

Examination of Immune Response Modifiers in Healthy Individuals as Compared to Refined Lacteal Complex

by Jesse A. Stoff, MD

Abstract

Background

Many substances are known to affect the structure and function of the immune system. Refined lacteal complex (RLC) has demonstrated in small clinical trials and reports from health care practitioners the apparent ability to optimize the modulation of the immune system.¹ This targeted induction of an immune response yields a new dynamic equilibrium of immune function with increased surveillance.

The modulating capability of RLC has been demonstrated by an *in vivo* animal study at the University of Arizona.² Various techniques were used to identify molecules in RLC and the other study materials, known to be relevant to the process of information transfer and immune awareness as described in pre-existing scientific research that has attained theoretical consensus.³⁻⁵

Objective

The comparative examination of substances, which are known to be supportive of immune function, to discern for each the presence of characteristics and capabilities that contribute to and support optimized modulation of the immune system

Design

Several different analytical technologies were employed to ascertain elements of the structure and function of the substances studied. These included the following:

Anti-bacterial testing

This testing allows for a semi-quantitative measurement of the bactericidal/bacteriostatic effect of the substances tested.

Electrophoresis

High-density electrophoresis gels were used to identify the presence and approximate molecular weights of proteins and peptides that the substances contained.

Cell culture

Leukocytes were studied in cell cultures, with the study substances added, to test for transfer factor effects.

Flow cytometry

Flow cytometry was done on whole blood, with the study substances added, to look for leukocyte activation in lymphocyte, monocytes, and granulocyte sub-populations.

Results

The study shows that RLC contains a significant concentration of specific transfer factors, defensins, and granulysins as well as other important nano-molecular immune system-supporting molecules. The specific concentrations are consistent with the bio-engineering of the compound utilizing the induction process. Their predictability and testability is essential to producing a consistent and reliable compound and appear in none of the other study materials in significant quantities.

All the study materials tested demonstrated immune stimulation capability at recommended serving sizes, but only RLC also demonstrated a strong and reliable capability to increase targeted immune surveillance and transfer immunological information among cell groups. These distinctive capabilities illustrate the dynamic and unique benefits of RLC.

Examination of Immune Response Modifiers

Introduction

The immune system is one of the primary and most critical systems. Among other functions, it helps to regulate the internal environment. It exerts its control by virtue of circulating components capable of acting at sites far removed from their points of origin. The immune system accounts for approximately one percent of the body's 100 trillion cells. The defending cells that comprise the immune system originally arise in the bone marrow and mature in the thymus, spleen, and lymph glands. The different cell lineages that develop all share one common objective: to identify and destroy all substances, living or inert, that are recognized as not being part of what "should be in the body," and then to remember those actions for the next challenge. The complexity of this system rivals that of the nervous system and, in fact, the similarities between the two are quite real in that both systems share many of the same bio-transmitters and cell receptor sites. Regulatory molecules, cytokines, and mini-cytokines are critical for optimal immune function.⁶

Reported benefits derived from the regulatory molecules in refined lacteal complexes date well back into the 1950s.⁷ The pursuit of deeper understanding and exploration of the reasons for such benefits began in earnest in 2001. The goals of this examination included a better understanding of the comparative benefit of RLC versus other substances designated to help healthy people optimize and sustain good immune function.

Methods

Laboratory:

Tiburon Diagnostic Laboratory
Tucson, Arizona

Test products

All products used for the testing, except RLC, were acquired in the general marketplace. A concerted effort was made to find the finest products available with special attention to avoiding products that contained additional chemical agents such as preservatives, fillers, etc. RLC was provided directly from its

manufacturing source to the laboratory as RLC. The selected products with their recommended serving sizes were diluted assuming 100% absorption into 5000 cc's (average adult blood volume) of blood and utilized as follows:

Substance	Recommended serving
RLC	50 mg
Lactoferrin.....	1000 mg
Whey isolate	3000 mg
Colostrum.....	1500 mg
Beta 1, 3 glucan	1000 mg
Astragalus	1 cc of the herbal extract
Maitake	1 cc of the herbal extract
Echinacea	1 cc of the herbal extract
Reishi	1 cc of the herbal extract
Goldenseal ...	1 cc of the herbal extract
Arabinogalactan	1500 mg

