particular reference to inflammation-related gene expression, human peripheral blood from normal donors was used to isolate PBMCs. Blood traverses through most organs and hence is a suitable overall physiological predictor. These cells were cultured under Earth-bound gravitational conditions in '1g' in T flasks or under modeled microgravity conditions in a rotating wall vessel for time periods of 24 and 72 hours. Cell samples were collected and subjected to gene array analysis using the Affymetrix HG_U95 array. Data was collected and subjected to a two-way analysis of variance. Different groups of genes related to the inflammatory response, were then analyzed.

The TRAF family is important in activating multiple inflammatory and immune related processes induced by cytokines such as Interleukin 1. These genes therefore represent strong candidate susceptibility factors for RA (Potter, et. al., 2007). Interleukins are a group of cytokines that were first seen to be expressed by white blood cells as a means of inflammation. The function of the immune system depends in large part on interleukins, and are rare deficiencies of a number of them have been described, all featuring autoimmune diseases or immune deficiency (Albensi, et. al., 1999). Nuclear

factor kappa beta controls many genes involved in inflammation. It is not surprising that NFKB gene is found to be chronically active in many inflammatory diseases such as inflammatory bowel disease, arthritis and asthma (Baltimore, et. al., 2002).

Table 1 shows the gene name and their abbreviations. The overall gene array results for PBMCs cultured in modeled microgravity are shown in Figures 2-9. The student's T test was used to determine the statistical significance of gene expression variation. These figures show that after statistical filtering for inflammatory response genes, key genes such as Nuclear factor kappa B1, Interleukin 4, Interferon gamma, Interferon alpha and Interferon beta were all found to be elevated significantly ($p<0.05$), while immune booster genes such as Interleukin 2 were found to decrease in expression. This scenario sets the stage for increased oxidative stress in the immune cell environment. The results were corroborated using real time PCR and western blotting for the interferon series of genes where up-regulation was observed in real time PCR ($p<0.05$) and western blotting ($p<0.001$), (unpublished data from Dr.Sundaresan). The error bars indicate standard deviation.

TABLE 1

Gene Name and Abbreviations for Figures 2-9

GENE NAME	ABBREVIATION
Nuclear Factor Kappa Beta-1	NF Kappa B1
Tumor necrosis factor receptor-associated factor	TRAF-1
Interleukin-1	IL-1
Interleukin-2	IL-2
Interleukin-3	IL-3
Interferon gamma-1	IFN gamma-1
Interferon gamma-2	IFN gamma-2
Interferon gamma-3	IFN gamma-3

FIGURE 2

Expression of Inflammation Trigger Gene NF Kappa B in Human Lymphocytes Cultured in mg at 48 hours

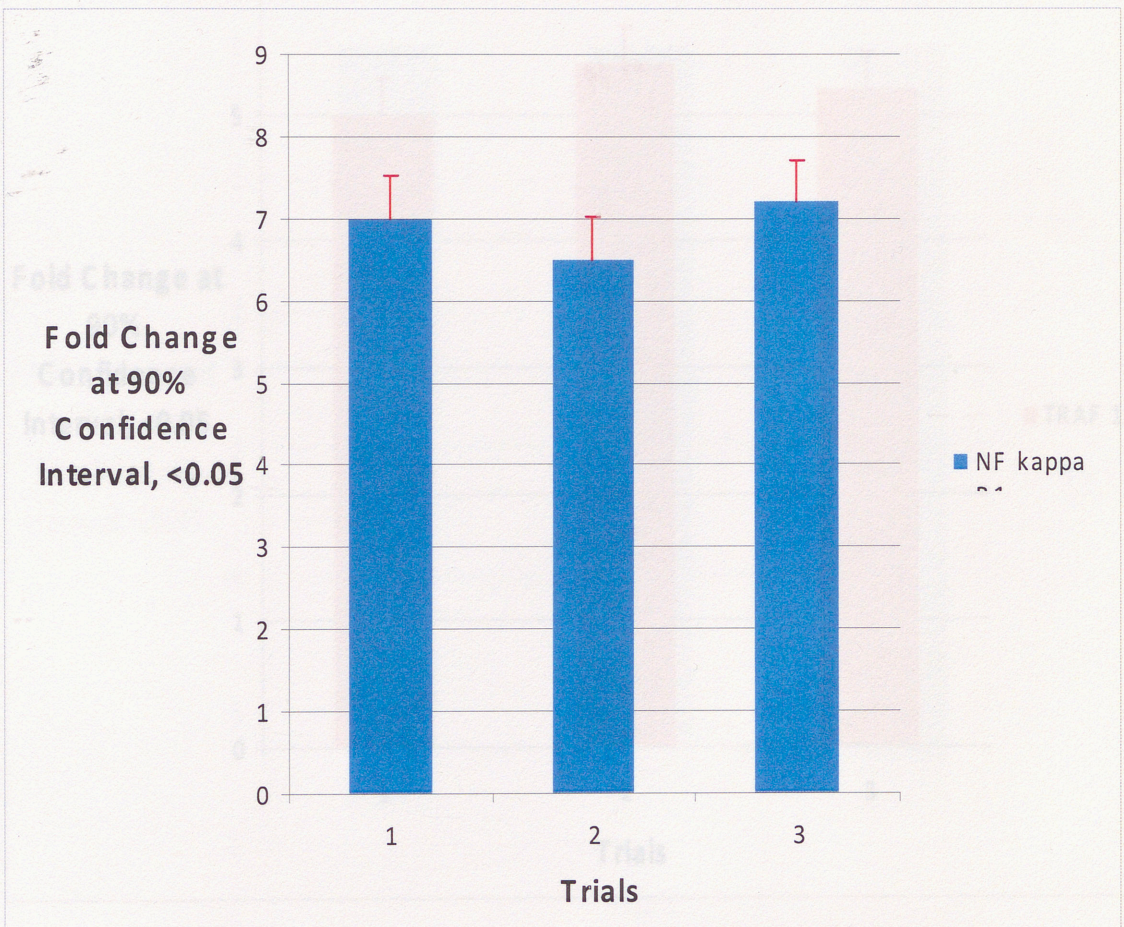


FIGURE 3

Expression of Inflammation Trigger Gene TRAF in Human Lymphocytes Cultured in mg at 48 hours

