

Lister V® Product Information

Indication

Lister V is intended for use in the management of viral infections and impaired immune function.

Lister V is a medical food that must be used under the active or ongoing supervision of a physician. Medical foods are developed to address the different or altered physiologic requirements that may exist for individuals who have distinctive nutritional needs arising from metabolic disorders, chronic diseases, injuries, premature birth associated with inflammation and other medical conditions, and pharmaceutical therapies.¹

Viral infections are the most common diseases afflicting humans. The effectiveness of the immune response to act as a defense against these infections and the cellular damage associated with them depends on an efficient mechanism of communication between the immune system and the neuroendocrine system. The immune system is linked to the central and peripheral nervous systems by the activity of neurotransmitters which facilitate the exchange of information between these systems through propagation of electrical impulses over specific neural pathways. These pathways create a bi-directional communication network through which information is transmitted to the neuroendocrine system from activated immunocytes at the site of infection triggering the release of immunomodulatory amino acids and peptides which modulate further activity of the immune system. **Lister V** is designed to provide a balance of neurotransmitters which support the communication between the immune system and the neuroendocrine system that is necessary to protect against cellular damage due to viral infections.

Ingredients

Lister V is a patented blend of neurotransmitter precursors and neurotransmitters (L-arginine, choline bitartrate, L-glutamine, L-lysine, L-cysteine, L-histidine); stimulators of precursor uptake (cinnamon bark); modulators of precursor utilization (L-lysine, L-cysteine); polyphenolic antioxidants (grape seed extract, green tea extract, cinnamon bark, cocoa extract); anti-inflammatory amino acids and precursors of anti-inflammatory molecules (L-histidine, L-lysine, L-cysteine, L-glutamine); immunomodulatory peptides (whey protein hydrolysate) and herbs (echinacea); adenosine antagonists (caffeine, cocoa extract); and an inhibitor of the attenuation of neurotransmitter production associated with precursor administration (grape-seed extract). **Lister V** is also a source of zinc which functions as a neural messenger, antioxidant, immunomodulator, and cofactor in numerous enzymatic reactions critical to immune function. The neurotransmitters and neurotransmitter precursors in **Lister V** have been carefully selected based on scientific support for their roles in the synthesis and activity of the specific neurotransmitters involved in regulating the immune response. These roles are summarized in this monograph in the section *Scientific Support for Use of Lister V in Management of Viral Infection and Impaired Immune Function*. The other ingredients in the formulation are functional components of the *Targeted Cellular Technology*[®] system.

¹ As defined in the guidelines issued by the Center for Food Safety and Nutrition, United States Food and Drug Administration (FDA).

All of the ingredients included in **Lister V** are classified as generally recognized as safe (GRAS) by the United States Food and Drug Administration (FDA). To qualify for GRAS status, a substance that is added to a food, including a medical food, has to be supported by data demonstrating it is safe when consumed in amounts obtained from these foods as they are typically ingested or prescribed.

Targeted Cellular Technology®

Lister V has been formulated using *Targeted Cellular Technology* (TCT), an integrated molecular system that facilitates the uptake and utilization of neurotransmitter precursors by target cells within the nervous system. This 5-component patented system consists of (1) specific neurotransmitter precursors; (2) a stimulus for the neuronal uptake of these precursors by specific neurons; (3) an adenosine antagonist that blocks the inhibitory effect of adenosine on neuronal activity (adenosine brake); (4) a stimulus to trigger the release of the required neurotransmitters from targeted neurons; and (5) a mechanism to prevent attenuation of the precursor response, a well known phenomenon associated with precursor administration.

Use of *Targeted Cellular Technology* improves the metabolic efficiency of neurotransmitter synthesis, thereby reducing the amounts of precursors needed to correct neurotransmitter imbalances. Use of *Targeted Cellular Technology* also ensures that the appropriate amounts of neurotransmitter precursors are delivered to the target neurons with the appropriate timing. As such, *Targeted Cellular Technology* synchronizes the availability of the precursor supply with the fluctuating demand for the corresponding neurotransmitters, which is especially important for processes that are regulated by circadian rhythms and are therefore sensitive to the timing of the synthesis and release of neurotransmitters such as acetylcholine, serotonin, nitric oxide, and histamine (1-5).

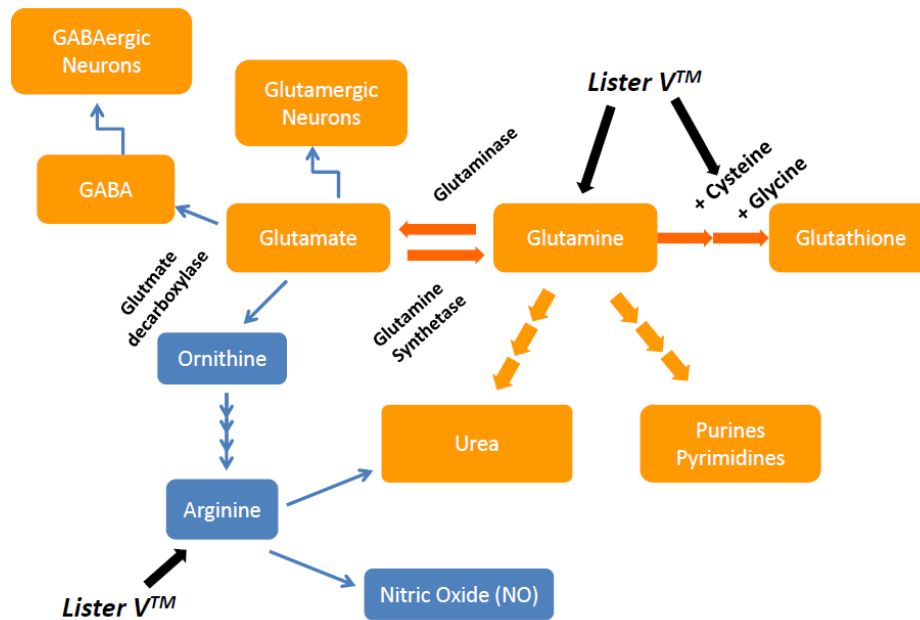
Previous attempts to provide an exogenous source of precursor amino acids and other biogenic amines in the quantities required to support neurotransmitter synthesis for individuals with specific needs necessitated that large amounts of amino acids be added to the formulations. For patients whose precursor requirements were considerably higher than normal, the amounts of exogenous amino acids that were needed were not practical to consume on a daily basis. Moreover, ingestion of large quantities of amino acids increases the potential for adverse effects. Metabolic efficiency is also decreased when large amounts of amino acids are delivered to the cells at one time because intestinal membrane transport receptors would be rapidly saturated resulting in a reduction in fractional amino acid absorption and thus attenuation of the tissue response to the supplemental amounts provided. Improving metabolic efficiency in uptake and utilization of neurotransmitter precursors by target neurons using *Targeted Cellular Technology* allows ingestion of smaller amounts of amino acids to elicit the same response as larger amounts, making daily dosing more feasible and reducing the potential for tolerance. Unlike pharmaceutical products which are not innate components of the processes that modulate the immune response and thus may lose their effectiveness in a relatively short period of time, the effectiveness of **Lister V** is not attenuated.

Metabolism

Lister V is a source of amino acids, biogenic amines, and other nutrients for patients with viral infections and impaired immune function. These patients require additional amounts of arginine, choline, glutamine, histidine, cysteine, and lysine to restore homeostasis. Under normal physiological conditions, these nutrients are considered nonessential because endogenous synthesis is sufficient to satisfy metabolic demand. When needs are altered by conditions that increase metabolic demand, the usual rate of synthesis is no longer sufficient and these nutrients become conditionally essential, requiring that supplemental amounts be consumed. Histidine has also been considered a nonessential amino acid for adults because blood levels can be maintained by breakdown of skeletal muscle and hemoglobin; however, there is no evidence of de novo histidine synthesis in mammalian tissues and thus an exogenous supply is important, especially during times of increased need to preserve muscle mass and plasma hemoglobin concentration. In contrast to nutrients which are nonessential under normal conditions, lysine is an essential amino acid which must always be provided in the diet, but the amounts needed are increased when the requirement for arginine is increased.

Glutamine. Under normal conditions, glutamine is synthesized from glutamate in virtually all tissues by the addition of an amino group (Figure 1). The primary function of glutamine is as a carrier of amino groups that are utilized in the synthesis of numerous compounds such as urea, which is produced in large amounts to dispose of excess nitrogen waste when protein catabolism is increased. Glutamine is also required for the synthesis of purines and pyrimidines utilized for nucleic acid synthesis and therefore demand is increased by the rapid rate of lymphocyte proliferation in response to an immune challenge. In addition, glutamine has neurotransmitter activity at N-methyl-D-aspartate (NMDA) receptor sites in the brain. It also contributes to the neurotransmitter pool through deamination to regenerate glutamate, the major excitatory neurotransmitter of the central nervous system, and is a precursor of GABA, the major inhibitory neurotransmitter of the central nervous system.

