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RESEARCH GUIDE

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INNOVATIVE INGREDIENTS TO **IMPROVE IMMUNITY IN CLINICAL PRACTICE**

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The immune system is well equipped to protect humans from illness because every aspect of the body is exposed to its surveillance. The immune system contains numerous types of cells and uses significant nutritional resources to carry out its health-protecting tasks.

When the immune system is operating efficiently, immune cells are mobilized to respond appropriately to invading allergens, pathogens, and toxins. Utilizing both innate and adaptive mechanisms, the immune system helps us survive our constantly changing environment.

Conversely, when the immune system is weakened, we are left vulnerable to a variety of illnesses including frequent infections, autoimmune conditions, and even cancer. Protecting and enhancing immunity in clinical practice is a primary goal for practitioners and patients alike.

An integrative approach to optimizing immune system function typically includes:

- dietary advice with possible modifications
- emphasizing lifestyle factors such as sleep and stress management
- recommending targeted dietary supplements

This research guide reviews 3 ingredients found in foods and dietary supplements that have been shown in the scientific literature to improve immune function:

- LC-Plasma (*Lactococcus lactis* strain Plasma) is a paraprobiotic that enhances immunity by stimulating plasmacytoid dendritic cells (pDCs).
- Glutathione is a potent intracellular antioxidant that stimulates natural killer (NK) cell function and promotes white blood cell activity.
- L-alanyl-l-glutamine is a fuel source for enterocytes, lymphocytes, macrophages, and fibroblasts.

Taken separately, in combination with each other, or with other immune-stimulating ingredients, these 3 ingredients can become an integral part of an immune-enhancing protocol. We will begin by taking a closer look at the novel ingredient LC-Plasma.

LC-Plasma

LC-Plasma has been shown to stimulate immune function in several intriguing ways. It is considered a "paraprobiotic," which is emerging as a distinct area of study in microbiome research. While live lactic acid bacteria have been shown to play a role in the health benefits of probiotics, the study of paraprobiotics is demonstrating that *inactivated* (nonviable) probiotics can produce health benefits as well. In the scientific literature, this is known as the probiotic paradox, where both live and inactivated probiotics act as biological response modifiers.

A 2020 review identified several key benefits of paraprobiotics including:

- convenient storage options
- longer shelf life
- safer for immune-compromised patients because there is a reduced risk of microbial translocation and sepsis