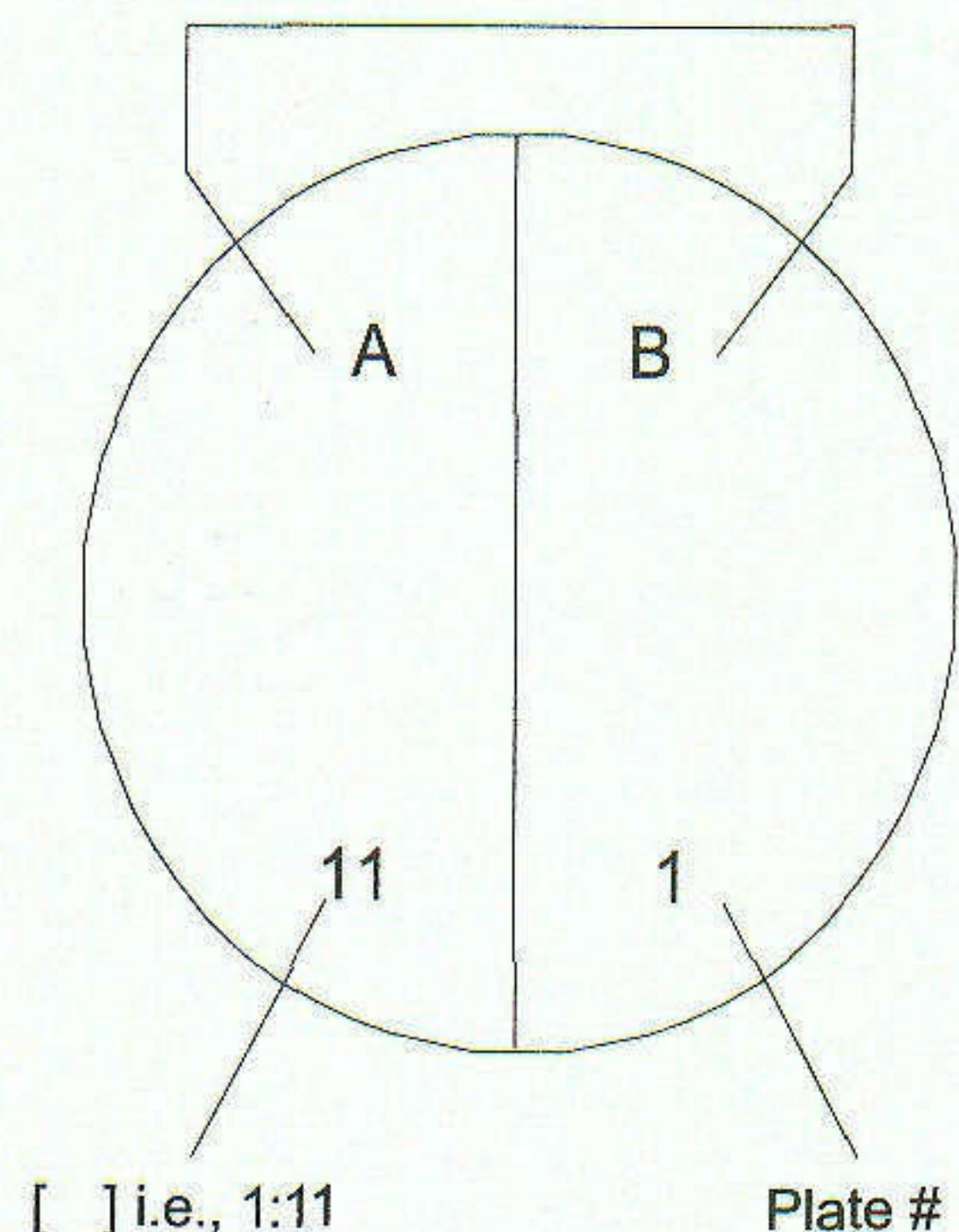
RLC was provided by Quantum Research

Whole Blood Sources

All blood utilized in the *in vitro* analysis was drawn at the laboratory. Over two dozen, asymptomatic, apparently healthy subjects were tested to find three participants whose blood profiles were within the normal ranges of laboratory tests including

Comprehensive Chemistry Panel, CBC, NK-cell panel, and T- & B-cell subset panels. These three participants agreed to support the research project by contributing blood for purposes of *in vitro* analysis. ➤

Figure 1 – Sample Petri Dish Layout to test two solutions



Definitions:

Refined lacteal complex: A refined lacteal complex as used herein is a "nano-molecular" complex derived by applying patented and proprietary fractionation processes to bovine secretions, generally referred to as milk, from cows that have received antigen stimulation to the teat, causing a shift in the nano-molecular content of the secretions.

Immune modulation: The intrinsic process of re-establishing the balanced regulation of immune function by restoring the cytokine communication pathways

Immune Reconstitution: The therapeutic process of repairing structural and functional damage to the immune system through the application of specific cytokines, cells, stimulants and/or suppressors from exogenous sources

Transfer Factor: A less than 5kD dialyzable extract of immune leukocytes that is capable of transferring a cellular immune response

Defensins: Defensins are small, basic unglycosylated proteins of 29-34 amino acids containing three intramolecular disulfide bonds that have anti-microbial effects.

Granulysins: Granulysins are small peptides produced by cytotoxic T-cells and Natural Killer (NK) cells, which reduce the viability of a broad spectrum of pathogenic bacteria, fungi, and parasites.

Lactoferrin: Lactoferrin is a small glycoprotein produced by a variety of cells and has antimicrobial effects.

Examination of Immune Response Modifiers

Study Protocols

Antibacterial Testing

Principle: This procedure is designed to test for a Bactericidal/bacteriostatic effect.

Reagents: ½ Normal Saline (NS)

Materials Required: TSA II 5% SRB Agar plate (BBL prepared media), ten to 30 plates, depending on the testing to be done
RLC and other substances to be tested
Active (in log phase) culture of a pathogenic strain of group A Beta hemolytic Streptococcus phenotype Streptococcus Pyogenes, ATCC # 19615
Small glass test tube for diluting the bacteria
1ul culture loops

Instrumentation: A Vitek Colorimeter is needed to approximately standardize the concentration of bacteria to be tested.

Calibrations: The Colorimeter is zeroed using ½ NS to align both ends of the instrument's scale.

Quality Control: A control plate should be run after all the test plates are done. A simple loop of the bacteria from the stock solution in the test tube should be plated out to ensure that the bacteria didn't die.

Procedure: The bacteria to be tested were previously plated and grown on a standard TSA II 5% SRB Agar plate (BBL prepared media) and incubated in a CO2 Napco model 5400 incubator at 37 C and 5.0% CO2. To prepare the stock solution (SS), bacteria were added to ½ NS until the concentration was down to 95% transmissible. The substances to be tested were mixed with increasing volumes of the SS to make the dilutions as follows:

- 10 uL I: 100 uL SS = 1:11
- 10 uL I: 200 uL SS = 1:21
- 10 uL I: 300 uL SS = 1:31
- 10 uL I: 400 uL SS = 1:41

The solutions are immediately plated (see below) (time 0) and then put into the incubator until needed for re-plating 15 minutes later. After 24 hours, the dishes were removed from the incubator and "read."

Reporting of Results: Sample Petri Dish Layout to test two solutions. For accuracy of interpretation, doing more than two test solutions per plate is NOT recommended. See Figure 1.

For the sake of consistency, plate 1 is 1:11 T0, plate 2 is 1:11 T15, plate 3 is 1:11 T30, plate 8 is 1:21 T0, plate 9 is 1:21 T15, etc., etc., etc. Since the loop used to plate the bacteria was 1 uL, count the number Colony Forming Units (CFU) seen and report as in the example of CFUs per ml.

Limitations: This test procedure will give qualitative information about one of its biological effects and some relative semi-quantitative information about the product being tested. For greater sensitivity, up to 300 CFU/ml, only one substance should be tested per plate.

Electrophoresis

Principle: This procedure is designed to separate the mixture of proteins, peptides, and nucleoproteins found in the processed infusion product while still maintaining biological activity.