Figure 1. Competing Pathways of Glutamine Metabolism



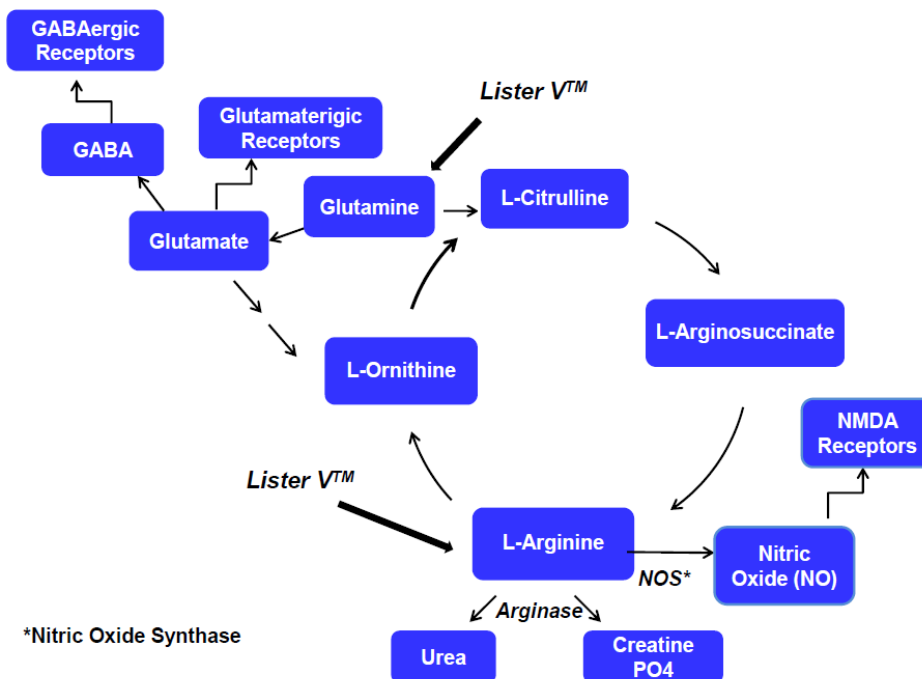
If glutamine intake is not sufficient, then the endogenous supply will be depleted and synthesis of glutathione and nucleic acids will be compromised. A decrease in the supply of glutamine will draw on the available supply of glutamate and will thus compromise functions dependent on glutamate. **Lister V** improves metabolic efficiency by providing supplemental glutamine to ensure that there is a sufficient supply of this amino acid, thus sparing glutamate for other important metabolic roles such as contributing to the tissue arginine pool and to the synthesis of glutathione (γ -glutamylcysteinylglycine). Glutathione is a potent intracellular antioxidant which prevents cellular damage from the reactive oxygen species produced by cytotoxic immune cells to facilitate viral destruction.

Cysteine. Cysteine is a conditionally essential amino acid which can normally be synthesized from methionine and serine in a transsulfuration reaction, thus an exogenous source is usually not required to satisfy demand. Cysteine is utilized as a precursor of acetyl CoA and for production of glutathione, taurine, and inorganic sulfate. The largest proportion of the cysteine body pool is used for production of glutathione. Cysteine is the rate-limiting substrate in glutathione synthesis, thus intake must be sufficient to ensure that sufficient amounts of glutathione are produced. Supplemental cysteine becomes important under conditions where glutathione demand is increased. **Lister V** provides cysteine to support glutathione synthesis during cellular cytotoxic activities when antioxidant protection becomes increasingly more important. Cysteine also has specific effects that contribute to cell-mediated immunity.

Arginine. The metabolic pathways which generate arginine are also normally sufficient to ensure an adequate supply of this amino acid. Because arginine can be synthesized from glutamine and glutamate, it is not considered an essential nutrient. A critical role of arginine is as a precursor of nitric oxide, a

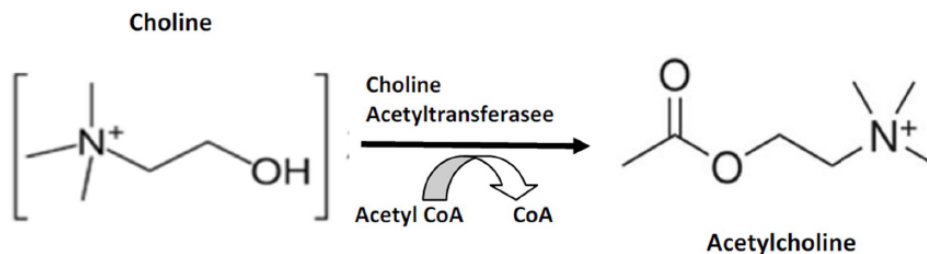
neurotransmitter which also functions as a vasodilator, immunomodulator, and intercellular/intracellular messenger. Arginine is also utilized as a precursor of polyamines, urea, and the high-energy storage compounds creatine and creatine phosphate (Figure 2). When the demand for nitric oxide is increased, arginine is diverted from the synthesis of these other compounds. To compensate for the decrease in arginine available to these competing pathways, glutamate and glutamine are converted to ornithine and citrulline which are then utilized by the urea cycle for synthesis of arginine. Consequently, a prolonged inadequate intake of arginine can eventually deplete glutamate and glutamine reserves. **Lister V** improves metabolic efficiency by ensuring that there is a sufficient amount of arginine available for production of nitric oxide while also ensuring that the demands of competing pathways are met. An adequate supply of arginine is also necessary to prevent depletion of the glutamate body pool which maintains a balance in the production of nitric oxide and GABA. By maintaining plasma and tissue glutamate levels, supplemental arginine also ensures that sufficient amounts of glutamine are available to meet cellular needs when demand is increased

Figure 2. Competing Pathways of Arginine Metabolism



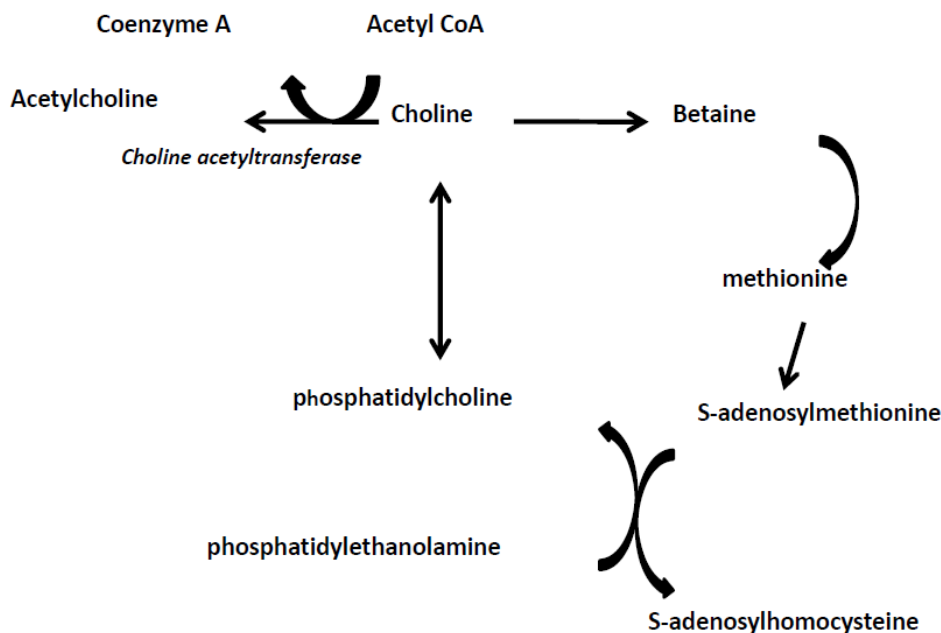
Choline. Choline is also considered a nonessential nutrient under normal physiological conditions. When the demand for choline is increased to supply additional precursor for synthesis of acetylcholine, supplemental amounts of choline are needed. Acetylcholine is produced from choline in an acetylation reaction catalyzed by choline acetyltransferase with acetyl coenzyme A (CoA) as the acetyl group donor (Figure 3).

Figure 3. Biosynthesis of Acetylcholine



The primary source of choline normally utilized in the synthesis of acetylcholine is phosphatidylcholine (lecithin), a membrane phospholipid which serves as a reservoir to supply choline for short-term needs (Figure 4). When the demand for acetylcholine exceeds the amount of choline that can be supplied by the hydrolysis of membrane phosphatidylcholine, dietary choline becomes an increasingly more important source. **Lister V** provides additional amounts of choline to meet the increased needs for acetylcholine if demand is elevated for an extended period of time. By supplying an exogenous source of choline, **Lister V** prevents the depletion of membrane phosphatidylcholine, thus preserving the structural integrity of the cell.

Figure 4. Sources of Acetylcholine



Lysine and Histidine. Lysine and histidine are both essential amino acids which must be consumed in increased amounts when demand is increased to meet the needs of the immune system. Because these amino acids cannot be synthesized endogenously, the amounts consumed determine the amounts available for competing pathways of utilization. One of these pathways is the synthesis of connective tissue in which lysine is incorporated into collagen and elastin fibers where it undergoes posttranslational hydroxylation to allow formation of cross-linkages between fibers. These linkages give connective tissue

the tensile strength that determines the integrity of scar tissue which protects wounds from infection and facilitates healing. Lysine also regulates intracellular levels of arginine by competing for binding sites on a common membrane transporter resulting in decreased cellular uptake. In addition, lysine is a precursor of carnitine, a substrate utilized in the synthesis of acetyl-L-carnitine which promotes the synthesis and activity of acetylcholine. Lysine is provided by **Lister V** to support tissue repair and wound healing, maintain intracellular arginine levels, and enhance acetylcholine-dependent cellular activities. Histidine is provided by **Lister V** as a precursor of histamine which is synthesized and released by neurons and by lymphocytes, basophils, and mast cells. The immunoregulatory activities of histamine depend on the particular tissue receptor subtype that is activated.