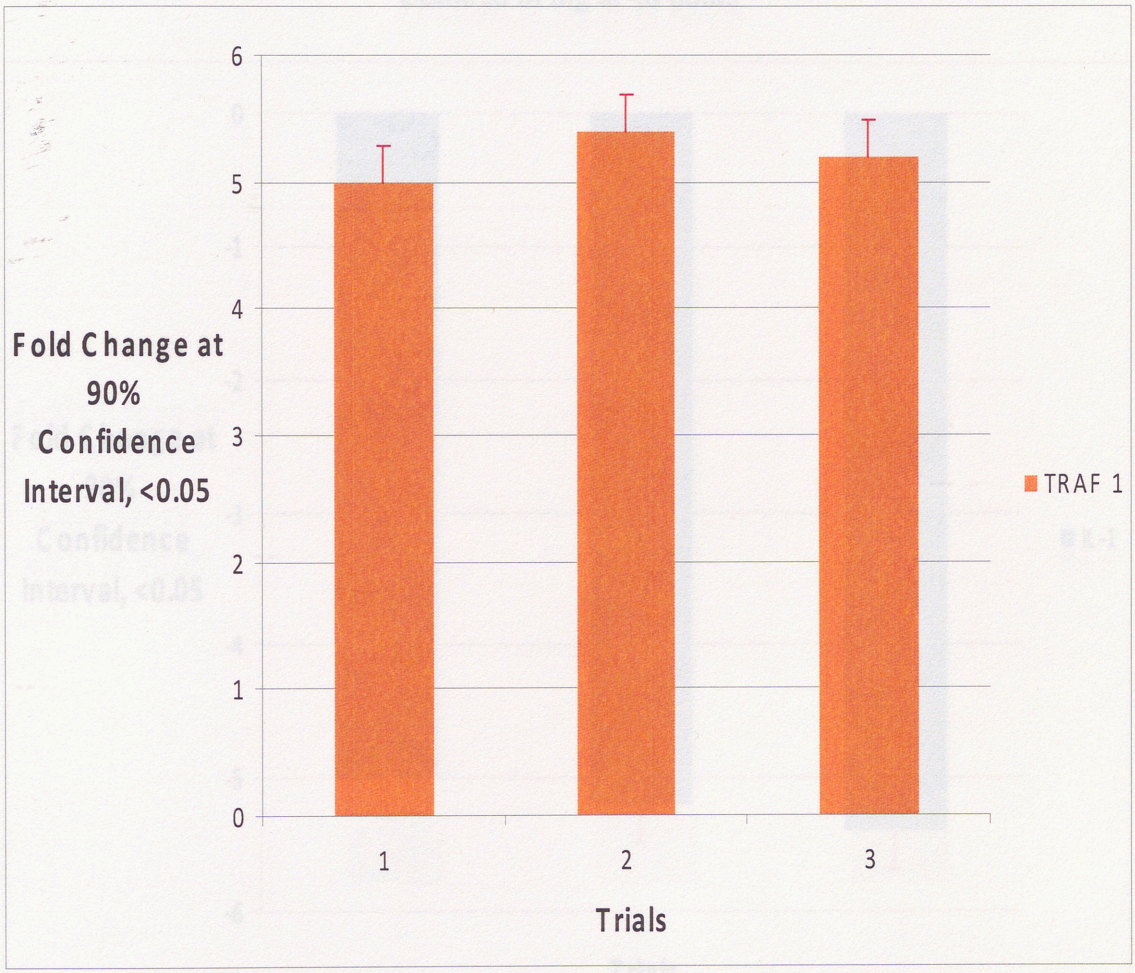


FIGURE 4

Expression of Inflammation Trigger Gene IL-1 in Human Lymphocytes Cultured in mg at 48 hours

