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**Clinical Science Docket
Summary of Research**



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Human Clinicals - Research Summary

#	Benefit	Study/Scientific Basis	# patients	Duration	Publication	Title & Summary
1	Increases macrophages	Peripheral mononuclear cells exposed to AG showed two to three times the cellular proliferation rate of Echinacea purpurea.	22	6	Proprietary Human Clinical Study - University of Minnesota	Causey 1998 (University of Minnesota) Monocytes, precursors of immune cell macrophages, proliferate at increasingly steady rates as AG incubation levels increase, surpassing rates demonstrated by Echinacea at equal concentrations.
2	Increases White Blood Count	Human <i>in vivo</i> circulating monocytes increase significantly as a direct result of AG oral consumption.	500	12 weeks	Proprietary Human Clinical Study - University of Minnesota	Causey 1998 (University of Minnesota) Monocytes, precursors of immune cell macrophages, proliferate at increasingly steady rates as AG incubation levels increase, surpassing rates demonstrated by Echinacea at equal concentrations.
3	Increases White Blood Count and lowers TNF Alpha	Confirmation of <i>in vivo</i> immune response in healthy adults is gained at much lower consumption levels. TNF alpha is found to decrease in healthy and HIV+ subjects.	48	4 weeks	Proprietary Human Clinical Study - SWC Research Institute Comp. Health Practice Review, V7, No. 3, S/S.	Kim (1) (SWCRI unpublished research) Healthy human adults consuming 1.5 to 4.5 g/d AG show increases in total white blood cell and monocyte levels in peripheral blood. Lower levels of TNF alpha are experienced with AG consumption, a positive indication given the association of high TNF alpha levels with human immune disease states.
4	Increased neutrophils and eosinophils	Confirmation of immune response in humans with free-living diets at SWCRI is gained with dogs under controlled diet conditions at same consumption levels, that is, 20 or 60 mg/kg/day.	33	6 weeks	Proprietary Human Clinical Study - SWC Research Institute Comp. Health Practice Review, V7, No. 3, S/S.	Grieshop, 2002 (Journal of Nutrition, 132: 478-482) Consumption of AG led to increased levels of circulating neutrophils and eosinophils with increasing trends in lymphocytes and monocytes.
5	Anti-bacterial and anti-viral effects	Consumption of AG increases circulating complement properdin in healthy female adults consuming 1.5 g/day.	24	4 weeks	Proprietary Human Clinical Study - SWC Research Institute Comp. Health Practice Review, V7, No. 3, S/S.	Kim (2) (SWCRI unpublished research) The complement component of the human immune system appears to be stimulated, as indicated by circulating properdin levels, by AG consumption. This increases phagocytic anti-bacterial/viral and microbial effects, activating the immune system in infections and other immunologically challenged events.
6	Increased immune response	Confirmation of immune function results from Slavin 1998 is achieved in preliminary results from Slavin 2000, wherein all test cells report increases in circulating monocytes.	100	6 Months	Proprietary Human Clinical Study	Slavin, 2000 (University of Minnesota) Healthy human adult subjects consuming either 1.4, 4.2 or 8.4 g/d show a time and dose dependent immune response to consumption of AG at one month. SF-36 health index and cold/flu symptoms are being followed over this 6-month study.
7 (NEW!)	Increased immune response	Consumption of Resist-Aid Arabinogalactan with polyphenols provides a greater immune response than AG alone	To be announced Oct 2009 (Orlando, Florida)		To be announced in October 2009	Human clinical results to be published in major peer-reviewed journal - TBD
8 (NEW!)	Increased immune response	Confirmation of Resist-Aid positive impact on specific immune functions when presented with a challenge	To be announced Oct 2009 (Orlando, Florida)		Annual Conference - American College of Nutrition. Oct 1 - 4th, 2009	Pre-publication seminar and poster presentation at the American College of Nutrition Conference. Manuscript in review.