Zinc. Zinc is also an essential nutrient which is incorporated as a structural component into tissue proteins or involved as a cofactor for more than 300 enzymes including those important to the activities of the immune system. Zinc is also responsible for synthesis of immunoregulatory proteins and thus is essential for maintaining normal immune function. Most transcription and replication factors are zinc-dependent, thus rapidly proliferating cells such as immunocytes are especially sensitive to zinc status and the numbers produced are diminished in the absence of zinc. **Lister V** provides supplemental zinc to ensure that adequate amounts are available to support the increase in zinc-dependent metabolic activity required by the immune system.

Dosage

The recommended dose of **Lister V** is 2 capsules taken 2 to 3 times daily. **Lister V** should be taken at the first signs of viral infection and continued for 5 days. **Lister V** should be taken at the onset of herpes-induced cold sores or incipient viral infection and continued until symptoms have disappeared. As with any medical food, the best dosing protocol should be determined by a physician based on assessment of individual needs.

There are no known interactions between **Lister V** and any medications or herbal supplements.

Patients taking pharmaceutical agents to treat other conditions may continue to take these medications with **Lister V**. These patients should be monitored by a physician and therapeutic doses modified based on clinical response. **Lister V** can be used with antiviral agents such as amantadine. Patients taking antiviral agents with **Lister V** should maintain the dosage of these drugs as directed by a physician.

The amounts of each ingredient consumed at the recommended doses of **Lister V** are presented in Table 1.

Table 1. *Lister V* Composition

Ingredient	mg/kg body weight¹
L-arginine	0.6 – 4.6
L-glutamine	0.4 – 3.1
L-histidine	0.4 – 3.1
L-lysine	0.4 – 3.1
L-cysteine	0.4 – 3.1
Choline bitartrate	1.0 – 7.7
Echinacea	0.2 – 1.5
Grape seed extract	0.2 – 1.5
Green tea extract	0.2 – 1.5
Cinnamon bark	0.2 – 1.5
Cocoa powder	0.2 – 1.5
Whey protein hydrolysate	0.6 – 4.6

¹ Dosing range of 2 capsules taken 2-3 times daily

Side Effects and Contraindications

As with any amino acid therapy, headache, nausea, or dry mouth may be experienced in some people after beginning treatment with **Lister V**. These symptoms are mild and temporary, and readily managed by increasing fluid intake. The development of side effects with use of **Lister V** can be minimized by careful titration of the dosage. All of the ingredients in **Lister V** are regularly consumed in amounts normally found in foods or dietary supplements; therefore development of an adverse reaction to **Lister V** is not expected.

Lister V is contraindicated in patients who may be hypersensitive to any component of an arginine-containing preparation. **Lister V** contains small amounts of L-arginine which has been associated with side effects when taken alone as a supplement. These effects are not observed at low doses when consumed from formulations containing other amino acids. The adverse effects associated with L-arginine supplementation also appear to depend on the dosage regimen being more frequently observed when taken as a single dose (6).

Side effects specific to oral supplementation with L-arginine have been reported at doses between 3 and 100 g/d, which is approximately 12 to 400 times the amount provided by **Lister V** in the recommended daily dose (126 mg/2 capsules). At doses of up to 15 g/d, L-arginine is generally well-tolerated. The most common adverse reactions have been observed at intakes of 15-30 g/d and include nausea, abdominal cramps, diarrhea, and vomiting. Some patients may experience symptoms at lower doses. Most of the side

effects associated with arginine supplements were reported at doses >9 g (>140 mg/kg), particularly when the total daily dose was >30 g/d (>174 mmol/d). Doses of 3 to 6 g rarely provoked side effects.

Patients testing positive for HIV-1 infection must not take arginine as a single supplement, but may take it in combination with lysine as provided in **Lister V**. Long-term safety studies have not been conducted with L-arginine. Because it may stimulate growth hormone (GH) production, pregnant and nursing women should avoid L-arginine supplementation. Individuals with renal or hepatic failure should exercise caution in the use of supplemental L-arginine. Oral supplements of arginine and citrulline can increase local nitric oxide production in the small intestine which may be harmful under certain circumstances.

The potential for adverse reactions to arginine obtained from **Lister V** is low. A 2-capsule dose contains 126 mg of L-arginine provided in a balanced formula with other amino acids (including lysine) and dietary factors be taken 2 to 3 times daily.

Abbreviations and Definition of Terms

The definitions for the abbreviations and terms referenced in this monograph are summarized in Table 2.

Table 2. Abbreviations and Definitions of Terms

Term/Abbreviation	Definition
Antigen-Presenting Cells	Prepare antigens for recognition by T-cells and B-cells; include monocytes/macrophages, Tc, NK cells
Antioxidants	Molecules or enzyme systems that inhibit injury to cells from reactive oxygen species
Cholinergic	Neurons that synthesize, package, and release acetylcholine
COX-2	Inducible form of cyclooxygenase, the controlling enzyme in the synthesis of proinflammatory prostaglandins; activated by macrophages at the site of inflammation
Cytokines	Intracellular immunoreactive proteins that bind to multi-unit receptors on surfaces of target cells to elicit a specific response; produced by all nucleated cells with the largest quantities activated immunocytes
Excitatory Neurotransmitters	Molecules released from presynaptic cells at terminal nerve endings that transmit action potentials between neurons by depolarization of postsynaptic cell membranes, which decreases the stimulus threshold for firing, and thus increases the frequency and rate of transmission
Glucocorticoids	Stress hormones secreted by the adrenal gland as the endproduct of the activation of the HPA axis; inhibits activation of the HPA axis by negative feedback
Glutamatergic	Neurons that synthesize, package, and release glutamate; neurons that contain storage vesicles for zinc
Glutathione	Potent cellular antioxidant synthesized from glutamate, cysteine, and glycine
HPA axis	Hypothalamus-pituitary-adrenal axis; hormone system that facilitates bi-directional communication between the neuroendocrine and immune systems
Inflammatory mediators	Modulate the inflammatory response; includes cytokines and prostaglandins

Term/Abbreviation	Definition
Inhibitory Neurotransmitters	Molecules released from presynaptic cells at terminal nerve endings that transmit action potentials between neurons by hyperpolarization of postsynaptic cell membranes, which raises the stimulus threshold for firing and thus decreases the frequency and rate of transmission
Neuropeptides	Polypeptides which function as neurotransmitters but are more widely diffused and have longer-lasting effects; may also function as hormones, e.g., prolactin, vasopressin
Neurotransmitters	Amino acids, biogenic amines, and other molecules that facilitate communication between the peripheral nervous system, spinal cord, and brain by generating a series of action potentials which are transmitted between neurons
NMDA receptor	N-methyl-D-aspartate; subfamily of glutamatergic receptors which require a co-agonist for activation
iNOS	Inducible enzyme isoform of nitric oxide synthase; catalyzes the synthesis of nitric oxide from arginine; induced by IFN- γ (gamma interferon) and TNF (tumor necrosis factor) released from activated immunocytes in response to viral infections
Prostaglandins	Inflammatory mediators synthesized from 20-carbon fatty acids by COX enzymes; may have proinflammatory (omega-6 fatty acids) or anti-inflammatory (omega-3 fatty acids)
Targeted Cellular Technology®	A patent-pending process that facilitates endogenous production, uptake, and utilization of neurotransmitter precursors

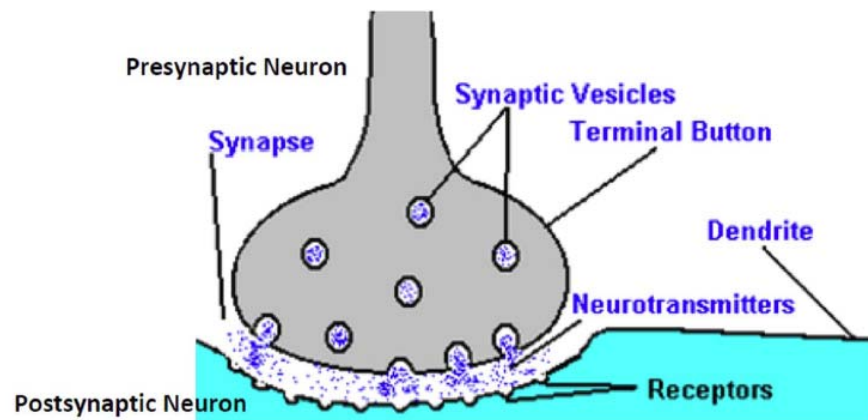
Mechanism of Action

Lister V has been formulated to provide a balance of neurotransmitters with well-defined roles in modulation of immune system responsiveness. Immune defenses are weakened by environmental factors that disrupt the communication network between the neuroendocrine system and the immune system and interfere with the intercellular links among immunocytes, resulting in disease. Homeostasis can be restored by supplemental amino acids and biogenic amines which support the activity of the neurotransmitters involved in the exchange of information between these systems. An intact communication network is an essential element of a healthy immune response.