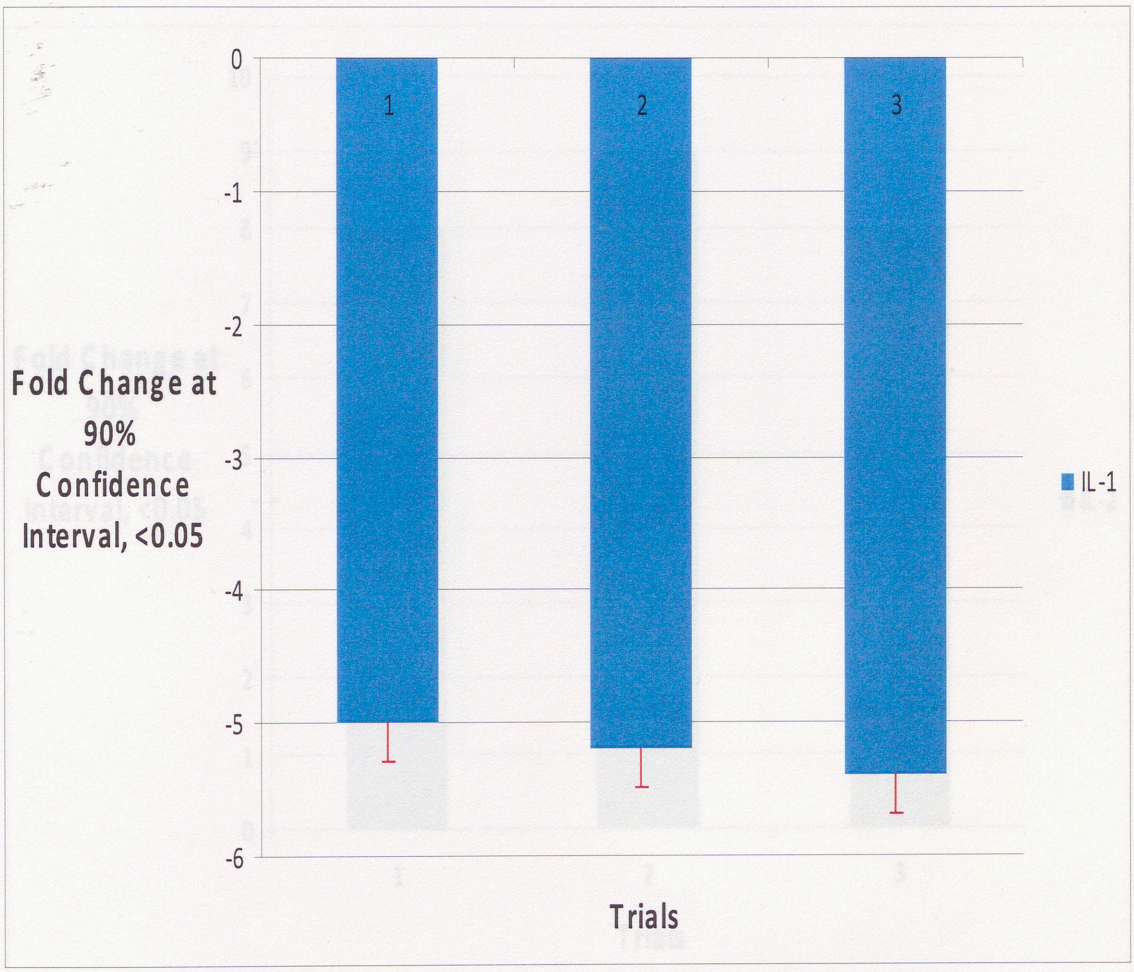


FIGURE 5

Expression of Inflammation Trigger Gene IL-2 in Human Lymphocytes Cultured in mg at 48 hours

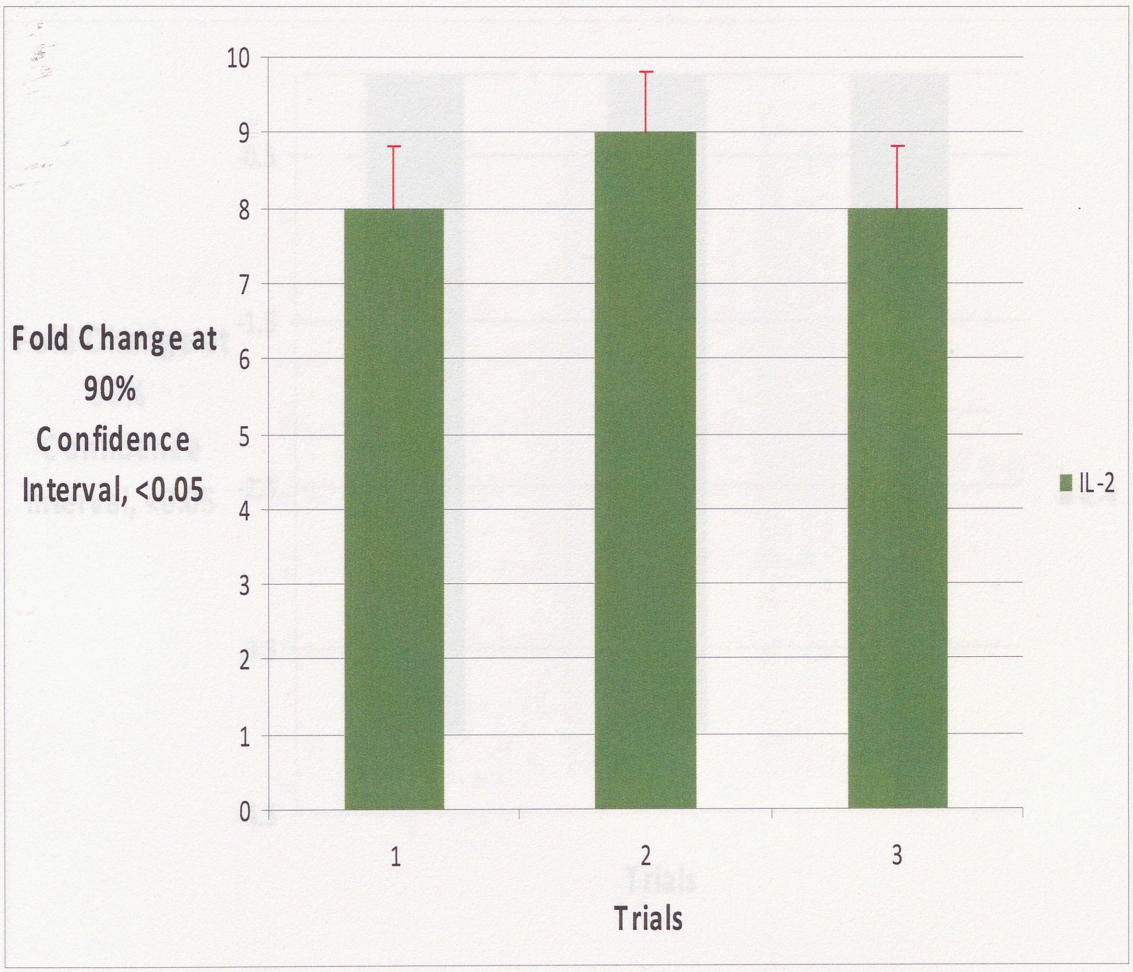


FIGURE 6

Expression of Inflammation Trigger Gene IL-4 in Human Lymphocytes Cultured in mg at 48 hours

