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Examination of Immune Response Modifiers in Healthy Individuals as Compared to the Refined Lacteal Complex Ai/E¹⁰[®]

Berlett Dietary Ingredients LLC

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ABSTRACT

Background:

Many substances are known to affect the function of the immune system in healthy individuals. Ai/E¹⁰[®], a refined lacteal complex, has demonstrated in small clinical trials and reports of health care practitioners the apparent ability to optimize the modulation of the immune system through the deliberate induction of an immune response that yields a new dynamic equilibrium of immune function with increased surveillance.

Objective:

The comparative examination of substances, including Ai/E¹⁰[®], presented to the marketplace for support of immune function and to discern for each the presence of characteristics and capabilities that contribute to and support optimized modulation of the immune system.

Design:

The overall modulating capability of a refined lacteal complex such as Ai/E¹⁰[®] has been established by in-vivo animal study at the University of Arizona. References to that study are included herein.

Various techniques were used to identify molecules in Ai/E¹⁰[®] and other study materials that are 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.

Stimulation capability was measured in-vitro using flow cytometry to identify the responses of lymphocytes, granulocytes and monocytes to Ai/E¹⁰[®] as compared to other substances in the marketplace. Recommended and comparable serving sizes were evaluated.

Studies were conducted to confirm oral absorption.

Results:

All of the study materials demonstrated immune stimulation capability at recommended serving sizes, but only Ai/E¹⁰[®] also demonstrated a strong and reliable capability to increase immune surveillance and transfer immunological information among cell groups. These distinctive capabilities illustrate the dynamic and optimal modulation benefits of Ai/E¹⁰[®].

KEY WORDS:

Ai/E¹⁰[®], colostrum, maitake, arabinogalactan, whey isolate, lactoferrin, reishi, goldenseal, astragalus, echinacea, Beta-1,3-glucan, immune modulation, immuno dynamic, transfer factor, immune system, dynamic equilibrium.

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Introduction

Scientific research reveals that immune system function plays a critical role in the maintenance of health and that optimum immune function is a desirable component in the pursuit of a long and healthy life. The public has become interested and proactive in taking responsibility for their health as awareness that optimum immune function may differ from traditional measurements of acceptable function and that unavoidable lifestyle and environmental factors can influence optimum immune function.

This proactive response by healthy people manifests in their attention to nutrition and dietary supplements to sustain and/or boost immune function. The motivation to take dietary supplements appears to range from the desire to maintain good health to disease prevention to a quest to re-establish health. Numerous foods and supplements may contribute to sustaining immune system function but there has been no apriori reason to believe that Ai/E¹⁰[®] is the most effective and important dietary supplement available for this purpose. This examination explores this matter from multiple perspectives using numerous techniques.

Reported benefits derived from refined lacteal complexes date well back into the 1950's. The pursuit of deeper understanding and exploration of the reasons for such benefits began in earnest in 1993.

In choosing a formula for a refined lacteal complex to be presented to the public, reliance was placed on the experience of clinicians and researchers. A unique refined lacteal complex was produced using the finest technology available at the time and was introduced to the public at the end of 1993. Minor changes would eventually result in the development and standardization of Ai/E¹⁰[®].

As the dietary supplement industry advanced during the 1990's demand for additional information supporting Ai/E¹⁰[®] was requested by the marketplace. To facilitate that demand Ai/E¹⁰[®] was used in several small clinical trials for the purpose of demonstrating its effects on immune function as evidenced by changes in natural killer (NK) cell activity, T and B cell subsets and immune modulation. These studies all demonstrated a highly favorable benefit.

Ai/E¹⁰[®] was utilized extensively by Jesse A. Stoff MD in clinical practice as a dietary supplement for people suffering from significant immune compromise. Dr. Stoff has written extensively and included discussions of benefits he noted in

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this regard in a booklet titled The Ultimate Nutrient and a book entitled The Prostate Miracle. Dr. Stoff has also lectured internationally in regard to his experience with Ai/E¹⁰®.

Though no major medical journals have published studies regarding Ai/E¹⁰® or a similar refined lacteal complex, many of the studies have been published by smaller peer reviewed and non-peer reviewed journals. The product has been well received in many nations and over 100,000,000 million servings have been safely consumed through 2004.

After significant consideration the process of laboratory examination of Ai/E¹⁰® began in earnest in 2001. The goals of this examination included a better understanding of the comparative benefit of Ai/E¹⁰® versus other products in the marketplace designated to help healthy people sustain good immune function. Further research was designed in consideration of assay techniques, potential new technology relative to production and quality control, understanding the product's mechanism of action and complementing the existing data supporting the product's successful reputation.

METHODS

Laboratory:

Tiburon Diagnostic Laboratory, Tucson, AZ

Test products

All products used for the testing were acquired in the general marketplace except Ai/E¹⁰®. 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. Ai/E¹⁰® was provided directly from its manufacturing source to the laboratory.