In-Vitro and Animal Data

1	Supports healthy immune function through enhancement of beneficial immune cells and cytokine activity.	AG increases specific cytotoxicity of natural killer cells against K562 tumor cells in vitro.			Cancer Immunotherapy	Hauer, 1993 (Cancer Immunotherapy) Cultures of human peripheral mononuclear cells showed enhancement of natural killer cytotoxicity, governed by the cytokine network (specifically interferon gamma), when pretreated with larch arabinogalactan. AG was also involved in an increased release of tumor necrosis factor alpha, interleukin 1 and 6.
2	Confirmation of active ingredients	Arabinogalactans isolated from medicinal plants including Echinacea purpurea, Baptisia tinctoria, Angelica acutiloba and Thuja occidentalis have shown activity against tumor cells and microorganisms.			Journal of Naturopathic Medicine	D'Adamo (Journal of Naturopathic Medicine) Common immunostimulatory polysaccharide identified in Echinacea and many other natural medicinal plants has been identified as arabinogalactan.
3	Cell behavior	Inhibition of liver metastasis in mice by blocking hepatocyte lectins with arabinogalactan infusions and D-galactose.			Journal of Cancer Research and Clinical Oncology	Beuth (J Cancer Research and Clinical Oncology) Preinjection and regular application of the receptor blocking agents D-galactose and AG prevented the settling of sarcoma L-1 tumor in the liver completely.
4	Vascular permeability	Enhanced vascular permeability induced in mice by larch AG.			Immunology	Kind (Immunology, 1970) Preinjection of AG caused increased vascular permeability in mice as evidenced by the blueing of ears when injected by AG and Evan's blue dye.

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Immune-Stimulating Properties of Larch Arabinogalactan

Arabinogalactan also has been reported to possess immune-stimulating properties, as evidenced by a number of *in vitro* studies (Hauer and Anderer, 1993; Causey et al., 1999; Choi et al., 2005).

Arabinogalactan and Increased Lymphocyte and Macrophage Activity

Choi et al. (2005) conducted an *in vitro* study to investigate the immunomodulating activity of arabinogalactan. Isolated mouse spleen lymphocytes and peritoneal macrophages were incubated with 10 to 100 µg/mL of larch arabinogalactan for a period of 24 hours. Lymphocyte and macrophage cytotoxicity to YAC-1 and B16 melanoma cells were then assessed. Arabinogalactan was reported to significantly increase lymphocyte and macrophage cell viability, and to significantly enhance tumoricidal activities of lymphocytes and macrophages to YAC-1 and B16 melanoma cells, respectively.

Increased Immune Response without Inflammatory Response

Incubation with arabinogalactan also significantly increased phagocytosis, lysosomal enzyme activity, and increased production of nitrite, hydrogen peroxide, tumour necrosis factor alpha (TNF-α), and interleukin-6 (IL-6), while having no significant effect on IL-1β. The authors postulated that the immunomodulating effects of arabinogalactan may occur through the production of the free radicals and cytokines.

Cytokine Activity

Arabinogalactan pre-incubation (up to 5 mg/mL) of human peripheral blood mononuclear cells (PBMC) and cultures of pre-separated peripheral non-adherent cells (PNAC) and monocytes for 48 to 72 hours generally resulted in an increased release of various cytokines including interferon γ (IFNγ), interleukin-1β (IL-1β), tumour necrosis factor α (TNFα), and IL-6 (Hauer and Anderer, 1993). The authors however, stated that IFNγ was the only cytokine involved in this enhancement of natural killer cytotoxicity against K562 tumour cells in this study.

4X Increase in Monocyte Activity

Causey et al. (1999) conducted an *in vitro* study in which isolated human monocytes were treated with 100 µg/mL of larch arabinogalactan. The authors reported that incubation with arabinogalactan resulted in a significant 4-fold increase in the number of monocytes, thereby indicating that arabinogalactan may have immunomodulating properties.

Significant Increase in Monocyte Activity

In a clinical study, Causey et al. (1999) reported that subjects that consumed 1.5 g arabinogalactan/day for a period of 3 weeks had a significant increase in the number of circulating monocytes relative to controls. At a dose of 3.0 g/day, there was a non-significant trend towards increased circulating monocytes.

Arabinogalactan increases CD8+ T-suppressor cells

In another clinical trial in which 4 g/day of larch arabinogalactan was consumed in orange juice, an increase in the percentage of CD8+ T-suppressor cells was reported in the arabinogalactan group relative to the control group (orange juice only) (Nantz et al., 2001).