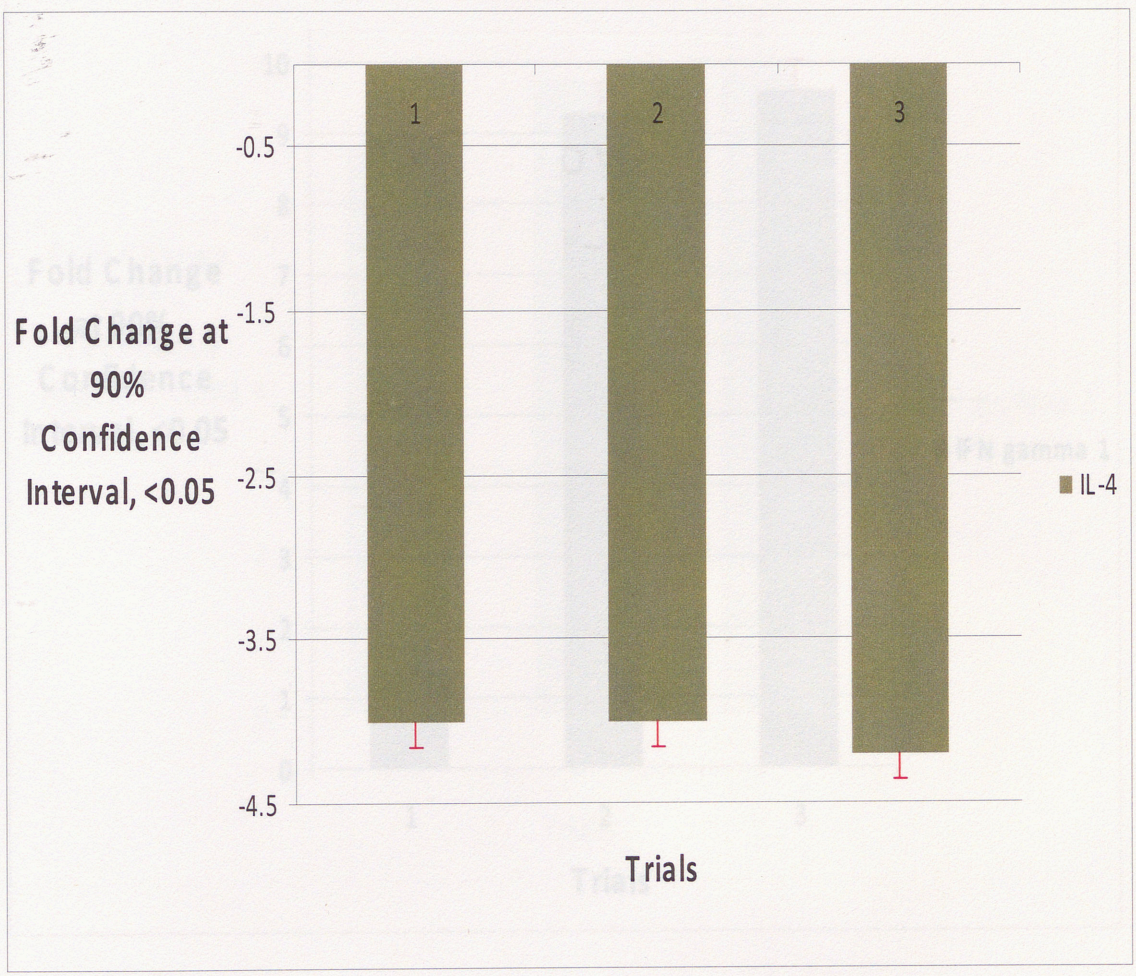


FIGURE 7

Expression of Inflammation Trigger Gene IFN Gamma-1 in Human Lymphocytes Cultured in mg at 48 hours