Reagents: SimplyBlue SafeStain
Invitrogen – LC6060
Novex Tris-Glycine Native Sample Buffer (2X) – LC2673
Novex Tris-Glycine Native Running Buffer (10X) – LC2672

Materials Required: Novex 18% Tris-Glycine Gel 1.0 mm, 10 well EC6505
Sample loading tip – LC1001

Instrumentation: Xcell SureLock Mini-Cell – E10001
PowerEase 500 Power Supply
E18600

Calibrations: The power supply comes precalibrated from the factory with no further adjustment possible.

Quality Control: QC is a function of product preparation before it comes to the lab. Ideally, there should be no molecules above 100 kD as measured with a molecular ladder, i.e., Sigma ColorBurst Electrophoresis Markers C4105. In conjunction with the molecular weight vs. mobility calculations,⁸ ColorBurst can be used to estimate sample molecular weights, to monitor the progress of an electrophoretic run, or to confirm that an electroblot is complete. Brilliantly colored, exceptionally well-resolved, convenient, and stable, Colorburst protein molecular weight markers perform impressively in a variety of gel compositions and concentrations. ColorBurst Markers are composed of eight polypeptides that have been chemically reduced and conjugated to brilliantly colored dyes. Conveniently, Colorburst Markers require no resuspension, reduction, or heating prior to use.

Procedure: The samples were run on an 18% 10 well Tris-Glycine gel at 125 volts, 35 milliamps, and 5 watts for four-and-one-half hours. The samples were diluted in the Novex Tris-Glycine Native Sample Buffer and run in the Novex Tris-Glycine Native Running Buffer. The Gels were then carefully removed from the cells, stained for one hour in the SimplyBlue SafeStain, and de-stained in de-ionized water.

Tissue Cell Culture, Transfer Factor Effect Testing

Principle: This procedure is designed to test for a transfer factor effect. Since the RLC has a known semi-specific bactericidal/bacteriostatic curve, it is reasoned that, if leucocytes were induced to produce similar bactericidal/bacteriostatic molecules after incubation with RLC, it would demonstrate a transfer factor effect as there are no antigens present to otherwise induce an immunological reaction.⁹

Reagents:

1. RPMI 1640 complete culture media (Cellgro.com)
2. Human serum from the subject whose WBC's are part of the experiment
3. L-glutamine stock (200mM in DI H2O)
4. FACSlyse

Materials/Instruments Required:

1. Sterile pipettes/pipettor tips
2. Sterile 25mL culture flasks should be polypropylene (from Sarstedt) with a gas permeable cap
3. Biosafety hood
4. CO2 incubator set at 37°C
5. Phosphate Buffered Saline (PBS)
6. Centrifuge
7. 15mL conical tubes
8. Accuspin Lymphocyte separation tubes.(Sigma)
9. Sysmex SF-3000 (Hematology Analyzer)
10. BD 4 color Flow Cytometer
11. Flow Cytometer
12. "Green top" heparin tubes
13. "Purple top" tube
14. "Red top", serum separator tubes

Quality Control: Since bacteria and/or fungi can activate leucocytes and cause them to produce bactericidal/bacteriostatic molecules, culture plates should be done at the end of the experiment on the media from the flasks to ensure sterility.

Procedure: Whole blood was collected in "green top" tubes, and the peripheral blood leukocytes (PBLs) were isolated. The PBLs were put into a 25 ml culture flask with 10 uL of the test substance and 5 cc's of RPMI 1640 culture media, supplemented with ten-percent Human Serum (from the same donor) and containing 2mM of L-glutamine. This can be accomplished by adding 5 ml of Human Serum, 500 uL of L-glutamine stock [200mM] in DI. PBL's (2x10⁶ cells/ml) were cultured at 37° C with five-percent CO₂ for 18 hours. Separate cultures were done for each substance tested, and a control culture was done with no additional substance added. After the 18 hours, some of the supernatant was withdrawn (through a .2 micron syringe filter) from the culture and diluted as above in the bacteria stock solution for plating.

Examination of Immune Response Modifiers

Study Protocols

Flow Cytometry

Principle: By using an early activation marker (CD69), we can assess the ability of a substance to stimulate leucocytes (CD45) as they are broadly divided into lymphocytes, monocytes, and granulocytes and displayed by a flow cytometer.

Reagents:

1. FACSlyse
2. DPBS (Cellgro)
3. BD Biosciences CD 45 #
4. BD Biosciences CD 69 #

Materials/Instruments Required:

1. Sterile pipettes/pipettor tips
2. Sterile 3 cc 12 X 75 tubes, should be polypropylene (from Sarstedt) with a gas permeable cap
3. Biosafety hood
4. 5% CO₂ incubator set at 37°C
5. Phosphate Buffered Saline (PBS)
6. Sysmex SF-3000 (Hematology Analyzer)
7. Flow Cytometer
8. "Green top" heparin tubes
9. "Purple top" tube
10. CD 45 and 69 antibodies

Calibration/Quality Control: Normal calibration with calibration beads for the flow cytometer per the manufacturer's recommendations. Gating was done on light scatter and CD 45.

Procedure: Whole blood was collected in "green top" tubes; 20 uL of a substance to be tested was added to 480 uL of whole blood and incubated at 37° C in a five-percent CO₂ incubator for different lengths of time. At the appropriate time points 100 uL of the whole blood was aliquoted into a polystyrene tube with 10 uL of each of the two antibodies and put in a dark area for 15 minutes. Then FACSlyse was added, and the tubes returned to a dark place for 15 minutes. The cells were rinsed and re-suspended in DPBS and analyzed later that day on a three-color BD FacsCalibur Flow Cytometer, double gating on light scatter vs. CD 45 was used to separate leukocyte cell populations. See Figure 2.

Results

Antimicrobial Testing

Antimicrobial testing with *Streptococcus Pyogenes* showed six molecular bands of RLC illustrated by electrophoresis to have an *in vitro* antimicrobial effect. Colostrum and Lactoferrin each had two bands that were active against the *Streptococcus Pyogenes* bacteria. Lactoferrin is

recognized to have a general, non-specific, antimicrobial effect. To the extent that Colostrum contained lactoferrin, it too showed minimal antimicrobial effects from those bands.

Electrophoresis Testing

Electrophoresis was used to evaluate the presence of peptides and molecules below 100 kD in the study materials. The presence of specific small molecular peptides is known to provide value in supporting the immune system.