Sympathetic nervous system activity in the immune response. The sympathetic nervous system is the primary channel of communication between the neuroendocrine and immune systems (6-9). Autonomic innervation of the thymus, bone marrow, lymph nodes, and spleen links the brain to lymphoid tissues. Through this pathway, the brain can influence lymphocyte development by transmission of signals to peripheral nerve endings which are distributed within specific cellular compartments of the spleen and lymph nodes, particularly within zones of T-cells and macrophages (9-11). The immunomodulatory activities of the endocrine system are dependent on sympathetic nervous system activity to stimulate the secretion of hormone-releasing factors such as corticotrophin-releasing hormone (CRH) and arginine vasopressin (AVP) from the hypothalamus, and adrenocorticotropin hormone (ACTH) and thyroid-stimulating hormone (TSH) from the pituitary gland (10). The binding of these neuropeptides to receptors in the adrenal cortex, thyroid, and thymus triggers the corresponding release of the respective immunomodulatory hormones, glucocorticosteroids, thyroid hormone, and zinc-thymulin.

Mechanisms of neurotransmitter activity. The mechanism by which information is passed between the sympathetic nervous system, endocrine organs, and immunocytes involves the coordinated activity of neurotransmitters. Neurotransmitters are amino acids, biogenic amines, or amino acid derivatives which function as mediators of physiological responses to physical, chemical, or electrical stimuli. Neurotransmitters are amino acids, biogenic amines, or amino acid derivatives which function as mediators of physiological responses to physical, chemical, or electrical stimuli. Neurotransmitters are released from storage vesicles in presynaptic neurons in response to action potentials at the distal nerve endings where they bind to receptors on postsynaptic neurons (Figure 5). Neurotransmitter binding alters the resting membrane potential of postsynaptic neurons generating an action potential which is transmitted to the terminal ending of the neuron where the sequence of electrochemical events is repeated until the signal reaches specific processing centers in the brain. The same mechanism of neurotransmitter-mediated electrochemical events is involved in transmission of output from the brain to target effector tissues or organs, and in transmission of signals originating within different regions of brain over the internal circuits between these regions.

Figure 5. Neurotransmitter Activity in Presynaptic and Postsynaptic Neurons



The rate of signal transmission between presynaptic and postsynaptic neurons in the central and peripheral nervous systems is dependent on the chemical nature of the neurotransmitter involved (11). Excitatory neurotransmitters released from presynaptic nerve terminals depolarize postsynaptic cell membranes which lowers the stimulus threshold for firing and increases the frequency and rate of transmission. Inhibitory neurotransmitters have the opposite effect of hyperpolarizing postsynaptic membranes which raises the stimulus threshold and decreases the frequency and rate of transmission. Although neurotransmitters can be classified as excitatory or inhibitory based on the primary effects they have on resting membrane potentials, these classifications do not always predict the response of the effector tissue or organ. Excitatory neurotransmitters can suppress a response by activation of inhibitory mechanisms and inhibitory neurotransmitters can activate a response by suppression of these mechanisms.

General roles of neurotransmitters. The primary neurotransmitters with immunomodulatory roles are acetylcholine, nitric oxide, glutamate, and histamine. Although not considered a neurotransmitter by conventional definition, zinc satisfies several of the criteria that define a neural messenger in that it is stored in synaptic vesicles, released upon membrane depolarization, and active at various receptor sites (12). Zinc is localized in glutamate-containing synaptic vesicles in the hippocampus suggesting that it might be a co-transmitter at glutamatergic receptors (13-14).

Glutamate is the major excitatory neurotransmitter of the central nervous system. Acetylcholine, nitric oxide, and histamine can exhibit both excitatory and inhibitory effects in the central and peripheral nervous systems depending upon the specific types and location of their postsynaptic receptors. Imbalances caused by deficiencies in one or more of the excitatory and inhibitory neurotransmitters, or changes in their binding affinities to postsynaptic receptors, will determine the intensity and duration of the signals transmitted (15-18).

Inflammatory mediators. The immune response is triggered when a virus or other antigens (e.g., malignant cells, foreign grafts) are detected by macrophages or other phagocytic cells. The interaction between viruses and immune cells stimulates the release of inflammatory mediators which bind to receptors on endocrine organs to complete the neuroendocrine-immune circuit. Inflammatory mediators also bind to receptors on other immune cells establishing a pathway for intercellular communication between immunocytes.

The primary inflammatory mediators involved in the immune response are cytokines and prostaglandins. Cytokines are comprised primarily of interleukins (IL), tumor necrosis factors (TNF α and β), and interferon (INF- γ), which are intracellular signaling proteins that bind to multi-unit receptors on the surface of target cells. Although they are synthesized by all nucleated cells, cytokines are produced in the largest amounts by activated immunocytes, specifically T-helper cells, monocytes/macrophages, and glial and dendritic cells (8). Prostaglandins function in a wide range of physiological activities which include modulation of the inflammatory response (7). The proinflammatory prostaglandins are produced from the omega-6 fatty acid arachidonic acid (C20:n4) by the inducible form of cyclooxygenase (COX-2) which is activated by macrophages at the site of inflammation (19-20). Some evidence suggests that COX-2 may also be expressed constitutively in small amounts in the central nervous system by glutamatergic neurons where it may have a role in glutamate-mediated neurotransmission (21).

The hypothalamus-pituitary-adrenal (HPA) axis. Data from experimental and clinical studies implicate the hypothalamus-pituitary-adrenal (HPA) axis as the primary hormonal link between the nervous system and immune system (10-11, 22). Dysregulation of the HPA axis is associated with impaired immune function and with blunted responsiveness of the immune system in patients with sepsis (10-11). The HPA axis is activated by inflammatory mediators through acetylcholine-mediated stimulation of CRH and AVP in the paraventricular nucleus of the hypothalamus. Both of these neuropeptides bind to receptors on the corticotroph cells of the anterior pituitary and stimulate the release of ACTH which is then transported to the adrenal cortex where it binds to receptors that promote the release of glucocorticoids.

The HPA axis is also activated by histamine and nitric oxide (19-23). Activation is terminated by the glucocorticoid binding to receptors in the hypothalamus which suppress CRH release and to receptors in the pituitary gland which suppress CRH-stimulated ACTH release (29). CRH and ACTH can also be secreted by peripheral neurons, but neuropeptides which are released peripherally do not have the same effects on immune function as the corresponding neuropeptides released in the hypothalamus (25).

Cytokine regulation of neuropeptide activity. Cytokines influence the release of immunomodulatory neuropeptides in the hypothalamus and pituitary gland involving both neural and circulatory routes. Signals originating from interactions between cytokines and peripheral neurons adjacent to the site of infection are sent from the periphery to the hypothalamus while cytokine stimulation of vagus afferent fibers has a direct effect on cholinergic receptors in the hypothalamus (7, 26). Cytokines transported in the blood can also directly stimulate neuropeptide and neurotransmitter release in the brain and pituitary gland. Blood-borne cytokines cross the blood brain barrier with the assistance of specific saturable transporters or through points of entry caused by damage from viral or bacterial infections (10, 27). In contrast, the pituitary gland is not protected by the blood brain barrier, and thus is readily accessible to blood-borne cytokines and more vulnerable to their effects (26).

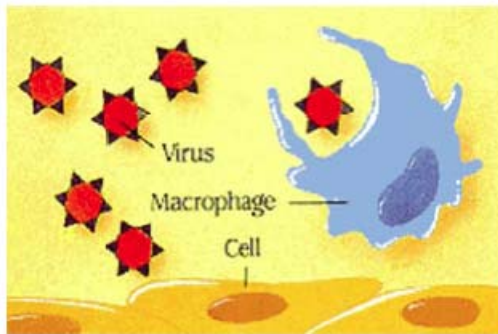
Neuroendocrine influence on the immune response. The pituitary gland, which is located at the junction between the autonomic nervous system and peripheral endocrine organs, has a critically important role in neuroendocrine homeostasis (22). The pituitary gland is a source of a majority of neuropeptides and hormones that modulate immune responses including GH, prolactin (PRL), thyroid stimulating hormone (TSH), and ACTH (26). The inflammatory mediator IL-1, which increases cholinergic activity in the hypothalamus and thus influences activity of the HPA axis, is also produced in the pituitary gland (28-29). Other cytokines secreted by the pituitary gland include TNF which activates the inducible isoform of nitric oxide synthase (iNOS) thereby increasing nitric oxide production.

Other endocrine organs which figure prominently in the immune response are the thymus and pineal gland. The thymus is the primary organ of the cellular immune system and the only source of thymulin, a zinc-dependent hormone which attracts stem cells to the thymus and controls the formation and differentiation of T-lymphocytes (22, 30-32). The production of thymulin is regulated by IL-1, glucocorticoids, GH, PRL, thyroid hormone, insulin-like growth factor, and nerve growth factor (10). The thymus is also a source of ACTH, GH, PRL, follicle stimulating hormone (FSH), leutinizing hormone (LH), and TSH. Innervation of the thymus gland by vagus fibers and the thoracic sympathetic chain provides a link between this organ and the autonomic nervous system (10, 22).

Thymic involution, which is associated with aging as well as a number of diseases of impaired immune function including HIV and AIDS, is due in part to a general decline in the function of the HPA axis and the loss of sympathetic innervation of the thymus gland and lymphoid (33). Thymic involution is also associated with altered function of the pineal gland which modulates cellular immunity through melatonin (N-acetyl-5-methoxytryptamine) production (10). Melatonin directly influences immune system activity by effects on zinc turnover which regulates the synthesis of zinc-thymulin (34). It also influences immunocyte activity through direct peripheral effects in addition to modulating GH and PRL secretion and the diurnal patterns of HPA activity (10).