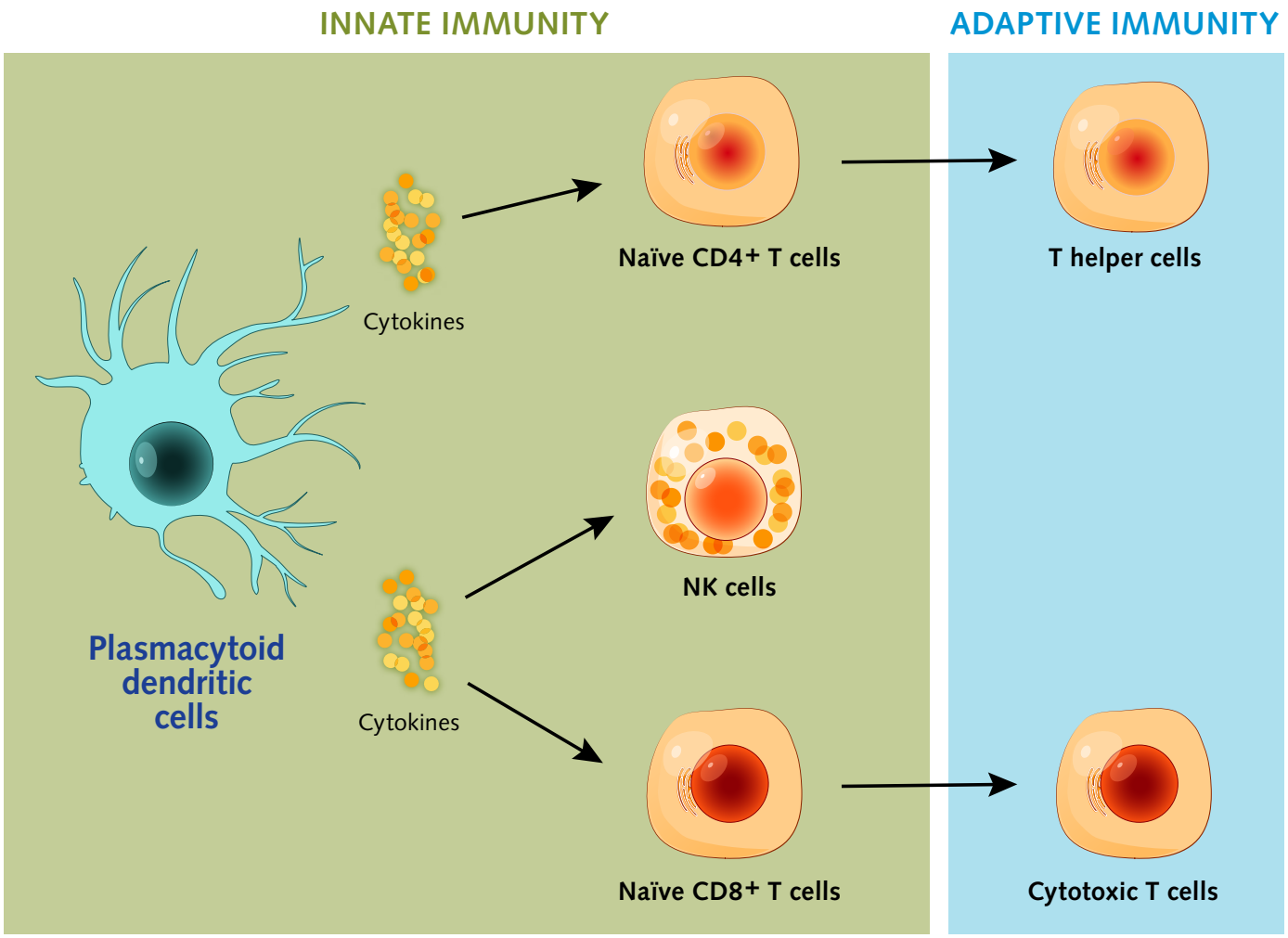
Mechanisms of Actions

LC-Plasma derives its name from the early discovery of its effects on a very specific segment of immune cells, called plasmacytoid dendritic cells (pDCs).

There are a variety of different cell types that make up the immune system. Most cells are either part of the innate or the adaptive immune response. Dendritic cells, however, straddle between innate and adaptive, orchestrating activity and communication between the two branches. Dendritic cells derive their name from their tree-like structure, with long projections able to stretch out between cells and provide an informative link between the first-line innate response and the long-term adaptive response. All dendritic cells play a crucial role in directing T-cell mediated responses throughout the body.

In addition to pDCs, there are conventional dendritic cells (cDCs). pDCs are dubbed "plasmacytoid" due to similarities to plasma B cells of the immune system, a trait that cDCs lack. pDCs play a pivotal role in bridging innate and adaptive immunity. In addition, pDCs are unique in 3 ways:

1. Once stimulated by a pathogen, they can rapidly secrete more interferons (IFN), sometimes up to 1,000-fold more, than other immune cells.
2. They are considered the most efficient antigen-presenting cells (APCs).
3. They can recruit or stimulate nearly every immune cell type including NK cells and B cells.



pDCs play a pivotal role in bridging innate and adaptive immunity and recruiting or stimulating nearly every immune cell type.

While pDCs have been studied since the 1990s, it's becoming even more apparent that enhancing pDC activity can provide potent immune system support. One way to stimulate these influential cells is with supplemental LC-Plasma, a unique strain of lactic acid bacteria that was isolated in the early 20th century by Dr. Orla-Jensen, a well-known researcher and authority on lactic acid bacteria. In the scientific literature, LC-Plasma is referred to as *Lactococcus lactis* JCM5805 or *Lactococcus lactis* strain Plasma. Both descriptions can be used interchangeably, and each refers to the same entity, LC-Plasma.