Not all studies were conclusive

Other clinical studies have demonstrated mixed results. Kim et al. (2002a) reported no significant effects on immune parameters, including white blood cell count, monocytes, serum interferon-*gamma* (IFN- γ), TNF- α , IL-6, following 4 weeks' supplementation with up to 4.5 g/day of larch arabinogalactan.

However, the authors acknowledged that limitations to their study methodology, including a small sample population (total of 21 subjects, 3 subjects/group), inadequate length of therapy, and a wide range of subject characteristics, rendered their results inconclusive.

6-week study Showed Significant Increase in CD8+ T-suppressor Cells

In contrast, in a study by Nantz et al. (2001) in which subjects consumed 4 g larch arabinogalactan/day for a period of 6 weeks in not-from-concentrate orange juice, the percentage of CD8+ T-suppressor cells was reportedly significantly increased relative to placebo; however, there were no significant effects on the levels of IgG, B cells and natural killer cells, and neutrophil oxidative burst activity. Increased lymphocyte proliferation was increased in both groups compared to baseline, which the authors suggested may be related to the immune-enhancing properties of the orange juice. The results of this study suggest that arabinogalactan may induce some immune-stimulating effects.

Unpublished Study Reports Stimulation of Monocyte Formation

In an unpublished study in which 60 subjects consumed 8.4 g larch arabinogalactan/day, or a rice starch placebo, for a period of 6 months, Causey-O'Brien (2002b) reported that larch arabinogalactan on immune parameters demonstrate that this ingredient may stimulate the formation of monocytes, thereby stimulating the immune system and providing support to maintain a healthy immune system.

References:

Causey JL, Robinson RR, Feirtag JM, Fulcher RG, Slavin JL. Effects of larch arabinogalactan on human peripheral blood mononuclear cells: results from *in vivo* and *in vitro* human trials [abstract]. *FASEB J.* 1999;13:A589. Abstract 457.7.

Purpose of Study

Human peripheral blood mononuclear cells, specifically immune cells, possess receptors on their surfaces that recognize and bind polysaccharide cell walls of foreign organisms. This facilitates phagocytosis and destruction of the foreign organisms. Purified cell wall polysaccharides such as glucans, mannans and xylans have been found *in vitro* to mimic the host-pathogen relationship resulting in dose and time-dependent increases in immune cell, specifically monocytes, proliferation and cytokine production. The purpose of this study was to determine if consumption of LAG would stimulate monocyte cell proliferation in healthy human subjects. Twenty healthy, adult human subjects consumed a placebo, 1.5 or 3.0 grams a day of LAG in a beverage vehicle for 3 weeks.

Findings

Blood draws indicated that consumption was followed by a statistically significant increase in circulation monocytes. Parallel *in vitro* studies indicated that freshly isolated human monocytes treated with LAG resulted in a 4-fold increase in cell numbers. The authors concluded that larch LAG enhances the mononuclear portion of the human immune system.

Kim L, Burkholder P, Waters R. Effects of low-dose arabinogalactan from *Larix occidentalis*: a randomized, double blind, placebo controlled pilot study. *Compr Health Pract Rev.* 2002;7:221-229.

Purpose of Study

The purpose of this Southwest College Research Institute study was to conduct a dose-response evaluation of the three major commercial grades of LAG on immune status and intestinal microflora in healthy adult humans at consumption levels significantly below those evaluated in the first University of Minnesota human clinical. LAG treatments of 1.5 or 4.5 g/day were consumed in a placebo controlled experimental design that evaluated the administration of LAG Grades 100, 1000 or 3000 as a dietary supplement. The treatment period lasted twenty-eight days with blood and fecal samples taken at baseline and immediately after the treatment period.

Findings

Dose-response trends were evidenced for circulating peripheral blood WBC and total monocytes with consumption of the commercially available decolorized LAG products. Human circulating TNF alpha cytokine levels decreased in a subject subpopulation at risk immunologically for HIV+. Total intestinal microflora and the number of subjects with countable Lactobacilli increased in the subject population. The authors concluded that consumption of LAG affected immune and gastrointestinal function with results dependent upon dose and type.