Electrophoresis demonstrated numerous molecular bands below

100 kD in RLC. The same number and density of these bands were not present in any of the other products tested. Colostrum demonstrated fewer bands of less apparent density than RLC.

See Figure 3 and Table 1

Results: See Table 2.

Microdensitometer Evaluation

A microdensitometer was used to objectively evaluate the banding pattern and can semi-quantitate the density of the bands in the electrophoresis gels. RLC presented significantly higher band density as

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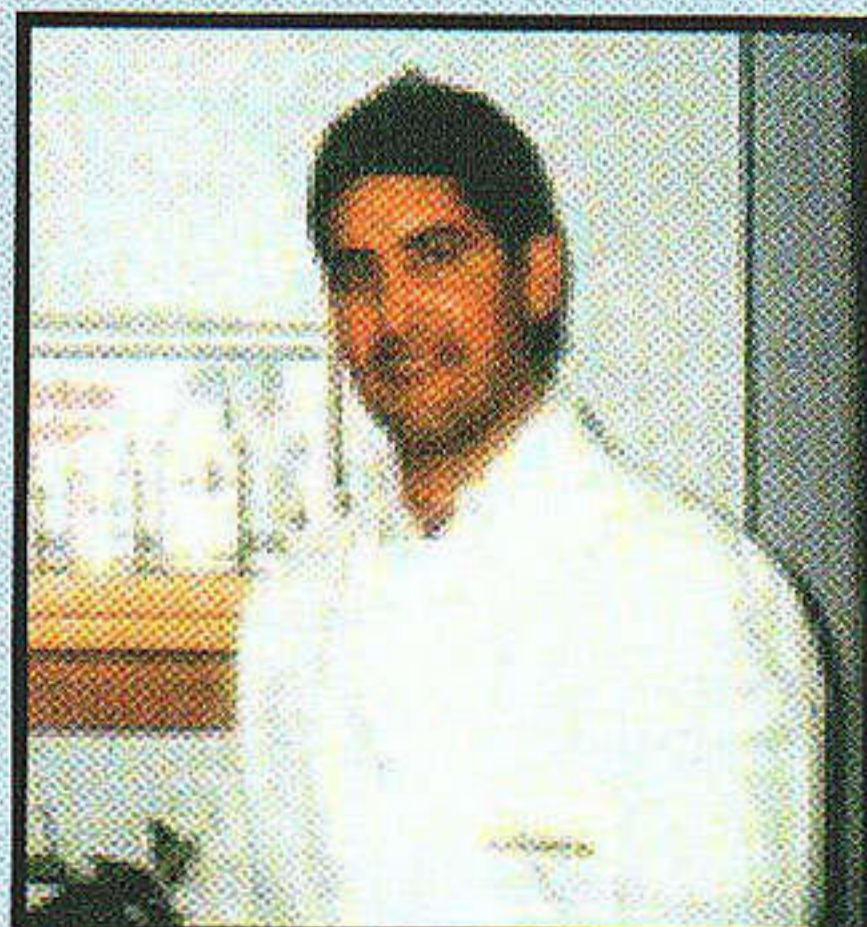
- Cancer
- Heart Disease
- HIV/AIDS
- Sinusitis
- Colds and Flu
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Figure 2 – Culture Tube Numbering

Tube Numbers

1 & 2	Negative Control
3 & 4	Goldenseal
5 & 6	Arabinogalactan
7 & 8	Astragalus
9 & 10	Maitake
11 & 12	Reishi
13 & 14	Whey
15 & 16	RLC
17 & 18	Colostrum
19 & 20	Lactoferrin
21 & 22	B-1,3-Glucan
23 & 24	Echinacea
25 & 26	+ Grans
27 & 28	+ Monos
29 & 30	+ Lymphs
31	Time 0, Negative Control

25, 26 = positive control for Granulocytes

27, 28 = positive control for Monocytes

29, 30 = positive control for Lymphocytes

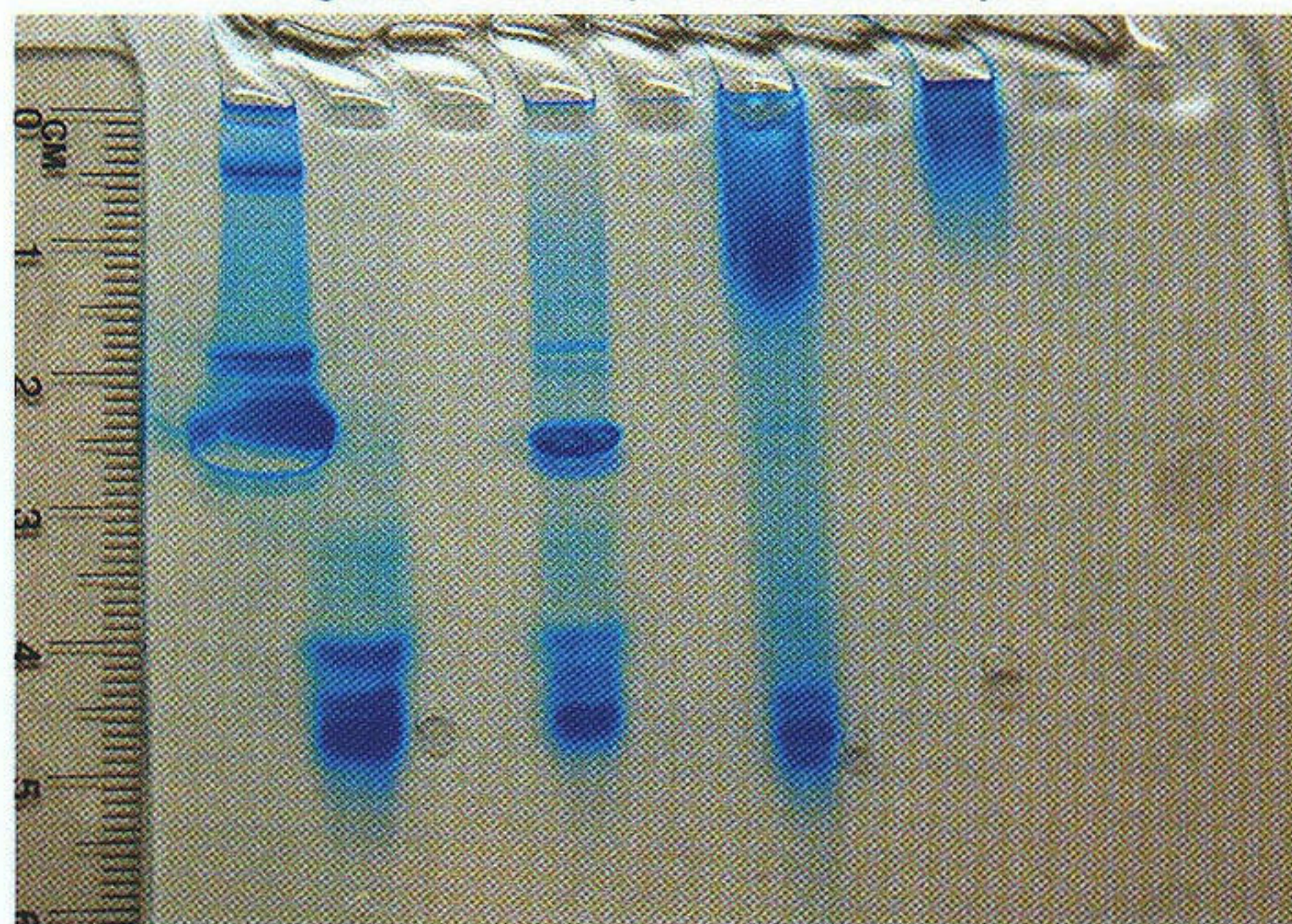
Odd numbered tubes will be tested at four hrs and the even numbered tubes at ~18 hrs.