Listed below are the selected test products and their recommended serving sizes. Standard laboratory techniques were utilized to provide test materials of strength equivalent to their recommended serving sizes for each study activity.

Ai/E ¹⁰ ®	50 mg
Lactoferrin	250 mg
Whey isolate	1500 mg
Colostrum	1500 mg
Beta 1, 3 glucan	250 mg
Astragalus	1 cc
Maitake	1 cc
Echinacea	1 cc
Reishi	1 cc
Goldenseal	1 cc
Arabinogalactan	1 cc

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Ai/E¹⁰⁰® given to participants, for in-vivo study and in-vitro measurements, was provided by Quantum Research, Inc.

Subjects

All blood utilized in the in-vitro analysis was drawn at the laboratory. Over two dozen subjects were tested to find participants whose blood profiles were within the normal ranges of laboratory tests including: Comprehensive Chemistry Panel, CBC, NK cell panel, T & B cell subset panels. Three participants agreed to contribute to the research project by taking a commercial form of Ai/E¹⁰⁰® and contributing blood at the beginning and end of the oral supplementation period for purposes of in-vitro analysis.

Study Protocols

Electrophoresis

Determine the presence of peptides and molecules below 100 kD.

Microdensitometer

Evaluate and graph molecular bands for semi-quantitative evaluation of weight.

Antimicrobial testing

Identify molecular bands for bactericidal activity.

Tissue cell culture

Determine the activity of transfer factor molecules.

Flow cytometry

Measurement of activated leucocytes.

RESULTS

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 Ai/E¹⁰⁰®. These same bands were not present in any of the other products tested. Colostrum demonstrated fewer bands of less apparent density than Ai/E¹⁰⁰®.

This was further confirmed with microdensitometer readings. See Microdensitometer Evaluation.

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Microdensitometer Evaluation

A microdensitometer was used to evaluate the banding pattern and density of the electrophoresis gels, providing a semi-quantitative evaluation of the molecules in the bands. Ai/E¹⁰[®] presented significantly higher band density as evidenced by the microdensitometer readings in comparison to the other study substances.

Colostrum presents high concentrations of large molecular weight proteins, much of which are antibodies made against microbes that the cow was exposed to while in her stall or grazing. Ai/E¹⁰[®] presents a majority of the proteins and peptides in the low molecular weight ranges including bands for Granulysins and Defensins/transfer factors that are not present in the colostrum. This shift in the pattern of protein/peptide production by the cow is entirely due to the patented and proprietary processes utilized in the production of Ai/E¹⁰[®]. The samples were adjusted to contain 7 mg/cc of solid material.

Antimicrobial Testing

Antimicrobial testing showed six molecular bands of Ai/E¹⁰[®] illustrated by electrophoresis to have an in-vitro antimicrobial effect. Only one of the other study materials, lactoferrin (one band), had a weak antimicrobial effect. Lactoferrin is recognized to have a general, non-specific antimicrobial effect. To the extent that other tested substances contained lactoferrin (FCS and colostrum), they too, showed minimal antimicrobial effects from that band.

Transfer Factor Testing

Leukocyte cell culture testing was done to successfully demonstrate a transfer factor effect from the study material produced using the specific induction of bactericidal molecules as an end point.

T Cells, B Cells and NK Cells Activation

Flow cytometry in conjunction with T & B cell subsets and NK cell measurements were used to determine if Ai/E¹⁰[®] activated T cells, B cells and NK cells. These studies revealed that Ai/E¹⁰[®] caused a high activation of these cells consistent with previous clinical trials and all subjects recorded significant increases in NK cell activity.

Immune stimulation

Immune stimulation was a key component of the study and was measured from multiple perspectives:

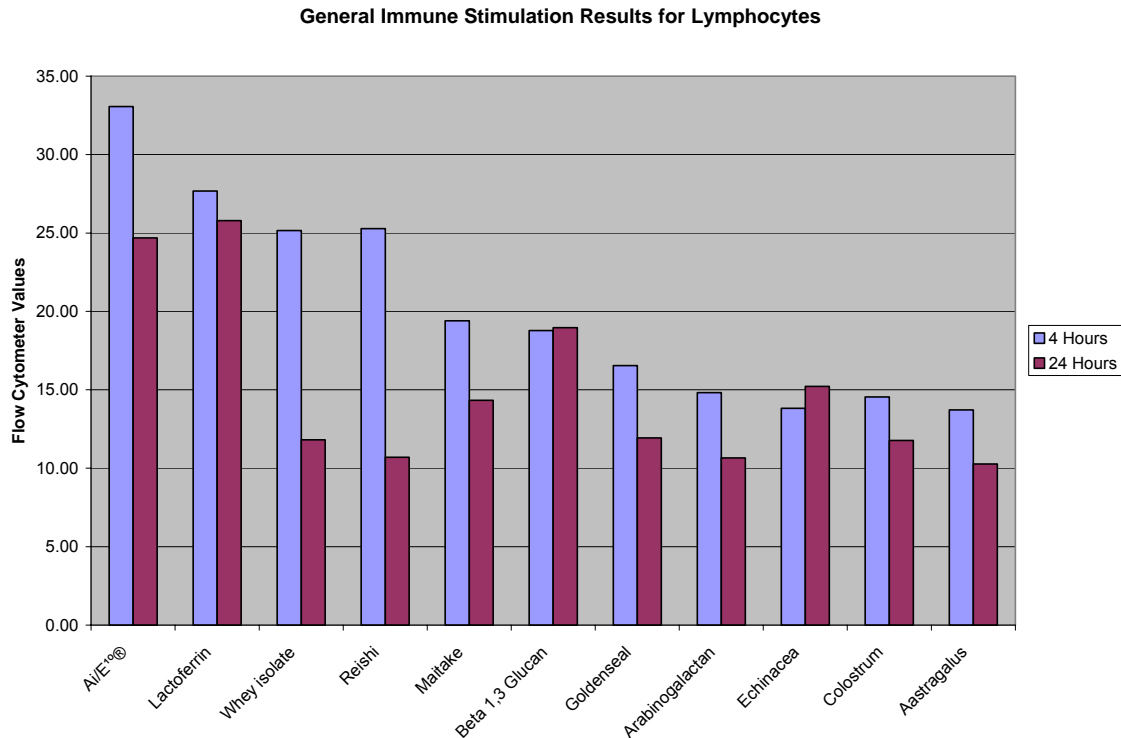
1. General immune stimulation using a single whole blood source for all study materials.
2. Hourly immune stimulation comparisons for Ai/E¹⁰[®] and colostrum were conducted with the whole blood of three new individuals using both the recommended serving sizes and comparable serving sizes.

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3. Determination of oral absorption and in-vitro activity was measured using whole blood extracted prior to a 14 day ingestion of Ai/E¹⁰® of 100 mgs three times per day. Though the earlier data indicated 50 mgs was ample to accomplish stimulation in-vitro, the higher serving size was utilized to shorten the compliance period and help overcome minor immunosuppressives from the environment and life style that may be present.

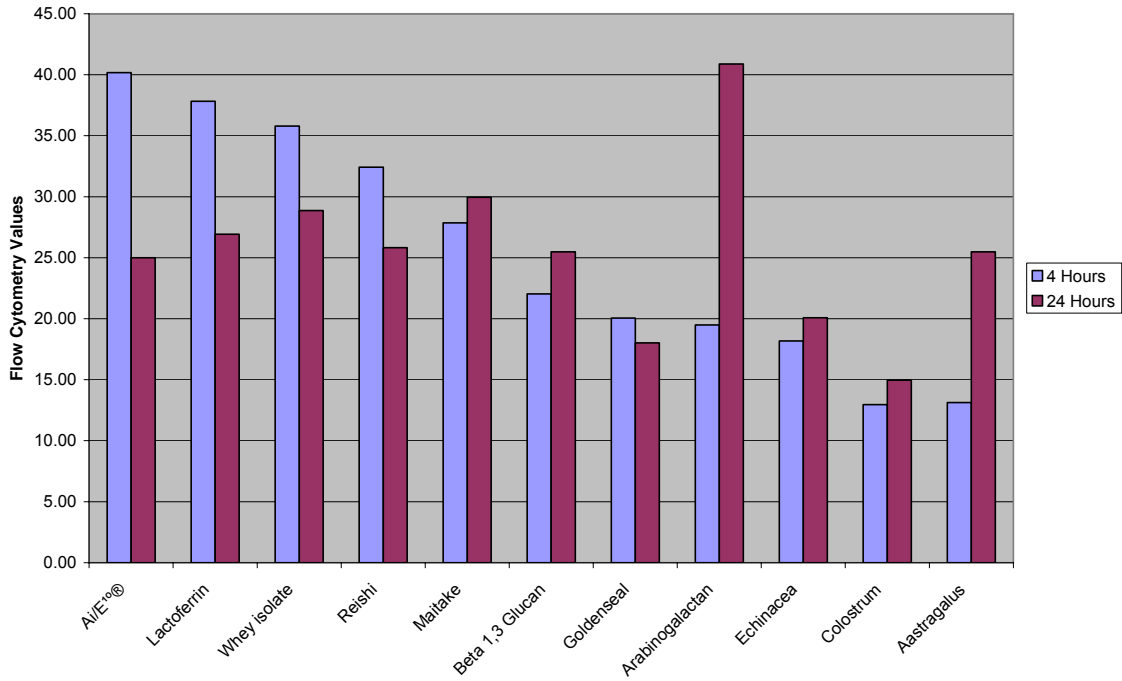
General Immune Stimulation Results

General stimulation measurements with all study materials using serving sizes as recommended for the products listed above and 50 mg of Ai/E¹⁰® produced the following results:

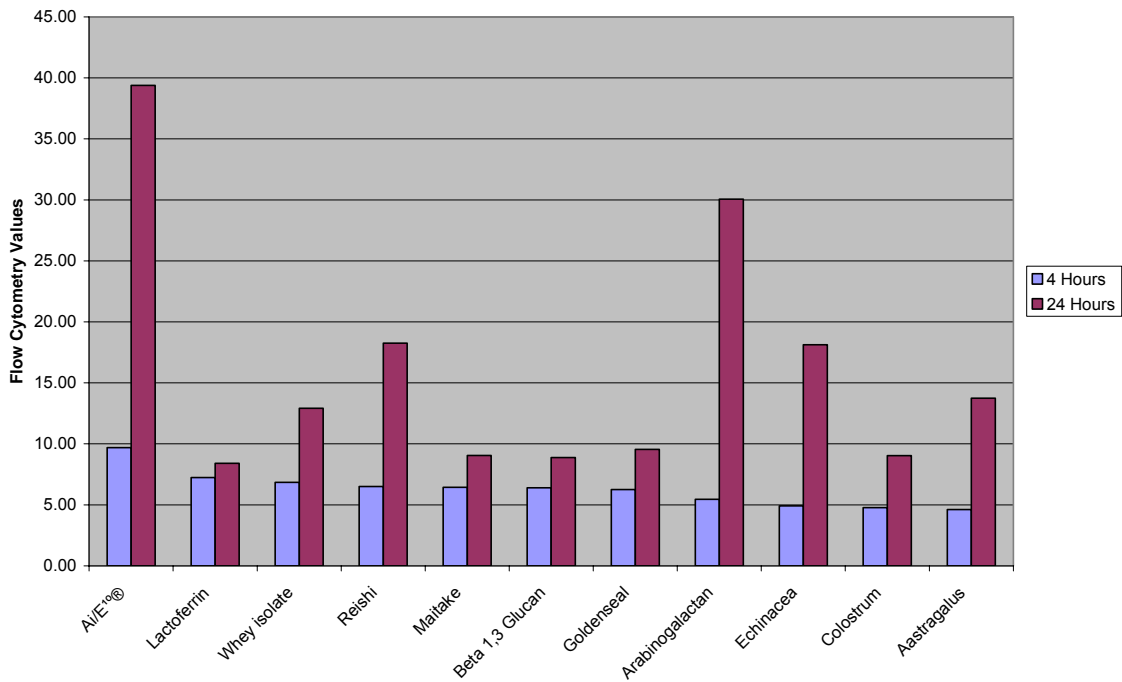


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General Immune Stimulation Results for Monocytes



General Immune Stimulation Results for Granulocytes



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Data tables for General Immune Stimulation Results

Lymphocytes

	4 Hours	24 Hours			
Ai/E ¹⁰ ®	33.06	24.69	Positive Control	45.45	46.94
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			
			Negative Control	11.91	10.48

Monocytes

	4 Hours	24 Hours			
Ai/E ¹⁰ ®	40.17	25.00	Positive Control	75.64	75.74
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			
			Negative Control	10.27	13.41

Granulocytes

	4 Hours	24 Hours			
Ai/E ¹⁰ ®	9.69	39.39	Positive Control	54.39	91.86
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			
			Negative Control	5.22	8.06

Assay confirms that Ai/E¹⁰® contains lactoferrin so further study of lactoferrin was not considered meaningful. Whey is a simple refined lacteal complex and further