"The key component of pDC activation is retained in the LC-Plasma's cell particle," explained researcher Shintaro Ichikawa, PhD, who is also Director of Technical Affairs with Kyowa Hakko, USA. "Since the heat treatment process does not break the cell particle, both the live and heat-treated LC-Plasma work the same way."

As stated, research demonstrates that LC-Plasma directly activates pDCs, which may be its primary mechanism of action. According to a 2018 review, other possible mechanisms include:

- binding to and directly inactivating viruses
- competing with receptors on surfaces of target cells
- producing antimicrobial and antiviral substances
- stimulating host-cell immune defense systems

The widespread adoption of paraprobiotics is still in its infancy, but they hold tremendous potential to be used across a wide range of products.

Human Clinical Trials

Several human clinical trials have shown LC-Plasma leads to immune stimulation, many validating the stimulation of pDCs and the ensuing production of IFN. To

date, there are 10 human clinical trials on LC-Plasma that have been published, including 8 efficacy studies and 2 safety studies.

A 2015 randomized, placebo-controlled, double-blind study looked at LC-Plasma and influenza symptoms. A total of 213 volunteers were randomly assigned to receive yogurt with or without LC-Plasma daily for 10 weeks. The cumulative days with cough and fever were significantly reduced in the LC-Plasma group compared to the placebo group. This study also measured IFN gene expression levels and found that IFN was significantly higher in the LC-Plasma group compared to the placebo group, which helps confirm a key mechanism of action.

A 2016 randomized controlled trial looked at cold and influenza prevention in a group of 396 volunteers. In this study, LC-Plasma at a daily dose of more than 100 billion cells was given as a capsule for 12 weeks. This was compared to a placebo that looked identical. There were significantly fewer days of cough and sore throat in the LC-Plasma group compared to the placebo group. This study again showed a significant increase in IFN gene expression levels in the LC-Plasma group compared to the placebo group.

A 2017 randomized, placebo-controlled, double-blind clinical trial with 111 volunteers also showed that LC-Plasma induced a significant antiviral response compared to the control group. There were improvements in both mucosal and systemic immune parameters in the LC-Plasma group compared to the placebo group.

Also in 2017, researchers looked at the ability of LC-Plasma to reduce the incidence of influenza in elementary and high school students. LC-Plasma in yogurt was associated with a two-thirds decrease in influenza compared to children who didn't eat the yogurt.