Table 1 – This Table shows the main bands of RLC and known molecular weight ladder. Distances from the base of the well are measured in mm's.

Reference Tris-Glycine 18%

AiE/10 Anti-microbial Bands	ColorBurst Bands	CB MW's kD
11	7	210
14	16	90
	19	65
	24	40
	25	30
36	37	20
41	44	13
51	53	8
56		

Figure 3 – Electrophoresis Gel Sample



Note: 1) All band measurements are in mm's.

2) Anti-microbial bands are determined by reverse electrophoresis and bactericidal testing (see above)

Table 3 – Colony forming units per plate of reverse electrophoresed material when tested for bactericidal activity

	1:11	1:21	1:31	1:51
Echinacea	TNTC	TNTC	TNTC	TNTC
Goldenseal	TNTC	TNTC	TNTC	TNTC
Arabinogalactan	TNTC	TNTC	TNTC	TNTC
Astragalus	TNTC	TNTC	TNTC	TNTC
Maitake	TNTC	TNTC	TNTC	TNTC
Reishi	TNTC	TNTC	TNTC	TNTC
Whey	TNTC	TNTC	TNTC	TNTC
Positive Control				
Original Source RLC	0	0	6	43
Cultured "RLC"	0	0	14	57
Colostrum	TNTC	TNTC	TNTC	TNTC
Lactoferrin	TNTC	TNTC	TNTC	TNTC
B-1,3-Glucan	TNTC	TNTC	TNTC	TNTC

TNTC = Too numerous to count, >300K colony forming units (CFU)

Table 2 – Results of Electrophoresis Banding and Bactericidal Testing

	Band at 11	Band at 14	Band at 36	Band at 41	Band at 51	Band at 56
Echinacea	NBP	NBP	NBP	NBP	NBP	NBP
Goldenseal	NBP	NBP	NBP	NBP	NBP	NBP
Arabinogalactan	NBP	NBP	NBP	NBP	NBP	NBP
Astragalus	NBP	NBP	NBP	NBP	NBP	NBP
Maitake	NBP	NBP	NBP	NBP	NBP	NBP
Reishi	NBP	NBP	NBP	NBP	NBP	NBP
Whey	NA	NA	NA	NA	NA	NA
RLC	A	A	A	A	A	A
Colostrum	A	A	NA	NA	NA	NBP
Lactoferrin	A	A	NBP	NBP	NBP	NBP
B-1,3-Glucan	NBP	NA	NBP	NBP	NBP	NBP

NBP = No Band Present

A = Active bactericidal/bacteriostatic effect against Streptococcus Pyogenes at 1:21

NA = Non-active bactericidal/bacteriostatic effect against Streptococcus Pyogenes at 1:21

These results were further confirmed with microdensitometer readings. See Microdensitometer Evaluation.

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Examination of Immune Response Modifiers

evidenced by the microdensitometer readings in comparison to the other study substances. See Figures 4, 5, and 6.

The Colostrum chart demonstrates high concentrations of large molecular weight proteins, much of which are antibodies made against microbes to which the cow was exposed while in her stall or grazing. The RLC chart demonstrates that the majority of the proteins and peptides present are found in the low molecular weight ranges, including a band for Granulysins and Defensins/transfer factors not present in the colostrum. This shift in the pattern of protein/peptide production by the cow is entirely due to the induction process used to make RLC, with specific antigens to produce a targeted response. The samples were adjusted to contain 7 mg/cc of solid material.