Figure 6 illustrates the interactions among immunocytes in the processes involved in detection and destruction of viruses.

Figure 6. Viral Destruction by the Immune System



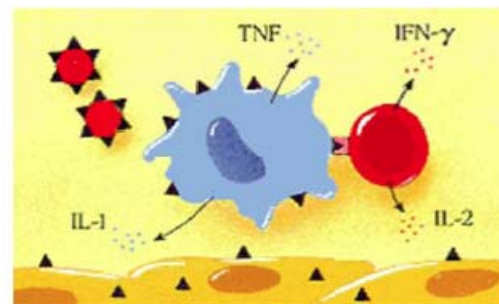
1. The immune response is initiated with detection of a virus by a macrophage which captures it and destroys it by phagocytosis. This process involves internalization and digestion of the virus. Viruses that escape these macrophages are available to infect nearby cells. Once viruses are localized intracellularly, they are accessible only to tissue macrophages and T-cells.



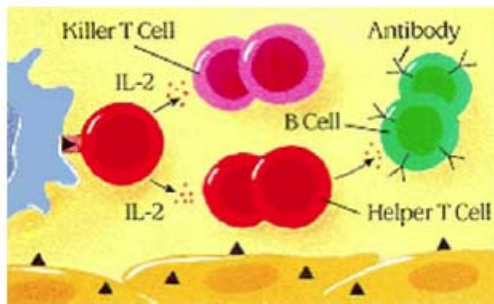
2. After the virus is digested, the macrophage functions as an antigen-presenting cell by displaying pieces of the virus on its surface as antigens.



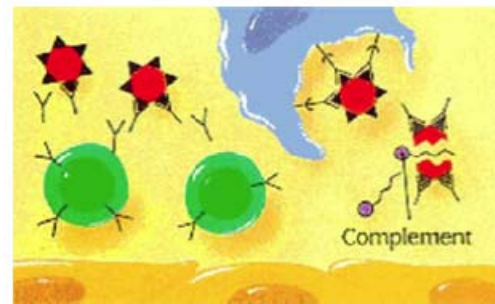
3. The surface antigens on the macrophage are then recognized by specific T-helper cells which bind at the antigen site.



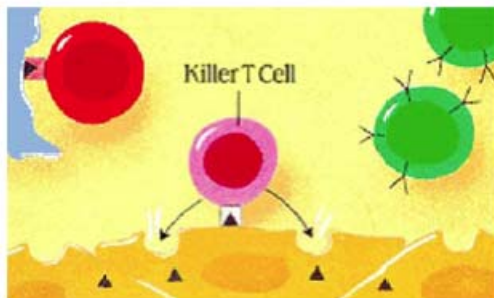
4. Formation of this bond stimulates production of cytokines which include interleukin-1 (IL-1), tumor necrosis factor-alpha (TNF- α) by the macrophage, and interleukin-2 (IL-2) and gamma interferon (IFN- γ) by the T-helper cell. These cytokines stimulate other immunocytes to initiate additional immune system activities that continue the process of antigen destruction.



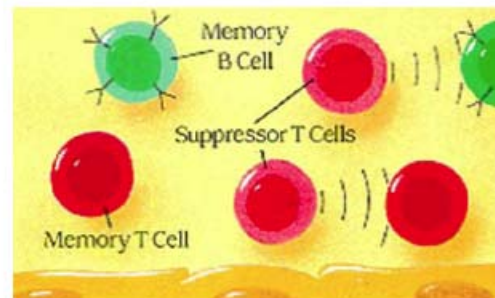
5. *IL-2 promotes the proliferation of T- helper cells and cytotoxic natural killer cells. The proliferating T-cells in turn promote the release of additional cytokines that stimulate proliferation of B-cells and production of immunoglobulins or antibodies by these B-cells.*



6. *The antibodies recognize the antigens on the surfaces of free-floating viruses and bind to them. The antibody-antigen complex facilitates destruction of the viruses by macrophages. This complex also activates the complement cascade which attracts leukocytes to the site of infection by chemotaxis. Complement also stimulates degranulation of mast cells to release histamine.*



7. *Host cells infected by viruses that have escaped capture by macrophages express antigens on their surfaces which are detected by natural killer cells. These cells release of cytotoxic substances such as nitric oxide and other reactive oxygen species which damage cell membranes and disrupt mitochondrial function resulting in necrosis and cell death.*



8. *Finally, as the infection is brought under control, T- and B-cells are inactivated by T suppressor cells. A few of these cells remain behind as "memory cells" which enable a more rapid response to subsequent exposure to the same virus.*

Scientific Support for Use of *Lister V* in Management of Viral Infections and Impaired Immune Function

The use of ***Lister V*** in the management of viral infections and impaired immune function is supported by experimental and clinical data which have identified specific roles for each ingredient in the neuroendocrine regulation of the immune response. Protection against cellular damage from viral infections requires a balanced production of acetylcholine, nitric oxide, glutamate, and glutathione which depends on the availability of choline, arginine, lysine, glutamine, and cysteine. Supplemental zinc is also

needed to support the accelerated rates of lymphocyte proliferation and differentiation that are key events in the initiation of the immune response.

Nitric oxide. Additional amounts of arginine are required by the immune system to support increased production of nitric oxide after exposure to an infectious agent. In addition to its role as a neurotransmitter, nitric oxide also mediates the regulation, proliferation, survival, and differentiation of neurons (12). Nitric oxide also has roles as intercellular and intracellular messengers in many tissues and in regulating transcription and DNA binding of $\text{nF-}\kappa\text{B}$, a transcription factor which controls cytokine synthesis (35). Diffusion of nitric oxide into the parvocellular cells of the hypothalamus activates COX-2 which stimulates the production of proinflammatory prostaglandins. These prostaglandins then act synergistically with nitric oxide to increase cholinergic, adrenergic, and histaminergic activity in the hypothalamus and stimulate the release of CRH and AVP which activates the HPA axis (19, 22-23, 26).

Induction of synthesis by pathogens. Nitric oxide synthesis is enhanced in the presence of pathogens which activate immunocytes to release the specific cytokines that stimulate the inducible isoform of nitric oxide synthase (iNOS). The release of IFN- γ and TNF from activated immunocytes is increased after exposure to a wide range of viruses including Herpes (36-37), adenovirus (38-40), HIV-1 (41-43), rhinovirus (44-45), rotavirus (46-47), and Coxsackie B3 (48-49). Increased replication of parainfluenza virus observed in the epithelial cells of patients with cystic fibrosis has been attributed to an inability of the affected cells to produce NOS (50). This possibility is supported by observations that overexpression of NOS or addition of a nitric oxide donor such as arginine are protective against increased viral replication in these patients. Although the constitutive isoforms of NOS expressed in neurons (nNOS) and endothelial cells (eNOS) also have roles in the immune response, only the inducible form of the enzyme is involved in inflammatory processes (23, 51-53).

Cytotoxic properties. Nitric oxide is a potent cytotoxic effector molecule which controls viral and bacterial growth, destruction and uptake, and demonstrates direct static and cidal effects on viruses, bacteria, fungi, and parasites (33). The cytotoxic properties of nitric oxide have been attributed to its characteristically high reactivity which can damage cellular DNA and thus inhibit cellular replication of pathogens. Although nitric oxide in large amounts is a potent inducer of apoptosis and necrosis in some cells, it provides powerful protection from cell death in controlled amounts (51, 54-56). Clinical outcome during infection is therefore determined by the balance in the production and degradation of nitric oxide (56-57). If excess nitric oxide is allowed to accumulate in tissues, it may combine with superoxide to form peroxynitrite or other reactive oxygen and nitrogen species which can inactivate cellular proteins or have other detrimental effects (52-55). Since nitric oxide is also a powerful vasodilator, high plasma concentrations can lead to severe hemodynamic instability (53-55). Chronic continuous exposure of the pituitary gland to blood-borne cytokines may lead to accumulation of nitric oxide in surrounding neurons resulting in apoptosis and reduced hormone secretion (22).

Homeostasis and immune activity. Nitric oxide production is closely regulated by competitive demand for the available supply of arginine between iNOS which increases nitric oxide production and arginase which decreases it (55). The competition for arginine by these enzymes appears to be a key mechanism in regulation of inflammatory processes (52, 56). A relative increase in arginase activity will divert arginine

from synthesis of nitric oxide to production of urea and ornithine (Figure 2). Patients with infection and sepsis show increased arginase activity which is likely responsible for the lower levels of nitric oxide observed for a given level of blood arginine in these patients (57-58). Supplementation with arginine and antioxidants can therefore restore nitric oxide production to levels consistent with a normal immune response (53). Patients with viral infections and compromised immune function should benefit from **Lister V** which is designed to enhance production of nitric oxide by decreasing arginase activity. Lysine is also added to **Lister V** to maintain balance in nitric oxide production by competing with arginine for sites on a common membrane transporter which modulates cellular uptake.