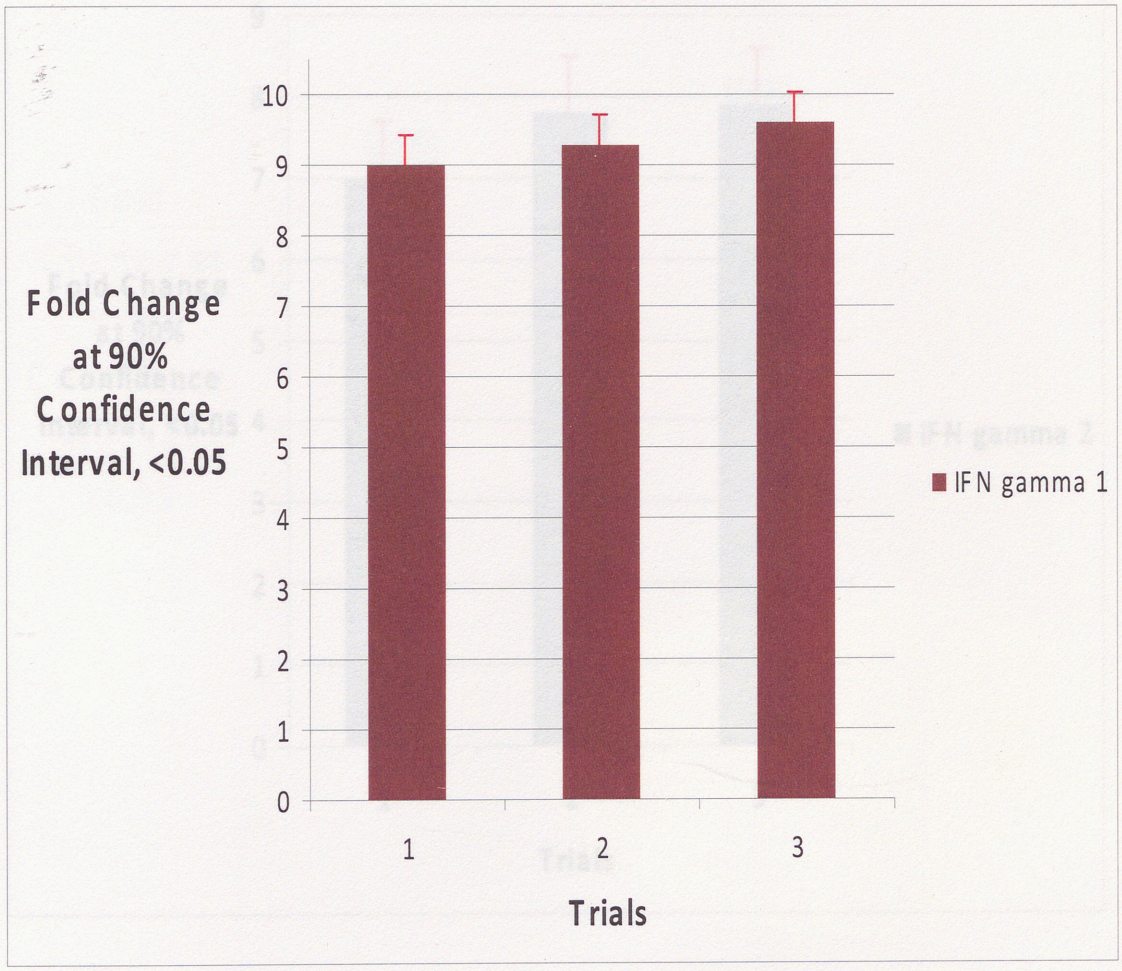


FIGURE 8

Expression of Inflammation Trigger Gene IFN Gamma-2 in Human Lymphocytes Cultured in mg at 48 hours

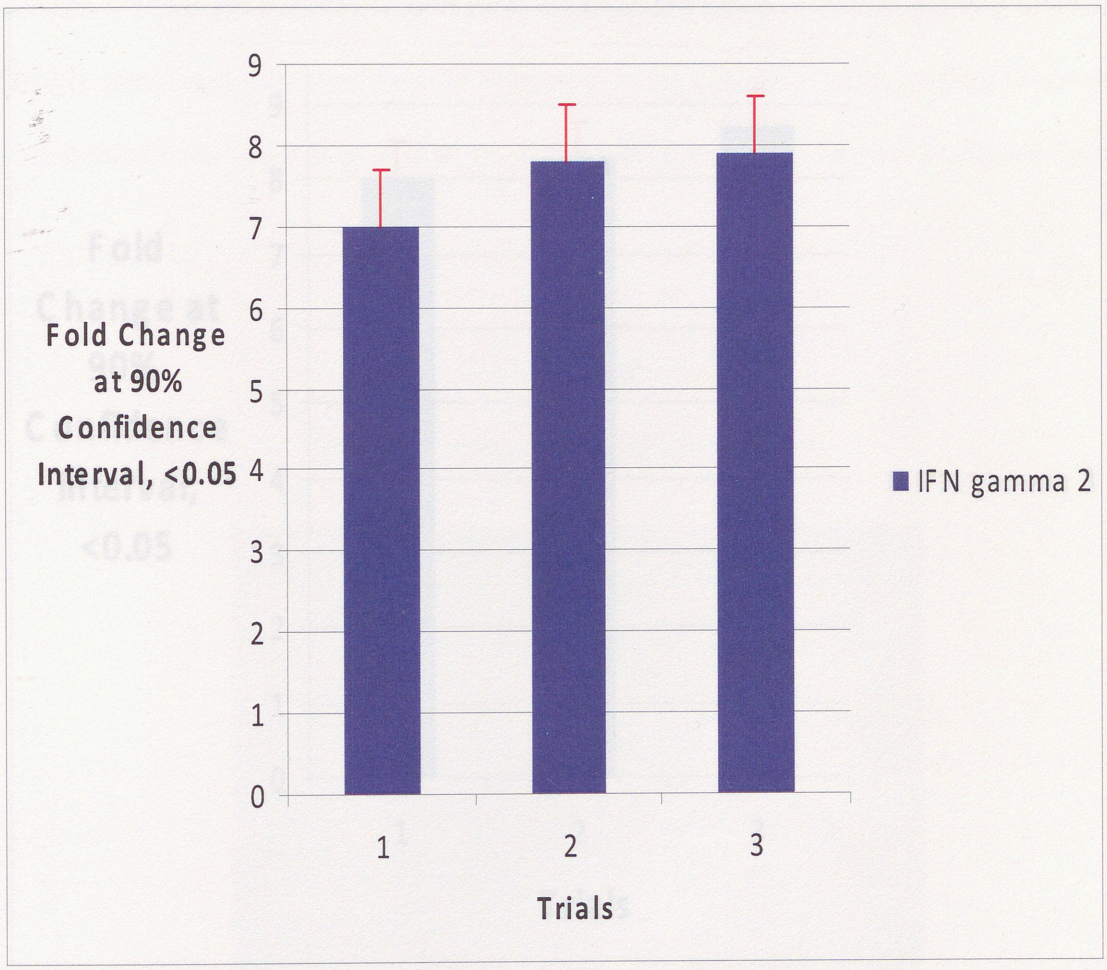
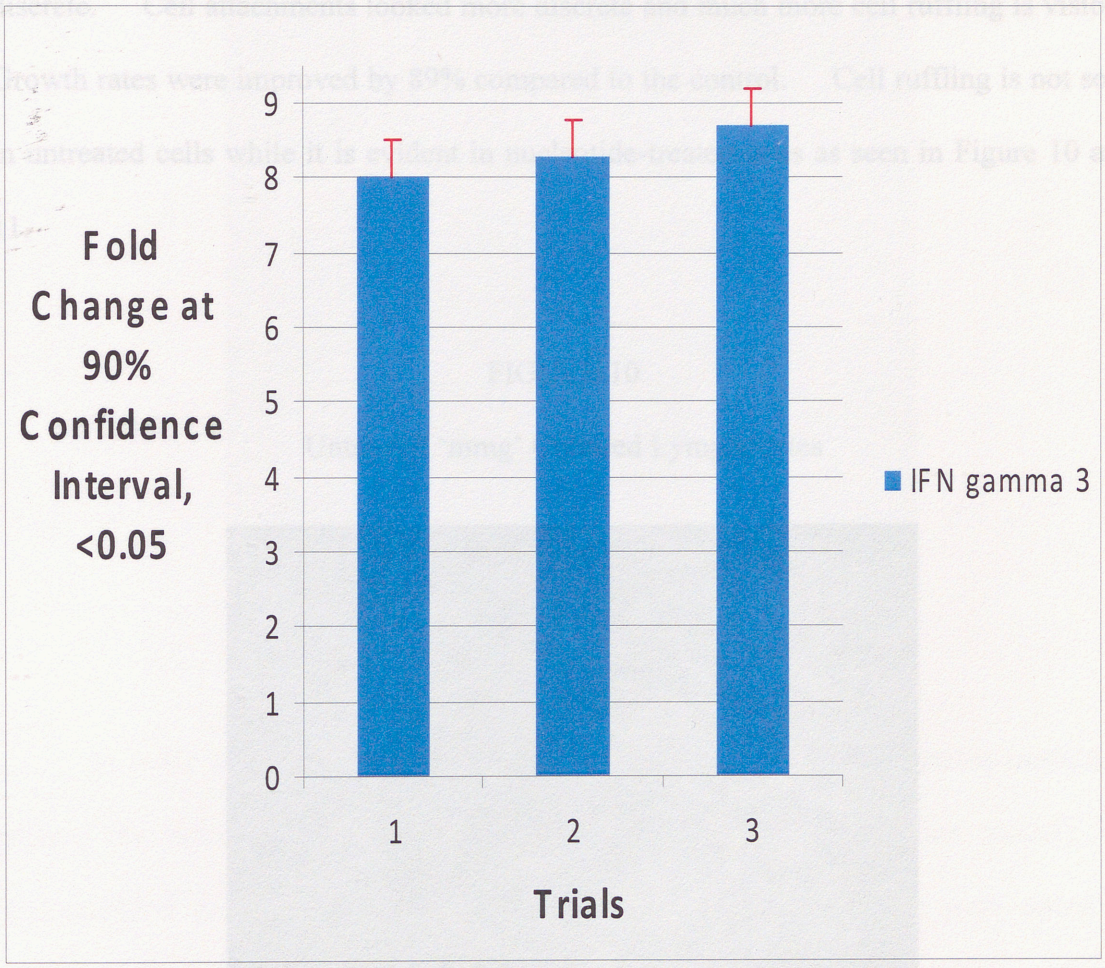