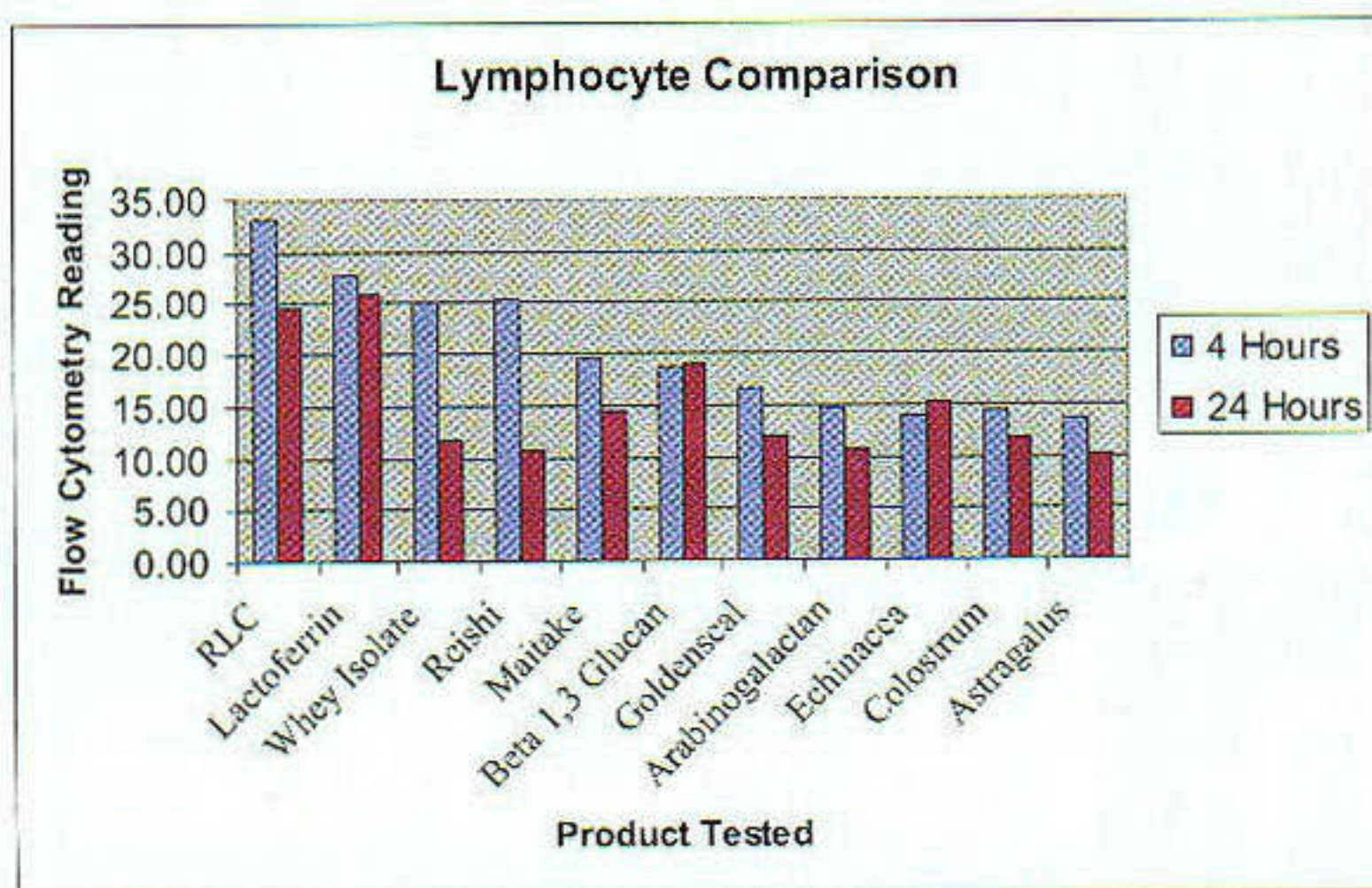


Figure 4 – General stimulation effect on lymphocytes of the substances tested by flow cytometry.

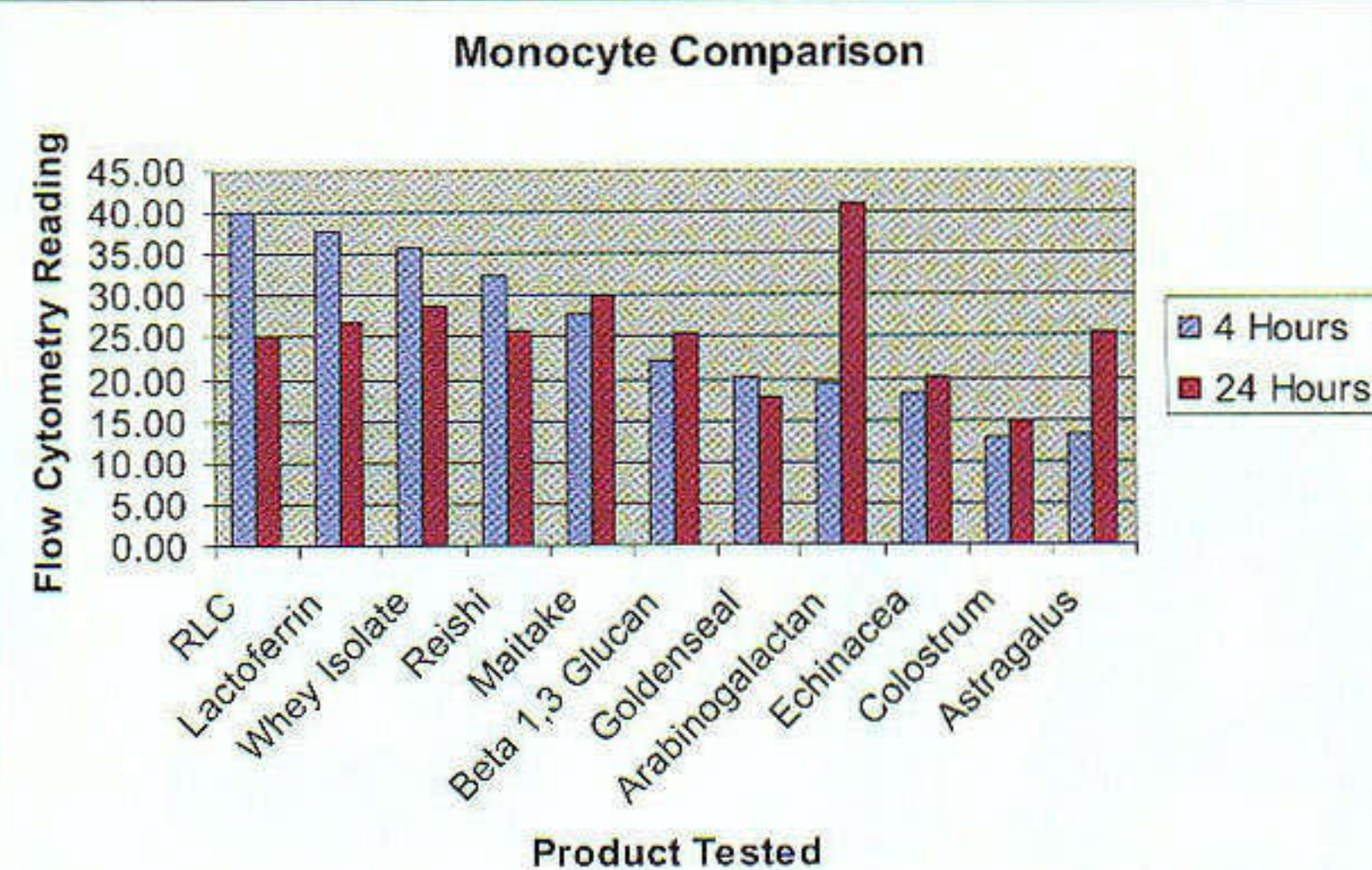


Figure 5 – General stimulation effect on monocytes of the substances tested by flow cytometry.