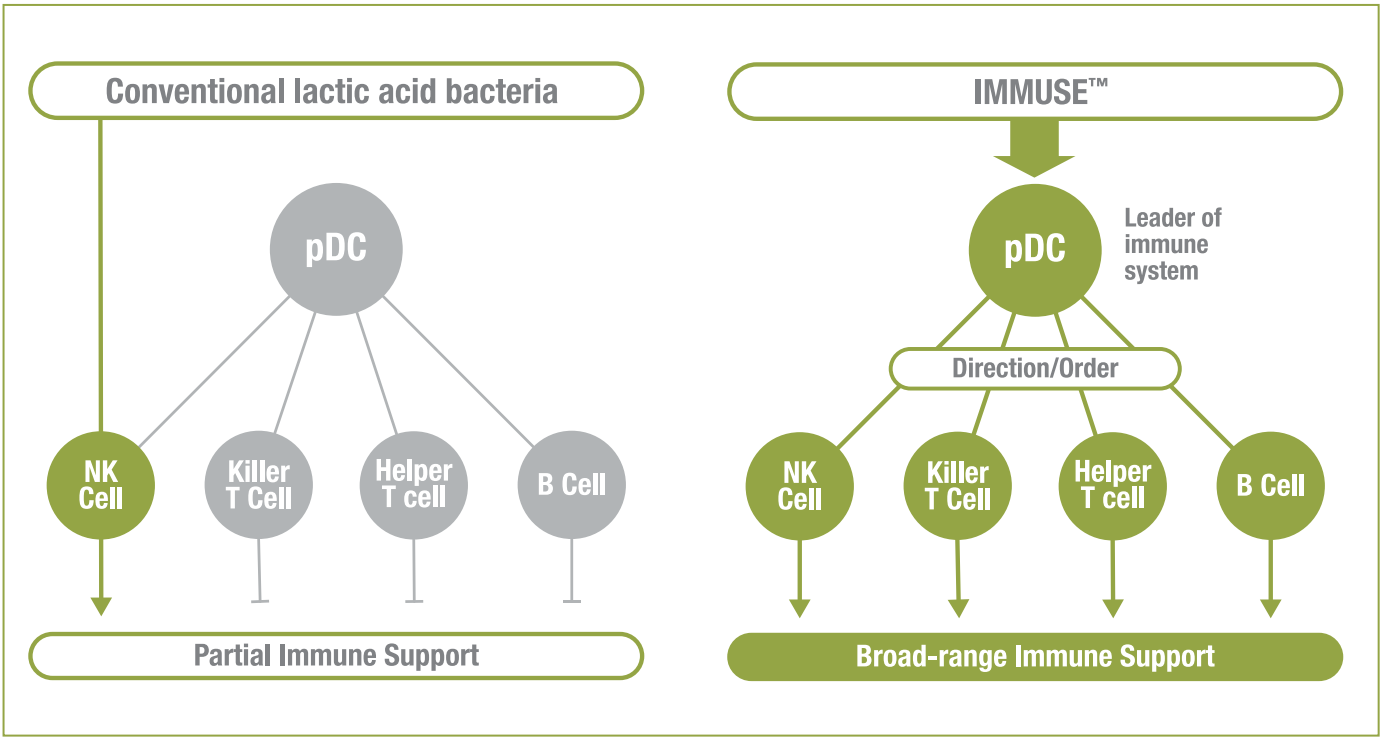
A 2018 randomized, placebo-controlled, double-blind trial looked at 51 high intensity exercising males and found that LC-Plasma significantly reduced symptoms of upper respiratory tract infections and also decreased fatigue compared to placebo. This study also identified pDC activation as a key mechanism of action.

Safety Data

The recommended daily dose is 50 mg/day of *Lactococcus lactis* strain Plasma which contains more than 100 billion inactivated cells. Studies looking at the safety of LC-Plasma in humans have used higher than clinically indicated doses over extended periods.

A 2015 excessive intake long-term use trial showed that LC-Plasma was safe at 3 times the typical dosage taken for 12 weeks. A 2018 randomized, double-blind,

IMMUNE SYSTEM ACTIVATION



placebo-controlled, parallel-group trial showed that LC-Plasma was safe at 5 times the recommended dose for 12 weeks.

Because there is also a long-term history of use in foods, this strain of *Lactococcus lactis* is considered safe. There is currently no information regarding potential contraindications or drug interactions with LC-Plasma.

The Patented Form

LC-Plasma is available under the brand name IMMUSE™. This patented form of the *Lactococcus lactis* strain Plasma is stable and can be used in a variety of applications including tablets, capsules, and food.

IMMUSE has been shown to activate NK cells via the same pathway as conventional lactic acid bacteria; however, its unique ability to activate pDCs results in more extensive immune stimulation with this patented form of *Lactococcus lactis* strain Plasma compared to other probiotic lactic acid bacteria.

"Activating NK cells is important, but that only provides partial immune support," explained Dr. Ichikawa. "This clinically studied form of the patented strain of *Lactococcus lactis* has been shown to activate pDC and is expected to provide broad-range immune support compared to conventional lactic acid bacteria."