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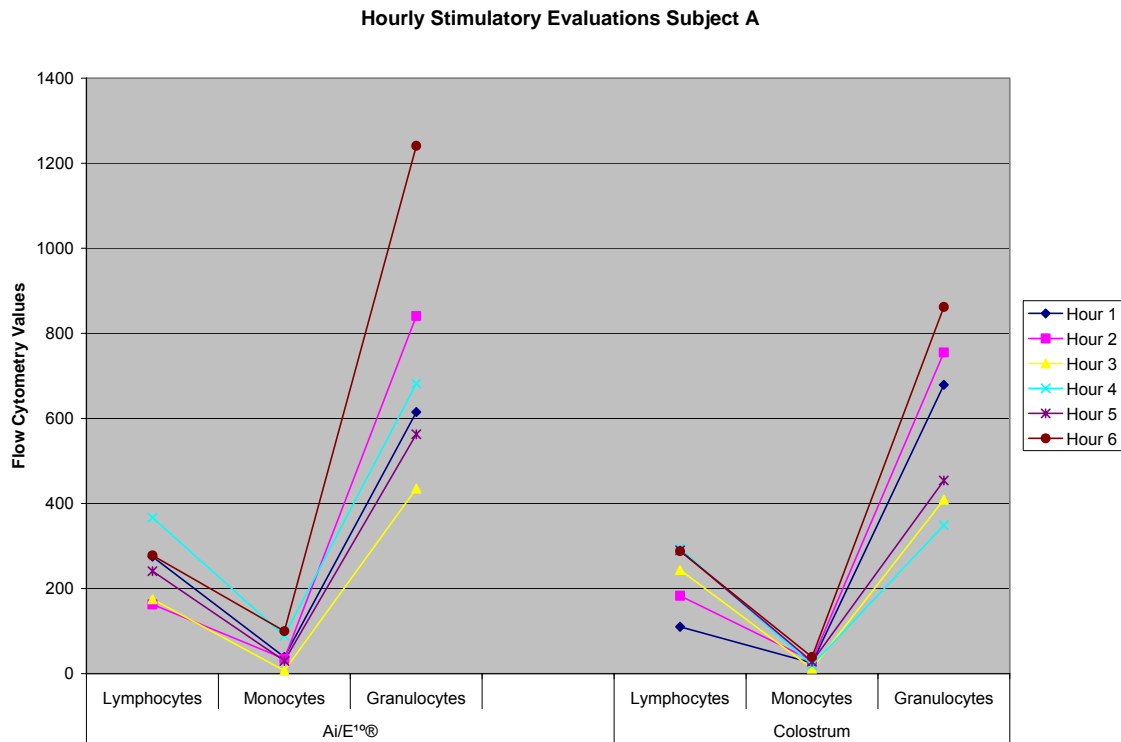
study was also not warranted. Their stimulatory capabilities were the only measurement deemed necessary.

The study demonstrated that no herb in the group was more stimulatory to all leukocyte populations studied than Ai/E¹⁰® so further study of those substances was terminated also. At the outset herbs were at a huge disadvantage as they contain no mammalian molecules carrying informational (targeting and modulation) data.

Theoretically, comparison of the stimulatory capability of herbs may have suggested vast superiority and demonstrated their value when used in conjunction with a mammalian product. No such superior capabilities emerged.

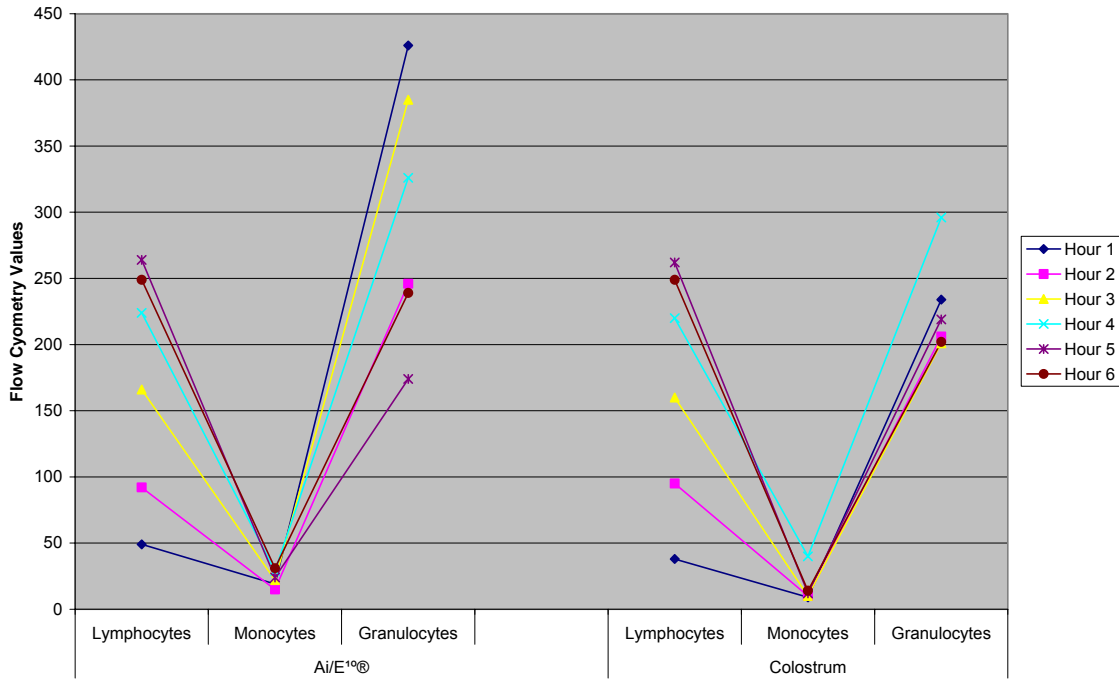
Hourly Immune Stimulation Comparisons

Evaluations by hour for six (6) hours of the stimulatory effects on lymphocytes, monocytes and granulocytes by Ai/E¹⁰® and colostrum were performed for the whole blood of three additional subjects (A, B, & C) using the criteria described earlier. The results were as follows:

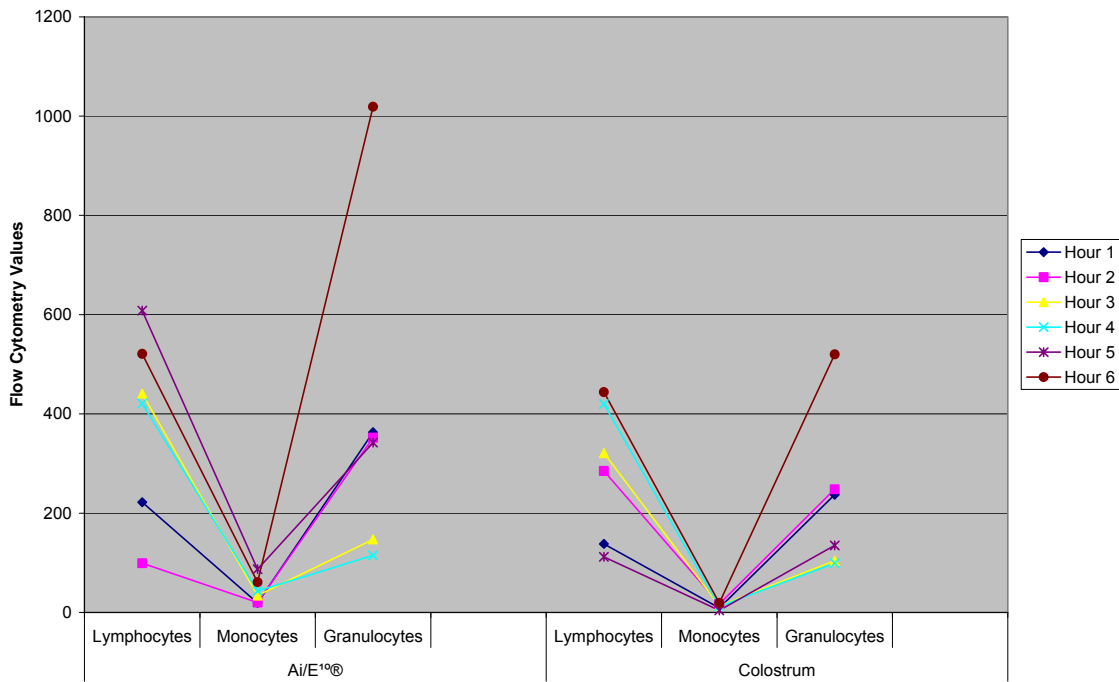


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Hourly Stimulatory Evaluations Subject B



Hourly Stimulatory Evaluations Subject C



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Data Table for Ai/E¹⁰[®]/Colostrum Comparison

Hourly Evaluations of Ai/E¹⁰[®] and Colostrum Using Recommended Serving Sizes

Subject A

		Hour 1	Hour 2	Hour 3	Hour 4	Hour 5	Hour 6
Ai/E ¹⁰ [®]	Lymphocytes	275	163	175	367	241	278
	Monocytes	39	35	7	89	30	100
	Granulocytes	615	841	435	682	563	1241
Colostrum	Lymphocytes	110	183	244	294	290	288
	Monocytes	25	31	12	20	28	39
	Granulocytes	679	755	409	349	454	862

Hourly Evaluations of Ai/E¹⁰[®] and Colostrum Using Recommended Serving Sizes

Subject B

		Hour 1	Hour 2	Hour 3	Hour 4	Hour 5	Hour 6
Ai/E ¹⁰ [®]	Lymphocytes	49	92	166	224	264	249
	Monocytes	19	15	22	29	24	31
	Granulocytes	426	246	385	326	174	239
Colostrum	Lymphocytes	38	95	160	220	262	249
	Monocytes	9	10	10	40	12	14
	Granulocytes	234	206	201	296	219	202

Hourly Evaluations of Ai/E¹⁰[®] and Colostrum Using Recommended Serving Sizes

Subject C

		Hour 1	Hour 2	Hour 3	Hour 4	Hour 5	Hour 6
Ai/E ¹⁰ [®]	Lymphocytes	222	99	441	421	608	521
	Monocytes	19	20	34	43	87	61
	Granulocytes	363	352	147	115	342	1019
Colostrum	Lymphocytes	138	285	321	420	112	444
	Monocytes	9	17	13	12	4	19
	Granulocytes	237	248	104	99	135	520