FIGURE 9

Expression of Inflammation Trigger Gene IFN Gamma-3 in Human Lymphocytes Cultured in mg - 48 hours



Scanning electron micrographs of lymphocytes cultured in vitro show that in lymphocytes that did not receive nucleotide treatment, the cells appear stressed and pinched looking (Figure 10). There appears to be erosion of cell structure. By contrast, nucleotide-treated lymphocytes (Figure 11) were rounded and had a healthier appearance. There is very little evidence of eroded cell structure and cell-cell attachments look more discrete. Cell attachments looked more discrete and much more cell ruffling is visible. Growth rates were improved by 89% compared to the control. Cell ruffling is not seen in untreated cells while it is evident in nucleotide-treated cells as seen in Figure 10 and 11.

FIGURE 10

Untreated 'mmg' Cultured Lymphocytes

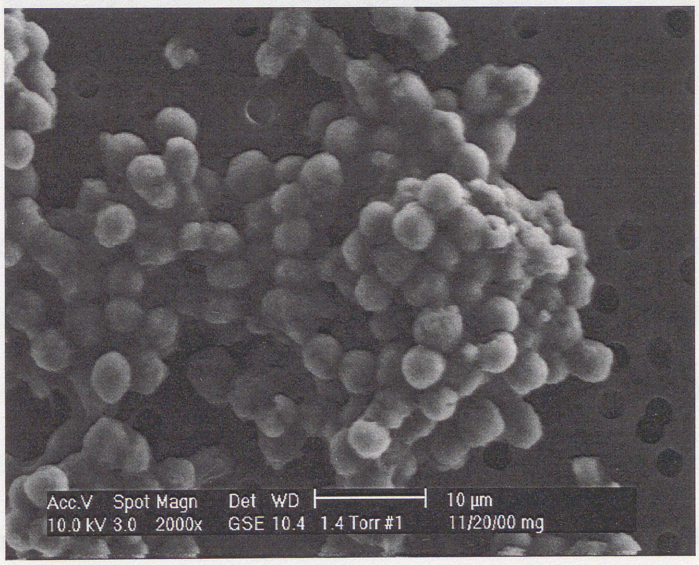
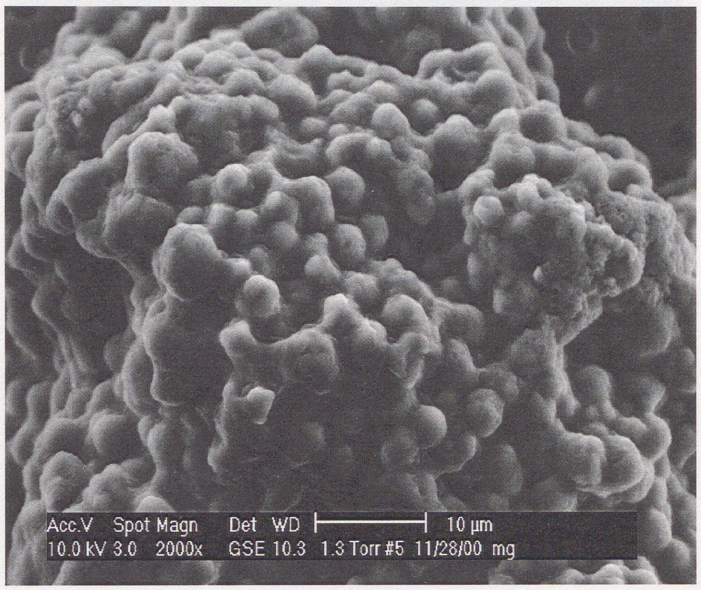