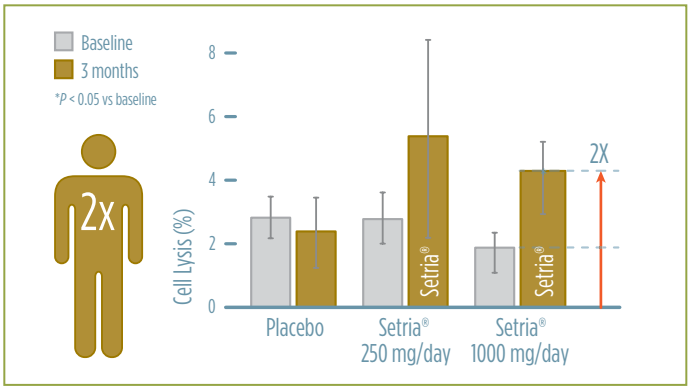
From a safety perspective, IMMUSE also has self-affirmed GRAS (Generally Recognized as Safe) status, which provides an extra level of safety.

Glutathione

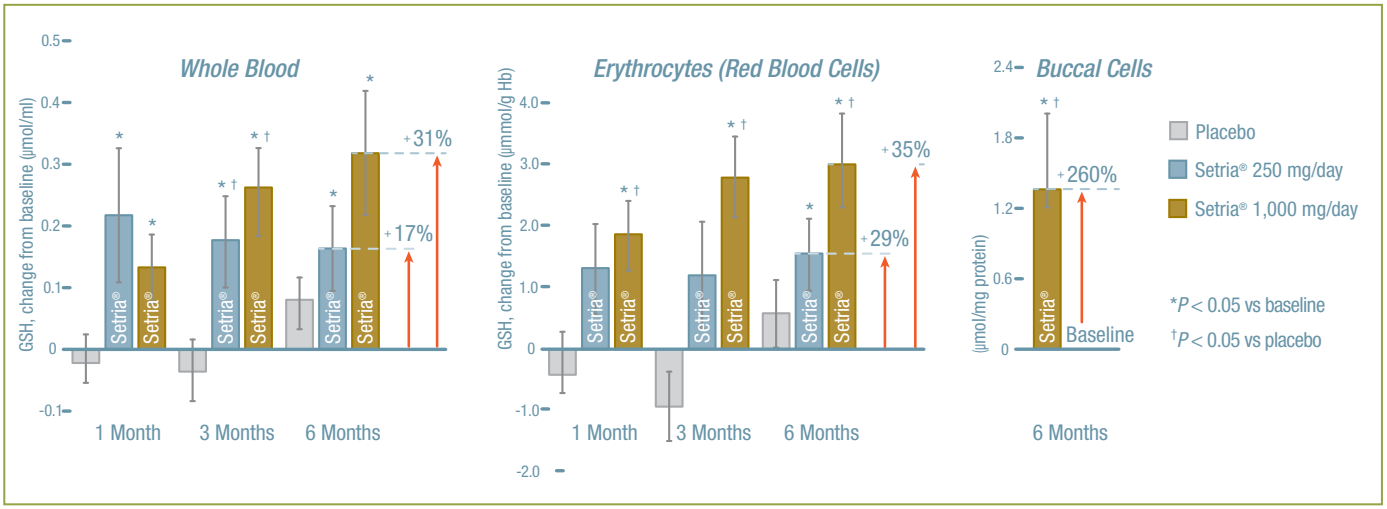
Glutathione is a tripeptide composed of glutamate, cysteine, and glycine. Reduced glutathione is the form that has earned it the title of being the body's most important redox regulator and master antioxidant. In addition to being a potent antioxidant on its own, glutathione also regenerates other antioxidants, including vitamins C and E. Specific to the immune system, glutathione stimulates NK cell function and promotes healthy T-cells and other white blood cell activity. Glutathione is also essential for both innate and adaptive immune responses.

According to a 2009 review, impaired glutathione synthesis and/or deficiency is associated with increased risk of a broad range of conditions including neurodegenerative diseases such as Alzheimer's and Parkinson's, as well as other diseases of aging like cancer and cardiovascular disease. A 2019 review described glutathione's immune-stimulatory benefits in patients with HIV infection and cirrhosis of the liver.