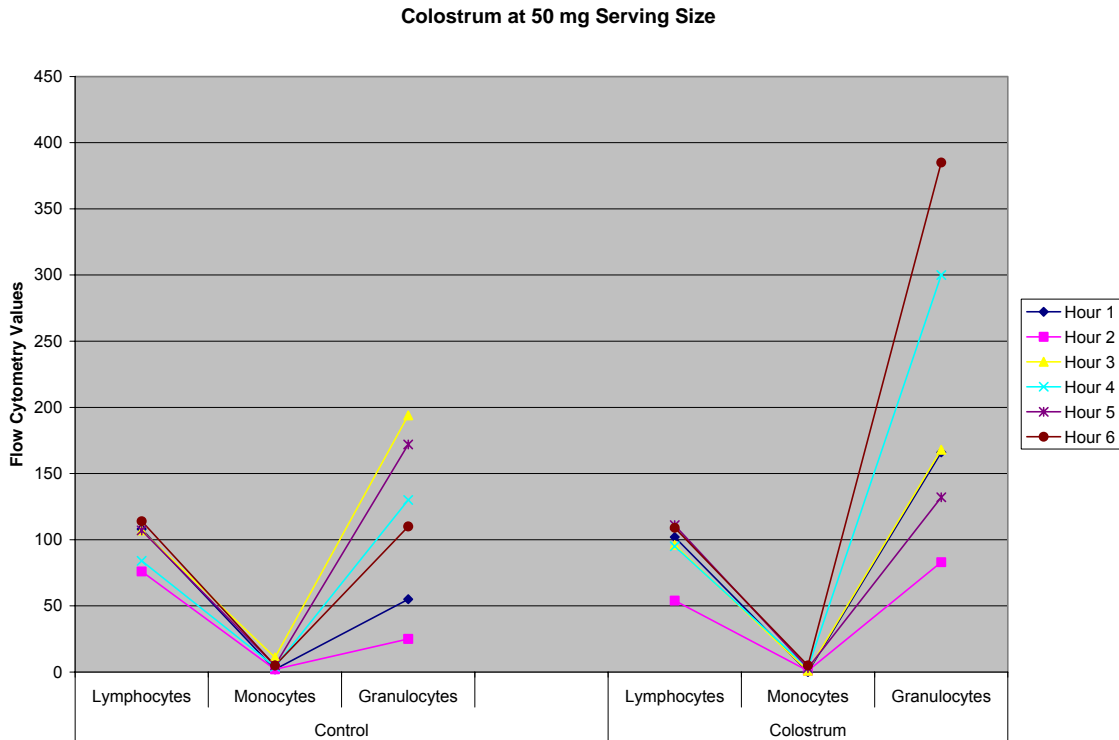
One consideration that became apparent in the study was the need for a colostrum serving relatively 30 times that needed for Ai/E¹⁰[®]. Since that issue raises concerns for various economic and utilization issues a study was conducted with flow cytometry for equivalent serving sizes. Since Ai/E¹⁰[®] outperformed colostrum in the earlier measurement, 50 mg of colostrum equal to the 50 mg of Ai/E¹⁰[®] were compared.

The data is worthy of further study and consideration, but suggests that the use of less than the recommended serving size of colostrum as studied by the marketer for serving size recommendations, or colostrum of specifications less than the very high quality used in the study may actually trigger immune

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suppression. This could result from interference with cytokine conduction by foreign proteins. At higher quantities this effect was overcome in the study data.

The following chart and data table illustrate this point through the charting of relevant flow cytometry data. The relative similarity and cases of diminished immune activity are highly apparent.



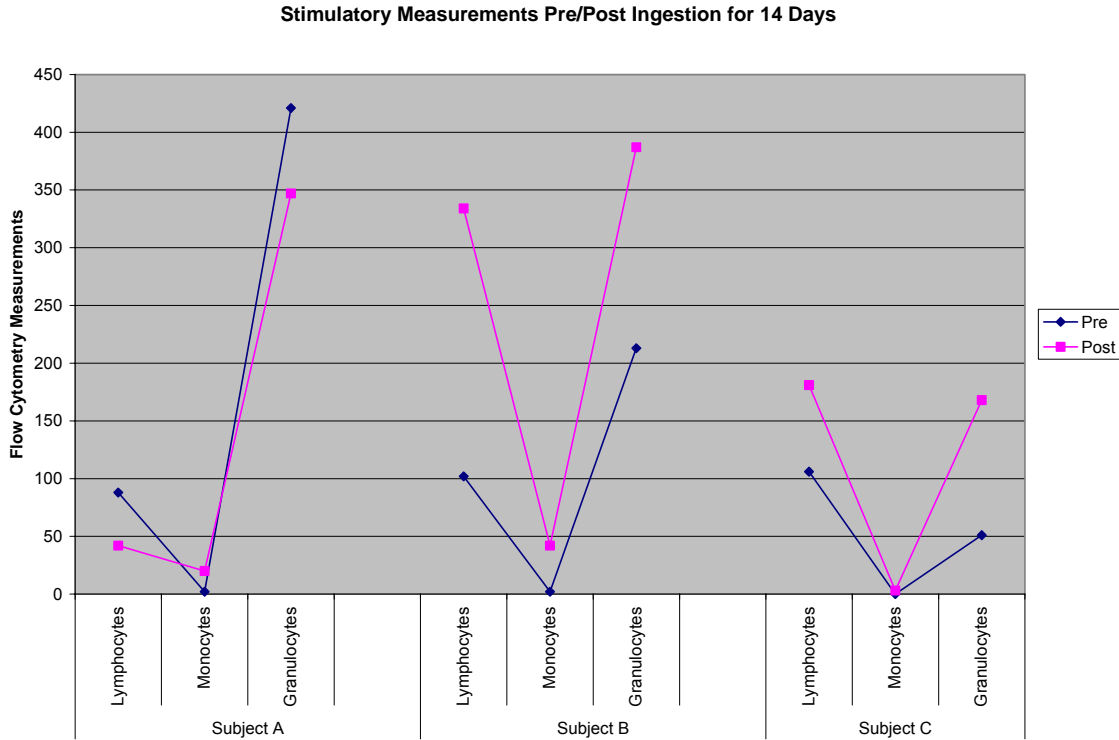
Hourly Evaluations of Ai/E¹⁰® and Colostrum Using Equivalent Serving Sizes