FIGURE 11

Nucleotide Treated 'mmg' Cultured Lymphocytes (12μM)



In order to augment osteoblast health and proliferation as a possible method to prevent the detrimental effects seen in microgravity (functional-deficit model), the recommended doses of the nutritional supplement isoflavone (5μM) were added to identically seeded human osteoblast cultures in triplicate in six well culture plates (Figure 12). Cell growth was observed by Trypan blue exclusion and the data are presented below. There was a 57.5% (statistically significant, $p<0.05$) increase seen in isoflavone-treated osteoblast growth over 48 hrs (doubling time for osteoblasts) when compared to controls.

FIGURE 12

Nutritional Treatment of Human Osteoblast with Isoflavones (5µm) in '1g'

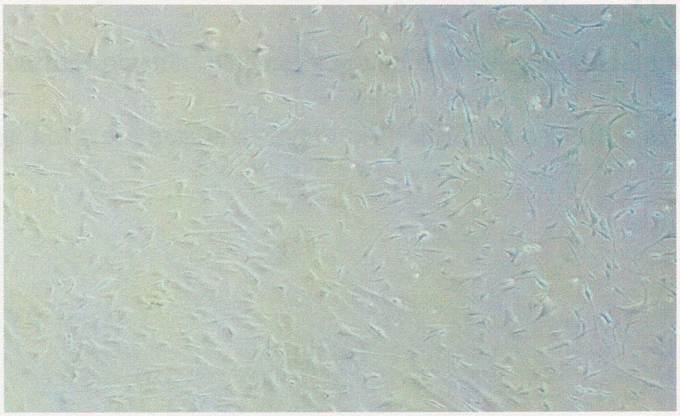


FIGURE 13

Nutritional Treatment of Human Osteoblast without Isoflavones (5µm) in '1g'

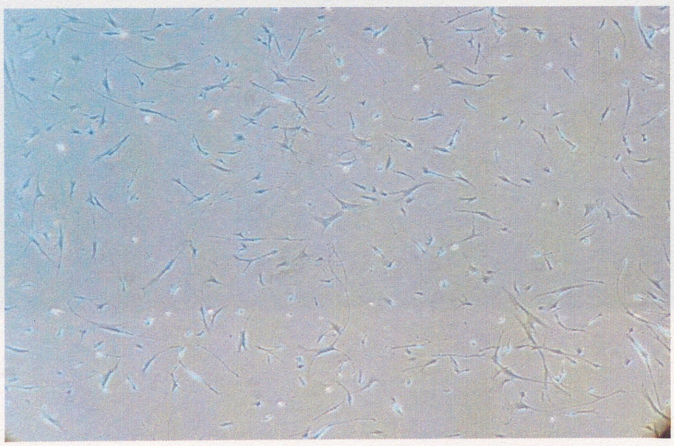


Figure 14 shows osteoblasts cultured in a standard tissue culture plastic flask while Figure 15 shows osteoblasts cultured with Cytodex 3 beads, coated with Type 1 collagen. Three dimensional culture of osteoblasts on Cytodex 3 beads, coated with Type 1 collagen showed better growth of osteoblasts by morphology as opposed to cells grown on standard tissue culture plastic. The cells are spindle shaped and appear to wrap around the beads, forming knitted structures eventually coming together in three dimensional clumps.