NK CELL ACTIVITY AFTER GLUTATHIONE SUPPLEMENTATION



GLUTATHIONE LEVELS AFTER ORAL SUPPLEMENTATION



A 2011 review described glutathione's anti-inflammatory role in lung conditions including viral and bacterial infections, as well as sepsis. The authors concluded that glutathione "is not just an inhibitor of inflammation but also a regulator of innate immunity." Glutathione deficiency is also associated with a variety of lung illnesses including chronic bronchitis, chronic obstructive pulmonary disease, idiopathic pulmonary fibrosis, and cystic fibrosis.

In addition to immune stimulation, glutathione also supports optimal cell health by continuously squelching free radicals inside the cell and its compartments.

Oral Absorption

It was previously thought that the only way to increase glutathione levels in the human body was to supply its precursors and cofactors for production. Research now shows, however, that glutathione can be orally absorbed.

In 2015, John P. Richie, PhD and colleagues published data from their long-term, randomized, placebo-controlled trial analyzing oral glutathione supplementation in 54 healthy non-smoking adults. Glutathione levels were tested in whole blood, plasma, erythrocytes, lymphocytes, and exfoliated buccal mucosal cells at baseline and after 1, 3, and 6 months. Ratios of oxidized to reduced glutathione were calculated to evaluate redox status. After a 1-month washout period, levels were tested 1 last time, for a total of 4 time points.

Glutathione levels increased significantly from baseline in whole blood and erythrocytes at 3 months and 6 months at a dosage of 250 mg and 1,000 mg daily. After 6 months, the 250 mg daily dose increased glutathione levels by 17% in whole blood and by 29% in erythrocytes. At the 1,000 mg daily dose, glutathione levels were increased by 31% in whole blood, 35% in erythrocytes, and 250% in buccal cells at the 6-month time point.

In addition, NK cell cytotoxicity increased more than 2-fold from baseline to 3 months in the 1,000 mg daily group.

Regarding the issue that oral glutathione may suppress endogenous production, this study found that neither the 250 mg or 1,000 mg daily dosage inhibited the body's endogenous production of glutathione. Specifically, there was no change in the activity of the rate-limiting enzyme glutathione cysteine ligase.

The Reduced Form

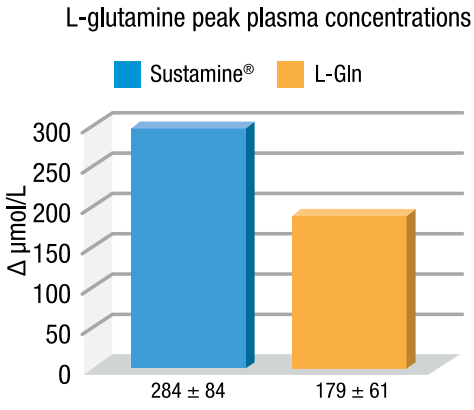
The form of glutathione used in the 2015 Richie absorption study was the reduced form that is manufactured using a patented fermentation process. The brand name of this clinically-studied reduced form of glutathione is Setria® manufactured by Kyowa Hakko USA, Inc.

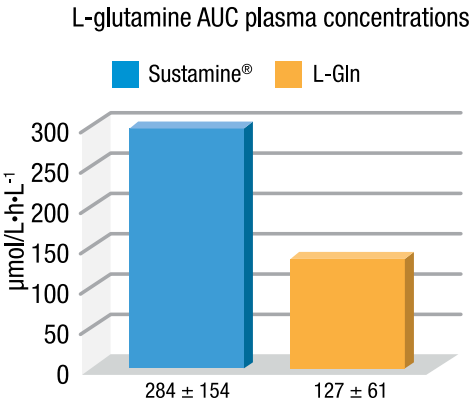
Setria is manufactured in compliance with Good Manufacturing Practice standards and meets specifications of the new USP monograph.