Kim LS, Waters RF, Burkholder PM. Immunological activity of larch arabinogalactan and Echinacea: a preliminary, randomized, double-blind, placebo-controlled trial. *Altern Med Rev.* 2002;7:138-149.

Purpose of Study

The purpose of this study was to determine the impact that standardization of whole herb Echinacea to its greatest immunostimulatory chemical component, LAG, would have on clinical immune markers and health related quality of life indices. Six study arms included; placebo, Echinacea extract, refined Echinacea, whole herb Echinacea, whole herb Echinacea standardized with larch LAG and larch LAG. Forty-eight healthy, adult females consumed 1.5g/day placebo, Echinacea, refined Echinacea, standardized Echinacea or LAG for a period of 28 days.

Findings

Blood samples pre and post indicated that the Complement Immune System was activated in treatment subjects as a result of consumption. The greatest response occurred in the standardized whole herb Echinacea and LAG groups. LAG treatment subjects reported through health related quality of life surveys an increased sense of well being, both physical and emotional (SF36). The authors concluded that activation of the complement immune system would appear to increase overall immune system response to infections and other immunologically challenged events.

Effectiveness of Orange Juice-based Dietary Supplements in Humans.
 Percival, S., The FASEB Journal (2001).

Purpose of Study

The purpose of this University of Florida study was to determine the impact of orange juice (positive control) and fortified orange juice on a number of aspects of the human immune system and it's functioning. Healthy adult humans participated in this six-week, double blind, randomized trial by consuming either one, eight-ounce serving of orange juice per day or the same amount of orange juice fortified with five grams of LAG. Fasting blood samples were taken at baseline, three and six weeks. Complete blood counts, CBC with differential/platelets and blood samples for functional immune assays were collected and measured. Immune markers measured from peripheral blood included respiratory burst activity, lymphocyte proliferation, IgG, NK cell activity and flow cytometry analysis.

Findings

CBC results indicated a trend towards increased white blood cells and oxidative burst activity with fortification; monocytes and NK cell activity trended higher in both groups. The author concluded that further research was necessary to determine the beneficial impact of orange juice and fortification with LAG on human immune system functioning.

Other References:

Causey J. Larch arabinogalactan increases monocyte counts in healthy humans.

Nantz MP, Painter AR, Parker ERB, McGill C, Percival SS. Evaluation of arabinogalactan's effect on human immunity. Paper presented at: Annual Meeting of The Federation of American Societies for Experimental Biology; 2001. Abstract 501.8.

Hauer J, Anderer FA. Mechanism of stimulation of human natural killer cytotoxicity by arabinogalactan from Larix occidentalis. Cancer Immunol Immunother. 1993;36:237-244.

Clinical Summary

Clinical Study Author (Date)	White Blood Cells	Monocytes	Neutrophils	Lymphocytes	NK Cell Activity	Respiratory Burst
Causey, 1999	✓	✓				
Hauer, 1993	✓				✓	
Causey, 1999	✓	✓	✓	✓		
Kim, 2002	✓	✓				
Kim, 2002	✓					
Causey In Review	✓	✓	✓		✓	
Nantz, 2001	✓	✓	✓		✓	✓
Causey In Review	✓	✓	✓	✓		✓

Animal – Immune Studies

Oral administration of arabinogalactan affects immune status and fecal microbial populations in the dog. Grieshop, C., Flickinger, E., Fahey, G., The FASEB Journal (2001).

Purpose of Study

The purpose of this University of Illinois Urbana Campus, study was to conduct a dose-response evaluation of the three major commercial grades of LAG on immune status and fecal microbial populations in the dog, a species widely used to predict equivalent human responses. LAG treatments at 20 or 60 mg/kg/day were administered along with a placebo control in a 7 X 7 Latin square design that evaluated the oral administration of LAG Grades 100, 1000 or 3000. Each treatment period consisted of a six-day adaptation followed by a four-day collection period. Blood and fresh fecal samples were collected on day ten of each period.

Findings

Overall, consumption of LAG products increased total lactobacilli and Bifidobacteria while decreasing clostridia. Consumption of LAG tended to have higher levels of peripheral blood circulating neutrophils and a trend towards higher levels of lymphocytes and monocytes. The authors concluded that specific forms of AG affected both fecal microbial populations and immune status of the dog.



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