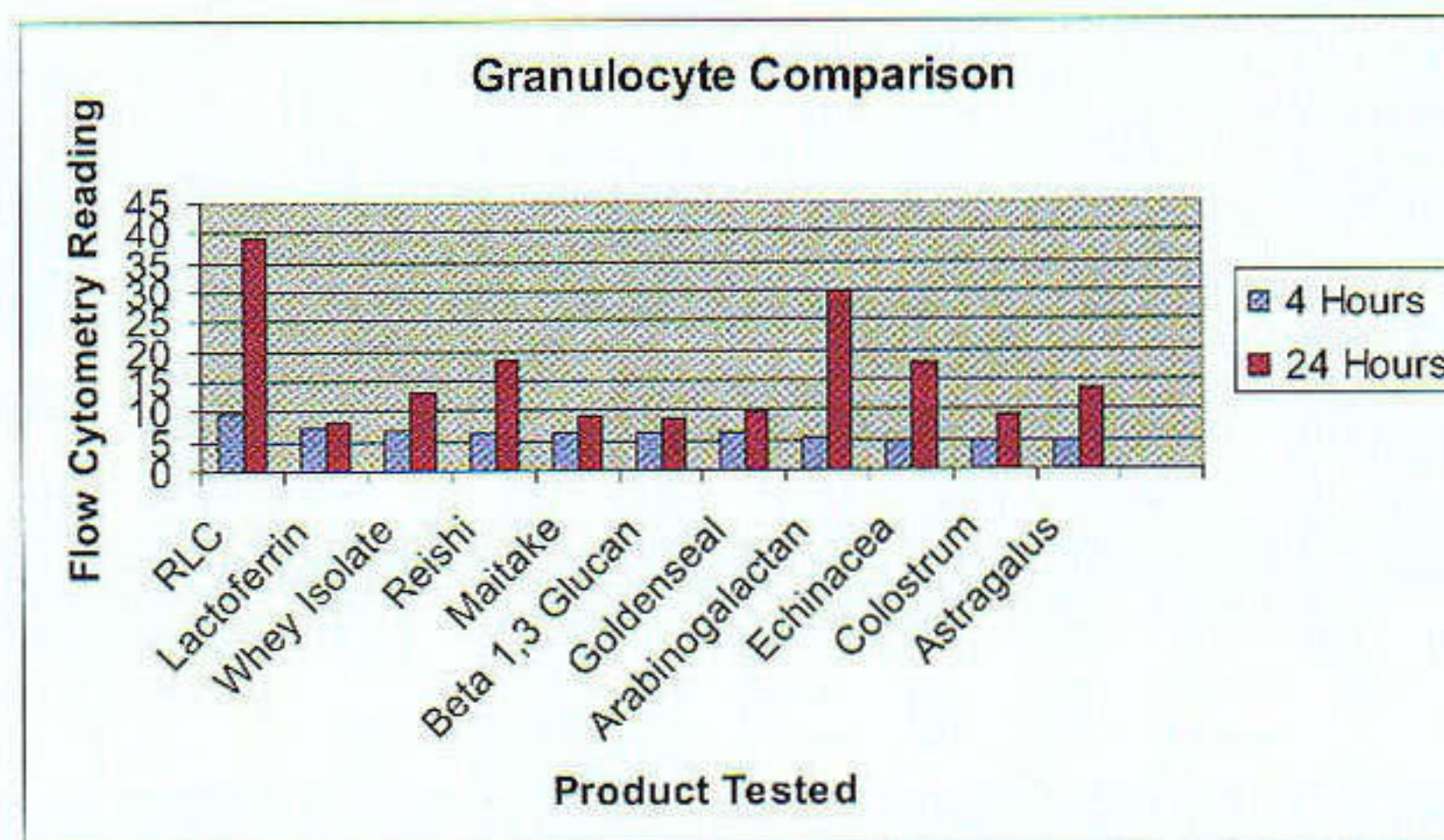


Figure 6 – General stimulation effect on granulocytes of the substances tested by flow cytometry.

IS ANYONE LISTENING?

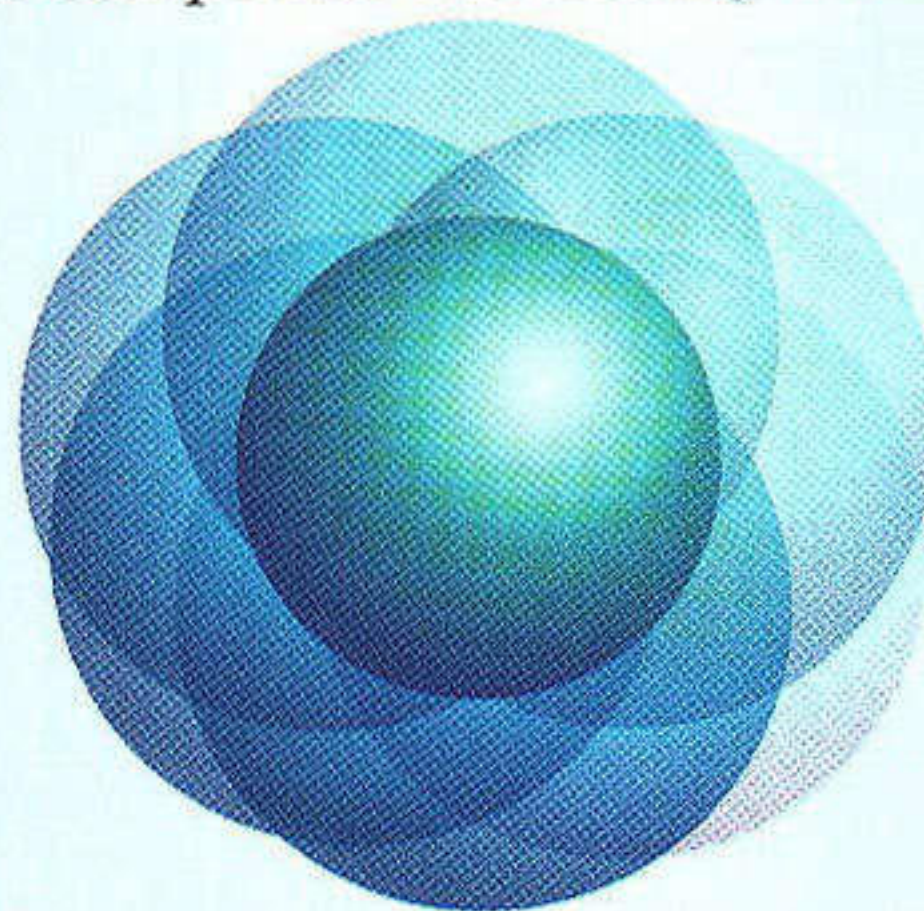
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Examination of Immune Response Modifiers