Dipeptide absorption research

A crossover study utilizing a placebo control measured plasma L-glutamine concentrations. The study compared L-glutamine absorption levels between water (the control), Sustamine® and straight L-glutamine (L-Gln) and measured both mean peak increase and mean area under the curve (AUC) levels.

- Sustamine® showed statistically significant increases in L-glutamine peak plasma absorption levels
- Sustamine® maintained L-glutamine levels in the plasma longer (up to 4 hours) than L-Gln (up to 2 hours)
- Mean AUC measurements revealed statistically higher plasma L-glutamine levels for Sustamine® than L-Gln





L-glutamine and l-alanyl-l-glutamine

L-glutamine is the most abundant amino acid in the body and is a primary source of metabolic fuel for enterocytes, lymphocytes, macrophages, and fibroblasts. It is also essential for glutathione production. l-glutamine is converted to glutamate, which is then enzymatically linked to glycine and cysteine to form glutathione.

Generally, there are high concentrations of l-glutamine circulating in the bloodstream, but it can become quickly depleted in times of high physiological need, such as traumatic injury, surgery, critical illness, or even strenuous exercise.

In addition to being an important fuel source for key immune system cells, l-glutamine plays a critical role in gut immunity by protecting epithelial tight junction integrity. It also helps regulate, repair, and maintain gut barrier function.

Enhanced Absorption

An ideal l-glutamine supplement should have optimal solubility, stability, and absorbability qualities. By combining l-glutamine with l-alanine, all of these qualities are significantly improved. Increasing the shelf-life and solubility allows for more versatile product formulations as well.

The l-alanyl-l-glutamine dipeptide requires less energy for digestion and absorption compared to longer peptide chains and is more efficiently absorbed than single amino acids. In fact, a 2012 study compared the absorption of l-alanyl-l-glutamine and an equivalent dose of l-glutamine, each dissolved in water, and found that the l-alanyl-l-glutamine led to higher peak plasma levels of circulating glutamine. Overall, l-alanyl-l-glutamine was absorbed up to 224% better than l-glutamine alone. Plasma levels were also maintained longer in those who consumed l-alanyl-l-glutamine.

Clinical Applications

Both l-glutamine and l-alanyl-l-glutamine offer immune health benefits. From a clinical standpoint, l-glutamine is best used in powder formulations, while l-alanyl-l-glutamine is more ideal for liquid formulations because of its stability.

Human clinical trials involving patients with multiple trauma, acute pancreatitis, and those undergoing surgery have shown that l-alanyl-l-glutamine can improve clinical outcomes. L-alanyl-l-glutamine has also been shown to enhance immune health in patients with HIV/AIDS. In most of the studies, l-alanyl-l-glutamine was added to Total Parenteral Nutrition therapy or as a stand-alone IV therapy.

To date, there have not been any clinical trials done that combine these 3 ingredients, however, given their varying mechanisms of action combining them may make sense.

L-alanyl-l-glutamine is available as a dietary supplement and has GRAS status, providing added confidence that it is a safe therapeutic ingredient.

In addition to immune stimulation, l-alanyl-l-glutamine has been shown to support rehydration, exercise performance, intestinal mucosal integrity, and intestinal barrier function.

Targeted Immune System Support

All 3 of these nutrients are branded ingredients that are available alone or in combination with other ingredients in dietary supplements and foods. To date, there have not been any clinical trials done that combine these 3 ingredients, however, given their varying mechanisms of action combining them may make sense.

"When it comes to keeping immune cells healthy, combining LC-Plasma with glutathione may have synergistic effects," said Dr. Ichikawa. "The glutathione will help with ongoing immune maintenance and the LC-Plasma will encourage optimal immune system activation."

Utilizing targeted dietary supplements to optimize immune system function is a central component of an integrative approach to health. Fortunately, many dietary supplements are available to integrative providers to help enhance clinical outcomes. LC-Plasma, glutathione, and l-alanyl-l-glutamine all have clinical outcome data showing they are well worth considering when creating personalized immune-supportive protocols.

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