Acetylcholine. In addition to arginine, choline is needed in increased amounts by the immune system to satisfy the demand for acetylcholine which has multiple roles in the immune response. The cholinergic-mediated anti-inflammatory pathway is a physiological neuroimmune mechanism responsible for innate immune function and control of inflammation (59). Acetylcholine also mediates the effects of CRH and AVP on activation of the HPA axis, potentiates the activity of nitric oxide, and supports autonomic regulation of inflammation through its role as the primary neurotransmitter of the sympathetic nervous system (19-20, 57, 59-61). Activation of the sympathetic nervous system by inflammatory mediators released from activated immunocytes localizes inflammation within infected tissues to protect healthy cells from the detrimental effects of inflammatory processes (8, 25). Recent studies indicate that acetylcholine can also function directly as an immunomodulatory cytokine which prevents macrophage activation through a receptor-mediated anti-inflammatory pathway (62). In addition, acetylcholine can influence immune system activity by controlling cytokine production through inhibitory effects on nF-κB (63).

Histidine. Supplemental histidine can favorably affect immune function by contributing to histamine production in lymphocytes and leukocytes (27). Histamine regulates T-cell activity, enhances secretion of the proinflammatory cytokines IL-1 and IL-6, and plays a role in activation of the HPA axis (19, 22, 27). The diversity of histamine effects on immune function may be explained by the different receptor subtypes expressed in various tissues. The H3 receptor, which is expressed by all immune cells except T and B lymphocytes, regulates antigen-presenting capacity and proinflammatory activity, and also controls neurogenic inflammation through local feedback loops between neurons and mast cells.

Glutamine. Glutamine is the primary respiratory fuel utilized by lymphocytes, macrophages, and neutrophils and thus supplemental amounts are needed in inflammatory states when immune cell proliferation and activities are increased (64-69). Glutamine modulates the inflammatory response by attenuation of inflammatory cytokine production (66). It also functions as a neurotransmitter at glutamate-dependent NMDA receptor sites in the brain. In addition, glutamine is utilized as a substrate in the synthesis of glutathione which is an antioxidant needed in increased amounts to protect immunocytes from elevated levels of oxidative stress associated with high rates of cytotoxic activities (65, 70). The absolute counts of T-helper cells (CD4⁺) have been directly correlated with intracellular glutathione concentration. Under some conditions, glutathione in its nitrosylated form (S-nitrosoglutathione) can also serve as an intracellular reservoir of nitric oxide. Cellular glutathione levels are dependent on the available supply of cysteine, the rate-limiting substrate in glutathione synthesis (70-71). Cysteine also has a direct

influence on immune function involving support for IL-2-dependent T-cell proliferation and decreased synthesis of nF-κB transcription factors (71).

Zinc. Zinc exhibits multifaceted effects on immune function including enzyme activation, cellular proliferation and apoptosis, cytokine expression, and activation of thymulin (30-31, 74-75). Zinc also has a stabilizing effect on DNA and preserves antioxidant enzymatic activities (31-32, 72). Other functions of zinc include activity as a transcription cofactor which stimulates lymphocyte proliferation, replication and differentiation, and as a cofactor for enzymes and hormones important to cell-mediated immunity (30, 73). Gene expression which is regulated by zinc impacts signal transduction, responses to various stressors including oxidative stress, and growth and energy utilization. Among the zinc-dependent genes identified which are involved in the immune response are those that regulate the synthesis of lymphocyte-specific protein tyrosine kinase, T-cell cytokine receptors, and the DNA damage repair and recombination protein-23B (76).

Zinc also promotes efficient communication between the neuroendocrine and immune systems and supports the interactions between the thymus gland and other components of these systems (31). In addition, the synthesis of nitric oxide and proinflammatory prostaglandins are also dependent on the availability of an adequate supply of zinc. Zinc depletion impairs all of the functions of monocytes, decreases cytotoxicity in NK cells, and reduces phagocytosis in neutrophil granulocytes. T-cell functions are also impaired In a zinc deficiency and B-cells undergo apoptosis (77).

The strongest evidence supporting a role for zinc as a neuromodulator is its association with the neurotransmitter activity of glutamate (13). Zinc ions are concentrated in the vesicles of specific glutamatergic terminals in the mammalian forebrain. The selective association of zinc with glutamate-containing synaptic vesicles suggests that it might be a co-transmitter at glutamate receptors (12). It is believed that during synaptic transmission, zinc is released from these vesicles and binds to receptors on the pre- or postsynaptic membranes (13). The finding that zinc chelators can double the amplitude of baseline NMDA responses suggests that zinc acts as an antagonist co-transmitter at the NMDA receptors (12, 14).

A summary of the roles of each of the ingredients in *Lister V* is presented in Table 3.

Table 3. Roles of *Lister V* Ingredients in Management of Viral Infections and Impaired Immune Function

Ingredient	Effector Molecule	Function	Role
Arginine	Nitric Oxide	Inhibitory and excitatory neurotransmitter; immunomodulator; cytotoxic effector	Functions as an intercellular and intracellular messenger; regulates DNA binding and transcription of nF-κB, functions as a transcription factor for cytokine synthesis; stimulates production of anti-inflammatory prostaglandins by activation of COX-2; activates HPA axis

Ingredient	Effector Molecule	Function	Role
Glutamine	Glutamine	Respiratory fuel Amino group transporter	Attenuates inflammatory cytokine production; precursor for glutathione synthesis; utilized for the synthesis of nucleic acids and urea; contributes to synthesis of arginine and glutamine; serves as a reservoir of nitric oxide under some conditions; increases CD4 ⁺ T-cell counts
	Glutamate	Excitatory neurotransmitter	Interacts with acetylcholine and zinc at glutamatergic receptors
	Glutathione	Antioxidant; Immunomodulator	Protects against cell membrane damage from cytotoxic activities
Histidine	Histamine	Excitatory neurotransmitter; Inflammatory mediator	Activates the HPA axis; regulates effects on antigen-presenting cells; influences T-cell regulation; enhances secretion IL-1 and IL-6; controls neurogenic inflammation at the H3 receptor.
Lysine	Lysine	Arginine uptake inhibitor	Modulates intracellular arginine concentration; regulates nitric oxide production; contributes to connective tissue tensile strength; utilized as a precursor of carnitine which enhances synthesis and activity of acetylcholine
Cysteine	Cysteine	Rate-limiting substrate for glutathione synthesis	Affects intracellular IL-2-dependent T-cell proliferation; decreases transcription factors for nF-κB.
Choline Bitartrate	Acetylcholine	Inhibitory and excitatory neurotransmitter	Acts as the primary neurotransmitter of the autonomic nervous system; activates the HPA axis; modulates circadian rhythms; potentiates nitric oxide activity; inhibits transcription factor nF-κB.
Zinc	Zinc Zinc-thymulin	Neural messenger; cotransmitter at glutamatergic receptors; enzyme cofactor; component of thymulin	Supports nitric oxide synthesis, lymphocyte proliferation, cytokine expression, monocyte function, NK cytotoxicity, and phagocytosis; promotes thymic T-cell differentiation and maturation through activation of thymulin
Cocoa Powder/ Cocoa Extract	Caffeine	Adenosine antagonist	Binds to adenosine receptors to disinhibit the adenosine brake which has an inhibitory effect on neuronal activity (78-79)
Echinacea	Echinacea	Immunomodulator	Influences phagocytosis and macrophage-derived cytokine concentrations; activates polymorphonuclear leukocytes and NK cells; affects numbers and activities of T- and B-cells; reduces the incidence and duration of the common cold and prevents symptoms after clinical inoculation (80-81)
Grape seed extract	Polyphenols	Antioxidant	Preserves receptor membrane integrity and prevents attenuation of responses to neurotransmitter precursors (82-83)

Ingredient	Effector Molecule	Function	Role
Green Tea Extract	Polyphenols	Immunomodulator Anti-inflammatory	Decreases production of nitric oxide and TNF- α ; modulates gene expression of COX-2; exhibits neuroprotective activity (83-84)
Cinnamon Bark	Cinnamaldehyde 2-methoxy-cinnamaldehyde	Inhibition of osteoclastogenesis	Reduces osteoclast-like cell formation; inhibits NFATc1 (nuclear factor of activated T-cell 1) (85)
Whey Protein Hydrolysate	Lactoferrin Lactoglobulin Lactalbumin Cysteine Glutamine	Immunomodulator Antioxidant Anti-inflammatory	Contributes to the supply of glutamine and cysteine for production of glutathione; provides antioxidants for protection against cellular damage from reactive oxygen species (86)

Nutritional Requirements of Immune Function

The nutritional requirements of most interest to patients with viral infections and impaired immune function are the nutrients and dietary factors that support the synthesis and activity of immunomodulatory neurotransmitters (arginine, glutamine, histidine, lysine, choline), promote cellular proliferation (glutamine, zinc), modulate the inflammatory process (arginine, glutamine, choline, histidine, zinc), protect against cellular damage from free radicals (arginine, lysine, glutamine, cysteine, choline), and facilitate intercellular communication among immunocytes (arginine, choline, zinc). Because the scope of nutrient-dependent immune activities is so broad, the immune system is highly sensitive to nutrient intakes so that tests of immunocompetence are widely used in clinical settings to assess nutritional status. Improvements in key indicators of immune function and reductions in morbidity and mortality have been demonstrated in patients with malnutrition and infectious disease who received supplemental amounts of arginine, glutamine, and cysteine (87-88); however, the most pronounced effects of supplemental intakes of any nutrient on immune status are observed in patients who show evidence of absolute or relative deficiencies of the particular nutrients (89-93).