Cell Culture Transfer Factor Effect Testing

Leukocyte cell culture testing was done to successfully demonstrate a transfer factor effect from the study material produced using induction – results of Bac-t testing at 18 hours and different concentrations as read after 90 minutes. See bac-t testing procedures above. See Table 3.

Flow Cytometry Immune stimulation

Immune stimulation was a key component of the study and was measured from multiple perspectives: general immune stimulation using a single whole blood source for all study materials per experiment.

Table 4 – Average Data Tables for General Immune Stimulation Results

	4 Hrs.	24 Hrs.
Lymphocytes		
RLC	33.06	24.69
Lactoferrin	27.68	25.79
Whey isolate	25.16	11.81
Reishi	25.28	10.70
Maitake	19.40	14.33
Beta 1,3 Glucan	18.78	18.96
Goldenseal	16.54	11.94
Arabinogalactan	14.82	10.66
Echinacea	13.82	15.22
Colostrum	14.54	11.77
Astragalus	13.72	10.27
Positive Control	45.45	46.94
Negative Control	11.91	10.48
Monocytes		
RLC	40.17	25.00
Lactoferrin	37.83	26.92
Whey isolate	35.79	28.87
Reishi	32.42	25.82
Maitake	27.85	29.96
Beta 1,3 Glucan	22.03	25.49
Goldenseal	20.05	18.02
Arabinogalactan	19.49	40.88
Echinacea	18.18	20.08
Colostrum	12.96	14.97
Astragalus	13.13	25.49
Positive Control	75.64	75.74
Negative Control	10.27	13.41
Granulocytes		
RLC	9.69	39.39
Lactoferrin	7.24	8.41
Whey isolate	6.85	12.92
Reishi	6.50	18.26
Maitake	6.44	9.05
Beta 1,3 Glucan	6.40	8.88
Goldenseal	6.26	9.54
Arabinogalactan	5.46	30.06
Echinacea	4.92	18.13
Colostrum	4.77	9.03
Astragalus	4.61	13.75
Positive Control	54.39	91.86
Negative Control	5.22	8.06

General Immune Stimulation Results

General stimulation measurements with all study materials using serving sizes as recommended for the products listed above and 50 mg of RLC produced the results shown in Figures 4, 5, 6, and Table 4.

Discussion

This study was undertaken to provide laboratory comparisons of various immune response modifiers (IRM) available in the marketplace for use by healthy people. An IRM used by healthy people should provide measurable, useful immune system support and assist a healthy person in sustaining the best immune system performance possible given their circumstances.

There are three immunologic imperatives that an IRM should address:

1. Recognize foreign substances, pathogen, and cells.
2. Respond effectively against foreign substances, pathogen, and cells.
3. Remember foreign targets.

Public discussion of the immune system has increased dramatically in the last ten years. This has been largely initiated by new scientific knowledge, AIDS/HIV, and expanded news coverage worldwide. The use of IRMs has grown simultaneously in popularity, as the public has come to realize that “adequate” and “acceptable” immune system profiles are not necessarily “optimum” when it comes to protecting our health. This is a trend that can be expected to expand massively in coming years given this experience.

Poor nutrition and stress are but two primary factors that affect the immune performance of healthy people. Regardless of effort, healthy people make compromises in diet and experience stress in their lives, which reduce their immune function in a manner that produces less-than-optimum protection. The long-term consequences to any specific individual as a result of their decisions and actions relative to diet and stress remain very difficult to quantify.

The RLC was obtained from a single source utilizing patented and proprietary processes for production. The product is classified as a “Generally

Recognized as Safe” (GRAS), an FDA designation, for substance and is marketed as a dietary supplement and/or a dietary supplement ingredient or as a dietary ingredient. As a result of bio-engineering technology, RLC contains significant concentrations of many active, targeted compounds that are naturally produced by the body and utilized by the immune system. When these compounds are present in the diet in an active form, they can enhance the immune system and help it overcome some of the adverse effects of poor nutrition and stress and achieve improved function.

In a previous study, RLC demonstrated a complex and integrated immune modulation response unlike any other known single substance. In this study, RLC demonstrated support for a combination of activation and specific, targeted, information transfer activities that appears to correlate to the observed benefits of the substance clinically. The other substances tested failed to replicate the RLC effects in totality. Without these factors in combination, as found in RLC, an immune system response would be greatly narrowed to as little as a one-dimensional, unregulated, immune stimulation “effect.”

Immune stimulation effects should not be confused or assumed to be of benefit, i.e., unregulated immune stimulation is related to many auto-immune diseases. One core benefit of supplementation with RLC is evidenced by the higher level of immune surveillance presented in the study data as evidenced by the “modulated” changes in key immune cell populations.

RLC provided a higher, quantitatively measured, stimulation of key immune cells *in vitro*, as measured by flow cytometry with whole human blood, than any other substance tested and currently available in the marketplace. In fact, on a weight-to-weight basis, nothing approaches RLC’s capabilities for stimulation of lymphocytes, granulocytes, and monocytes.

Qualitative and quantitative analysis shows that RLC contains a significant concentration of unique and specific transfer factors, defensins,



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➤ and granulysins as well as other important nano-molecular, immune system-supporting molecules. The concentrations and specificity is consistent with the bio-engineering of the compound utilizing the induction process. Their predictability and testability is essential to producing a consistent and valuable compound and appears in none of the other study materials in significant or useful quantities.

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Notes

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