		Hour 1	Hour 2	Hour 3	Hour 4	Hour 5	Hour 6
Control	Lymphocytes	108	76	107	84	107	114
	Monocytes	2	2	11	4	5	5
	Granulocytes	55	25	194	130	172	110
Colostrum	Lymphocytes	102	54	96	95	111	109
	Monocytes	0	1	1	3	3	5
	Granulocytes	166	83	168	300	132	385

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Measurement of In-Vivo Immune Stimulation Response

Subjects A, B & C were compliant with the oral ingestion of 100 mg of Ai/E¹⁰® three times per day for 14 days. Whole blood was tested by flow cytometry for lymphocyte, monocyte and granulocyte levels using the same controls to assess the absorption and effects on these three measurements of immune cell activity.



Data Table for Measurement of In-Vitro Immune Stimulation Response

		Pre	Post
Subject A	Lymphocytes	88	42
	Monocytes	2	20
	Granulocytes	421	347
Subject B	Lymphocytes	102	334
	Monocytes	2	42
	Granulocytes	213	387
Subject C	Lymphocytes	106	181
	Monocytes	0	3
	Granulocytes	51	168

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Discussion

This study was undertaken to provide laboratory comparisons of various immune response modifiers for healthy people. The use of immune response modifiers has grown in popularity as the public has come to realize that "adequate", "acceptable" and "normal" immune profiles are not necessarily "optimal" and that sustaining good immune function is an important health consideration.

Poor nutrition and stress are the primary factors that could be expected to affect the immune performance of healthy people. Regardless of effort, nearly every healthy person makes compromises in diet and experience stress in their life that reduces their immune function in a manner that produces less than optimum performance. 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.

An immune response modifier used daily by healthy people should provide measurable immune support and assist the user in sustaining the best immune system performance possible given their circumstances.

Ai/E¹⁰® is a refined lacteal complex obtained from a single source utilizing patented and proprietary processes for production. The product is classified as a GRAS (FDA designation for "Generally Recognized as Safe") substance marketed as a dietary supplement and/or a dietary supplement ingredient or as a dietary ingredient. Ai/E¹⁰® contains significant concentrations of many active 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 help the immune system sustain optimal function.

In this comprehensive examination, Ai/E¹⁰® demonstrated a complex and integrated immune modulation response unlike any other known substance. The substance demonstrates support for a combination of activation, modulation, and information transfer activities that appear to correlate to the observed benefits of the substance. Without the three factors in combination an immune system response would be greatly narrowed to as little as a one-dimensional immune stimulation "effect".

An immune stimulation effect should not be confused or assumed to be a benefit, i.e. un-regulated immune stimulation. One core benefit of supplementation with Ai/E¹⁰® is evidenced by the higher level of immune surveillance presented in the study data as "modulated" results of key immune cell populations.

A recently completed animal study at the University of Arizona demonstrated the very clear immune modulation benefits associated with the use a refined lacteal

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complex such as Ai/E¹⁰®. This capability in a dietary supplement has not been otherwise demonstrated.

Ai/E¹⁰® provides 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 known to be currently available in the marketplace. In fact, on a weight to weight basis nothing even remotely approaches Ai/E¹⁰®'s capabilities for hour by hour support of lymphocytes, granulocytes and monocytes.

The study shows that Ai/E¹⁰® contains a significant concentration of specific transfer factors, defensins, and granulysins as well as other important immune system supporting molecules. Oral absorption into the blood stream has been demonstrated and results achieved with oral utilization correlate with in-vitro results. The specific concentrations are consistent with the production expectations. 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.

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