Neurotransmitter balance. *Lister V* is formulated to provide the optimum balance of amino acids, antioxidants, and zinc to maintain neuroendocrine-immune homeostasis using *Targeted Cellular Technology* to control the timing of the release of these ingredients. Homeostasis ensures a strong immune response which not only protects against infection but also the cellular damage associated with it. Balance in the production and release of neurotransmitters is important to neurotransmission because it is the highly integrated functions and complexity of the multiple feedback loops between them that determine the net input received by the brain. These interactions explain why an imbalance in the intake of a nutrient or dietary factor which supports the synthesis or activity of any one neurotransmitter can influence the activities of the others, potentially inducing absolute and relative deficiencies (94-96). It also explains why both excess and deficient intakes of a nutrient or dietary factor can have similar adverse effects on immune function (76, 96-98). For example, alterations in zinc-dependent functions observed in a zinc deficiency are similar to those observed with excess zinc intake (76, 98).

Nutrient requirements in disease. The concept that nutrient requirements are modified by disease has been recognized for more than 30 years and is supported by studies which have shown changes in plasma, urinary, and tissue levels of nutrients associated with abnormalities in physiological endpoints reflective of specific pathologies (99). These requirements can be estimated by determining the level of intake at which a physiological response is improved indicating that the balance between intake and metabolic demand has been favorably modified. The nature of the pathological characteristics of a disease will determine the relative amounts of nutrients needed to restore balance between intake and demand. (2, 68, 100-109). The degree of coordination between the activities of different neurotransmitters is an important consideration in assessing the amounts of dietary precursor needed (100-101, 106-113).

Diseases with pathologies that involve imbalances in neurotransmitters will increase the requirements for certain amino acids and other dietary precursors to restore homeostasis (2, 99-104). For most of these amino acids and dietary precursors, uptake by target neurons is a concentration-driven process; therefore, intakes must be sufficient to increase the extracellular to intracellular concentrations to levels high enough to drive a rapid rate of uptake (100-102, 112-114). The rate of precursor uptake by target neurons is important to neurotransmitter synthesis because the enzymes involved are found only in these neurons and thus the amount of substrate available is the limiting factor in neurotransmitter production (100-101, 115-116). As blood levels of dietary precursors rise in response to increased intakes, the concentration-driven rate of precursor uptake by target neurons is increased, making more substrate available for neurotransmitter production and subsequent release (103-104, 111-112, 116-118). Changes in intakes of the dietary precursors of these neurotransmitters will therefore influence physiological responses by affecting neurotransmitter availability (91, 94-96, 100-108, 110-118). Uptake of dietary precursors such as arginine which depend on membrane transporters are less affected by extracellular concentrations. For these nutrients, competitive interactions with other nutrients will have a greater effect such as observed between arginine and lysine which bind to the same sites on a common membrane transport carrier (119-121).

A large body of peer-reviewed published data supports the basis for increased requirements of choline (100, 121-123), arginine (4, 55, 124-128), glutamine (91, 128), and histidine (129-130) in conditions which depend on neurotransmitter balance. Decreased blood levels of certain amino acids have been observed in patients with infections and with conditions such as fibromyalgia despite maintaining their usual protein intake indicating that the needs for these amino acids are selectively increased in these patients (90, 128, 131-132). This observation may be explained by the competitive demands for certain amino acids by different metabolic pathways which decrease the supply of neurotransmitters available to function in the other processes (See Section on *Metabolism* in this monograph).

Nutrient effects on neurotransmitter availability. Certain physiologic and biochemical mechanisms must exist in order for nutrient consumption to affect neurotransmitter synthesis (116). These conditions are listed below. The extent to which neurotransmitter synthesis in any particular aminergic neuron is affected by changes in precursor availability will vary directly with the firing frequency of the neuron. Consequently, precursor administration can produce selective physiologic effects by enhancing neurotransmitter release from some but not all of the neurons potentially capable of utilizing the precursor

for the particular effect. It is also useful in predicting when administering the precursor might be useful for amplifying a physiologic process, or for treating a pathologic state.

Conditions that Support Effects of Dietary Precursors on Neurotransmitter Synthesis

1. Absence of significant feedback control of plasma precursor levels
2. Ability of plasma precursor levels to control influx into or efflux from the central nervous system
3. Presence of a low-affinity (unsaturated) transport system mediating the flux of precursor between blood and brain
4. Low-affinity kinetics of enzyme that initiates conversion of precursor to neurotransmitter
5. Lack of in vivo end-product enzyme inhibition by the neurotransmitter

Several key clinical studies in immunosuppressed individuals (e.g., burn patients, individuals with cancer and HIV infection, and those undergoing surgery or who have experienced major traumas) have tested the hypothesis that supplemental arginine and/or glutamine are beneficial to immune function and clinical outcome (128). Plasma levels of arginine, glutamine, and choline, which are reduced in patients with infection and sepsis, returned towards normal as the infection subsided indicating that the demand for these nutrients had been increased (124, 129-136). The importance of an adequate intake of arginine for patients with infection is supported by the observation that a marked reduction in serum levels was predictive of mortality in these patients (53). The finding that patients surviving septic shock had higher plasma nitrate levels than non-survivors indicates that there is a critical need for nitric oxide in these patients and suggests that the incremental increase in demand for arginine imposed by this need would have to be satisfied by supplemental arginine in order to raise plasma levels sufficiently to increase nitric oxide production (126, 137)

Requirement for arginine. The primary determinant of plasma arginine levels and thus nitric oxide production is dietary arginine because endogenous synthesis does not increase sufficiently to compensate for depletion, increased turnover, increased requirement, or inadequate supply of the amino acid (51, 53, 55-56, 126-127, 138-141). Arginine requirements are therefore influenced by metabolic utilization and factors that affect rates of de novo synthesis (55, 126, 139-141). Utilization of arginine is increased by citrulline which upregulates iNOS and eNOS activity and by glutamate which increases iNOS and nNOS activity in the brain and other nervous system tissues (140). In a study of stable short-bowel patients, a decrease in plasma arginine levels was observed after 5 days of consuming an arginine-free diet and was accompanied by a decrease in levels of citrulline, indicating that synthesis of arginine from citrulline in the urea cycle had been increased, but the rate was not sufficient to maintain plasma arginine levels when intake was inadequate (54-55). Thus an increase in the demand for arginine to support increased nitric oxide synthesis would require an increase in arginine intake to satisfy the demand.

Since metabolic utilization of arginine impacts the dietary requirement for this amino acid, the need to support immune function during infection and sepsis would be expected to considerably increase this requirement (125). The decline in circulating arginine levels which is seen in patients with infection is similar to what has been observed in healthy humans consuming arginine-reduced diets indicating that the increased demands of infection induce a relative deficiency of arginine which can be reversed by increasing intake (4, 127-128). It has been demonstrated that arginine utilization and turnover is increased by infection and that formulations which provide supplemental arginine and restore blood arginine levels improve the clinical status of these patients (125-126, 136). A body of evidence also suggests that increased intake of arginine upregulates immune function and reduces the incidence of postoperative infection (53, 136, 141).

Requirement for glutamine. The arginine body pool is also influenced by intake of glutamine and glutamate which contribute to levels of ornithine and citrulline, intermediates of the urea cycle which can be converted to arginine (Figure 3 and Figure 4). Glutamine also plays a role in regulation of whole body arginine homeostasis through inhibitory effects on arginine utilization that reduce nitric oxide synthesis (55). As the primary donor of amino groups to arginine, glutamine also increases arginine utilization for synthesis of urea. Despite being the most abundant amino acid in the blood, glutamine levels are rapidly depleted by catabolic processes such as infection and injury. Endogenous glutamine synthesis and release from skeletal muscle is increased by as much as 2-fold during infection yet the intracellular pool is still depleted indicating that the rate of release exceeds the rate of synthesis (142).

Although a clearly defined glutamine deficiency syndrome has not been described, endogenous production is not sufficient to meet the increased and altered tissue demands imposed by trauma, sepsis, infection, and inflammation (142). Since most naturally-occurring food proteins contain 4% to 8% of their amino acid residues as glutamine, an average of less than 10 g of dietary glutamine is likely to be consumed daily. Studies in stressed patients indicate that considerably larger amounts of glutamine (20-40 g/day) may be necessary to maintain glutamine homeostasis (143). Treatment with exogenous glutamine has been found to be a highly effective approach for decreasing the incidence of infection in trauma and surgery patients, specifically in lowering the risk of post-surgical infections (64, 144).

Plasma glutamine levels do not appear to be affected by the increased release of glutamine from skeletal muscle during infection which suggests that glutamine availability is reduced by competition for uptake among tissues and thus becomes rate limiting for lymphocyte proliferation, phagocytosis, and antibody production (142-143). A decrease in availability of glutamine has been shown to impair antigen-presenting capability of monocytes, reduce phagocytic activity, decrease lymphocyte proliferative response to mitogens, and shift the Th₁/Th₂ ratio of T-helper cells to lower levels associated with a decrease in the cell-mediated immune response (65).