FIGURE 14

Osteoblast Culture on Plastic

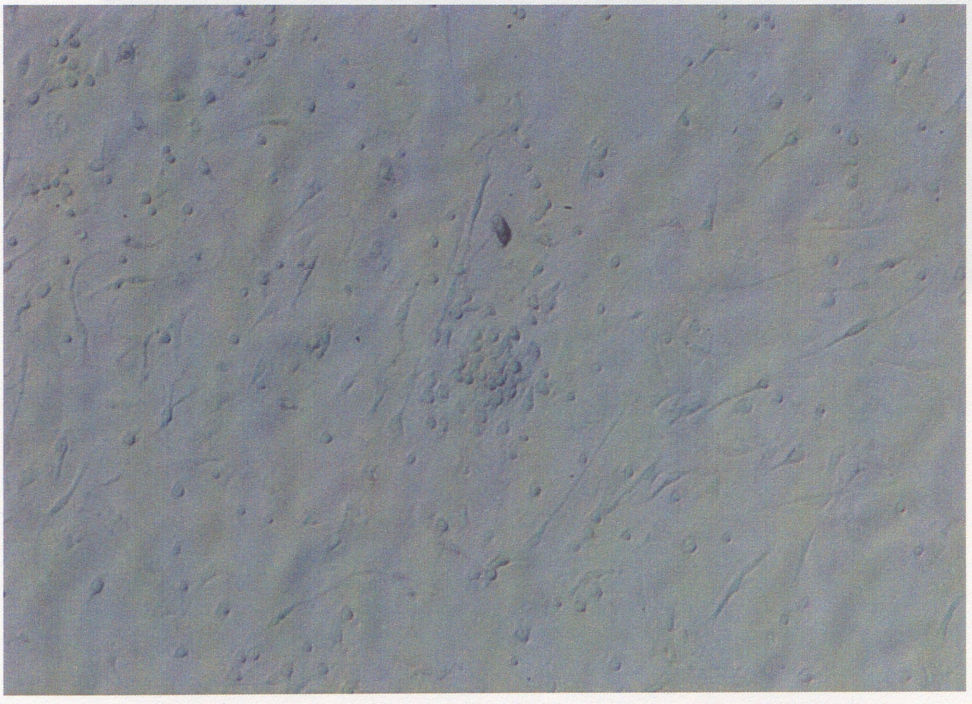
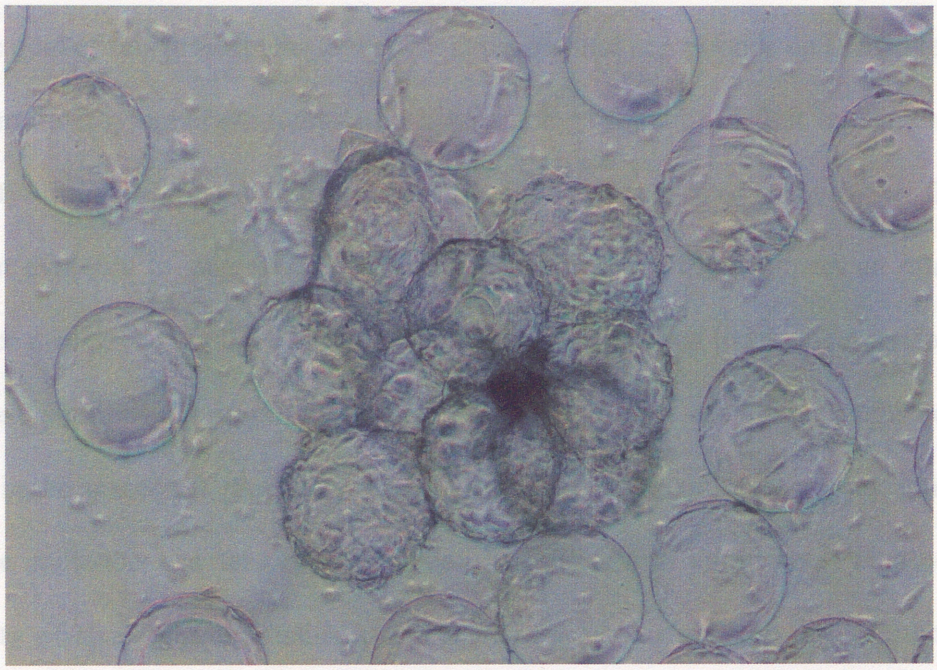


FIGURE 15

Osteoblast Culture on Cytodex 3 Beads



CHAPTER 5

SUMMARY, CONCLUSION, AND RECOMMENDATION

The goal of this project was to study and test natural compounds on their selective ability to improve lymphocyte function in a lymphocyte function-deficit model such as microgravity. Immune suppression is known to be a consequence of space flight. There is sufficient evidence to suggest that several aspects of space flight impact on immune performance measures. Firstly, stress attendant to space flight can induce neuroendocrine changes that significantly impact immune performance until the crew adapts to the conditions in the spacecraft. Secondly, layered onto that stress, and even after adaptation, exposure to radiation can temporarily or permanently affect immunity. Finally, the microgravity of space can dramatically affect hemodynamics and the flow lymph that traffics and disseminates lymphocytes throughout the body. An additional effect of microgravity is the effect of decreased gravitational force on the individual cells in the immune system. Therefore, there is a compelling need to address the risks involved and clinical significance of the decline in immune function.

Previous experiments by our laboratory and others have demonstrated that lymphocyte activation and locomotion are impaired in microgravity and modeled microgravity (MMG). In order to identify possible response suites in the genetic makeup of immune cell i.e. the lymphocyte in microgravity, gene array analysis was performed

on normal human peripheral blood mononuclear cells cultured under MMG conditions in a rotating wall vessel (RWV)-microgravity analog culture system, and under 1g (Earth-bound gravitational conditions in tissue culture flasks). The RWV was developed at NASA/JSC and is an accepted model for modeling some aspects of microgravity on the ground.

Since the lymphocyte is the primordial cell affected in inflammatory diseases such as arthritis, these were investigated for inflammation-related gene expression. Inflammatory genes appeared to be regulated by culture under MMG conditions over 24 and 72 hrs in comparison to '1g' (Earth-bound gravitational conditions). Inflammatory and oxidative stress inducers such as Tumor Necrosis Factor (TNF), Nuclear factor kappa B 1 (NF Kb), TRAF 5 and REL A, Interferon gamma 1, 2 and 3 genes were significantly up-regulated in modeled microgravity. In human lymphocytes -fold changes above '1' are considered significant since these are normal cells and not transformed. These results will set the stage for increased oxidative stress in the immune cell environment. Also, results prove that nucleotide treatment has a positive effect on lymphocyte growth in mmg. Previous experiments by our group and collaborates have also shown that nucleotides can rescue declined locomotion and activation in human and mouse lymphocytes in mmg. Isoflavones also have a positive effect on osteoblast growth augmentation. Further research is needed on the positive effects of isoflavone treatment on osteoblast cells. NASA is interested in the beneficial effects of natural compounds on oestoblast health. This corroborates results seen in other laboratories both in analog microgravity and spaceflight (J.Plumber, D.Ford and A.Sundaresan, 2008). The results seen in this study will serve as a platform for future experiments in three areas.

a) Nutritional supplementation for physiological augmentation as a countermeasure in inflammation and aging.

b) Using microgravity as a physical perturbation to study cell function deficits similar in occurrence to aging.

c) The effects of spaceflight and increased microgravity on the immune system.

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