An increased demand for glutathione to provide antioxidant protection from byproducts of immunocyte cytotoxic activities increases the need for glutamine as well as cysteine. Supplemental glutamine maintains tissue glutathione levels while both amino acids support the activity of the glutathione peroxidase antioxidant enzyme system (70, 145). The improvements in immune function that have been reported in clinical trials in which glutamine and cysteine supplements were given have been attributed to

the antioxidant potential of these amino acids as precursors of glutathione and to their inhibitory effects on inflammation (63).

Requirement for choline. Acetylcholine is produced in the terminal endings of cholinergic neurons and in regions of the brain where choline acetyltransferase is concentrated. Under steady state conditions, the brain enzyme is not completely saturated, thus the rate of acetylcholine production is driven by the availability of choline and acetyl CoA (146-148). The rate of choline transport across the blood brain barrier is increased by an amount proportional to the increase in serum concentration and determines the amount of acetylcholine subsequently released from cholinergic neurons (146). Dietary choline is the primary contributor to plasma choline levels accounting for a greater proportion of the plasma concentration than de novo synthesis (147, 149-152). Elevated levels of plasma choline promote the expression of high affinity choline transporters on cholinergic neurons which regulate the synaptic availability of choline and facilitate the release of acetylcholine from these neurons indicating that exogenous choline is utilized by central cholinergic neurons as a substrate for acetylcholine synthesis (153-154). Synaptic acetylcholine levels are regulated by a negative feedback mechanism in which accumulation of the neurotransmitter inhibits transporter activity on cholinergic neurons to prevent further uptake of choline. Anticholinergic drugs such as chlorpromazine, atropine, and cholinesterase inhibitors decrease acetylcholine release by inhibition of these transporters (155-157).

In the brain, choline is incorporated into the membrane phosphatidylcholine pool and released when the demand for acetylcholine is increased; however, the appearance of choline in cerebrospinal fluid confirms that there is a pool of free choline in the brain (153-154). In a normal physiological state, most of the choline utilized for acetylcholine synthesis is obtained from hydrolysis of phosphatidylcholine (150-155, 158-160, 170). When demand for acetylcholine is increased over prolonged periods, dietary choline becomes an increasingly more important source of precursor (147, 151, 154, 158, 161-163). If a supplemental source of choline is not provided to meet these increased demands, loss of membrane phosphatidylcholine will eventually compromise cell membrane function and trigger apoptosis (147-148, 164).

Although serum choline levels are decreased by a choline-free diet, brain choline levels remain relatively stable indicating that the brain is given metabolic priority when the amount of free choline available is limited (153). Brain phosphatidylcholine levels decrease in parallel with the decrease in serum choline which further suggests that brain choline concentration is maintained within narrow limits at the expense of larger tissue pools of phosphatidylcholine and other phospholipid precursors (serine and ethanolamine) (150, 153-154). Data from an experimental study in rats showed that brain choline concentration increased within 5 hours following oral administration of choline chloride (153). The consumption of a choline-free diet for 7 days lowered serum choline and brain phosphatidylcholine concentration suggesting that choline kinase, the controlling enzyme in phospholipid synthesis, is unsaturated with substrate in vivo and thus may serve as a modulator of the response of brain choline concentrations to alterations in the supply of circulating choline.

Clinical evidence of a human choline deficiency was first reported in adults receiving total parenteral nutrition (TPN) (165-166). These patients exhibited hepatic morphologic and aminotransferase

abnormalities which were reversed by choline-supplemented TPN. The effects of inadequate choline intakes on physiological endpoints are rapidly observed. Low blood levels of choline indicate that the requirements for the dietary precursors are not being met at current levels of intake (150, 152, 154-155, 167). In patients with infection, reduced levels of plasma acetylcholine are associated with suppression of the immune response and a reduction in lysosomal enzymes and phagocytic activity (168). The decrease in acetylcholine levels with infection has been attributed to the increased catabolism of the neurotransmitter caused by large amounts of acetylcholinesterase released into the plasma by parasites and other pathogens.

Clinical signs of choline deficiency have also been observed in men with otherwise normal nutritional status after consuming a choline-deficient diet for a period of < 3 weeks (165). Changes in blood and urine markers of organ dysfunction (muscle and liver enzymes) were also been reported in these men. Decreases in plasma levels of choline and phosphatidylcholine accompanied by elevated alanine aminotransferase, a biochemical marker of liver damage, and elevated creatine kinase, a biological marker of muscle damage, have also been observed with a dietary choline deficiency (162, 169-171). Serum choline levels are more responsive to increased choline intake than to a choline deficiency with increases of as much as 52% observed with choline supplementation compared with decreases of 20% with a choline-deficient diet (146, 154, 172).

Requirement for histidine. The increased need for supplemental histidine in inflammatory states can be attributed to the increased demand for histamine production. After 8 weeks of consuming a low histidine (10 mg/day), low nitrogen (6.3 g/day) diet, a significant decrease in 24-hour urinary free histidine was observed in 7 healthy men associated with a reduction in serum hemoglobin concentration indicating that the contribution of hemoglobin to the histidine body pool is limited (173). Addition of histidine to the diet resulted in an increase in serum hemoglobin concentration to baseline levels within 2 weeks that was associated with a corresponding increase in urinary histidine levels.

Requirement for zinc. Abnormal immune function and increased rates of infectious diseases have been widely reported in humans with zinc deficiency (174-175). In non-critically ill patients, zinc supplementation has been associated with an improvement in markers of immune function (176). Severe zinc deficiency can cause substantial impairment of cellular immunity resulting in infection and even death (76). Although the effects of mild to moderate zinc deficiency on immunity are considerably less severe, almost all immune cells show some impairment. Immunologic abnormalities characteristic of zinc deficiency are evident in HIV disease, most notably a reduction in the number of circulating T lymphocytes (177). The impairments in immune function associated with a zinc deficiency can be reversed by zinc supplementation when given in amounts adapted to the actual requirements of individual patients (76).

A summary of support for increased requirements of specific nutrients in patients with viral infections and impaired immune function is found in Table 4.

Table 4. Observations Supporting Increased Nutrient Requirements for Immune Function

Nutrient	Biochemical and Physiologic Observations	Clinical Observations
Arginine	Plasma levels reduced by infection and increased with dietary supplementation	Reduced plasma levels observed with infection and sepsis returned towards normal when infection subsided; restoration of blood levels associated with improved clinical status; elevated plasma nitrate levels in survivors of septic shock
Choline	Plasma levels reduced by infection and increased with dietary supplementation; associated with increased plasma acetylcholinesterase released from pathogens	Reduced plasma levels with infection and sepsis returned towards normal when infection subsided; associated with suppression of immune response and a reduction in lysosomal enzymes and phagocytic activity
Cysteine	Plasma levels reduced by infection and increased with dietary supplementation	Inflammation suppressed and glutathione levels increased with supplementation
Glutamine	Plasma levels reduced by infection and increased with dietary supplementation	Decreased the incidence of infection in trauma and surgery patients, and specifically post-surgical infections
Histidine	Anemia observed with deficiency	Low serum levels in patients with wounds associated with impaired healing; decreased hemoglobin levels within 20 days after beginning a histidine-free diet (178).
Lysine	Inversely related to nitric oxide levels	Arginine/lysine ratio lower in patients with sepsis than in ICU patients with noninflammatory conditions
Zinc	Reduction in T-cells with deficiency	Immunological impairments in HIV associated with deficiency reversed by zinc supplementation

Clinical Validation of *Lister V*

The relationship between intakes of dietary precursors and production of the corresponding neurotransmitters has been validated by observations of improvements in neurotransmitter-mediated clinical outcomes with supplemental intakes of these dietary factors (11, 96, 99, 105, 108, 113-116, 118-119, 122, 124-125, 127, 165, 173, 178-186). A change in the levels of a neurotransmitter in the blood and/or its metabolites in cerebrospinal fluid following ingestion of a dietary precursor from a medical food reflect the uptake and utilization of the nutrient or dietary factor for synthesis of the neurotransmitter by target cells, thus demonstrating the biological availability of dietary precursors and the clinical utility of the medical food as a source of these precursors (54, 91, 99-105, 107-108, 110, 112-113, 118, 122, 129, 147-148, 154, 163, 165, 169-170, 179, 182, 187-192).

The clinical benefits which may be obtained from medical foods can be validated by the observed changes in biological, physiological, and clinical endpoints following ingestion by individuals with specific conditions. If an individual with low blood arginine levels ingests a medical food containing supplemental arginine and subsequently shows an increase in blood levels (biological availability) accompanied by an increase in nitric oxide production (physiological response) followed by improvement

in an associated functional parameter (FEV₁) (clinical response), the clinical benefit of this medical food as a source of precursors of nitric oxide has been validated.

Several open label trials have been conducted with the **Lister V** formulation which showed a reduction in the incidence of viral infections and improvements in the onset, duration, and severity in patients with these infections. The data presented in Figure 7 demonstrate that compared with placebo, treatment with **Lister V** resulted in a reduction in the duration of herpes simplex-induced cold sores expressed as the number of days from onset of symptoms to loss of scab. In the **Lister V** group, recovery was complete for all subjects after 12 days compared with in 60% of patients in the placebo group over the same time period. Recovery was first noted after 2 days in the **Lister V** group, but signs of recovery were not apparent in the placebo group until after 4 days.

Figure 7. Effect of *Lister V* on Clearance of Cold Sores

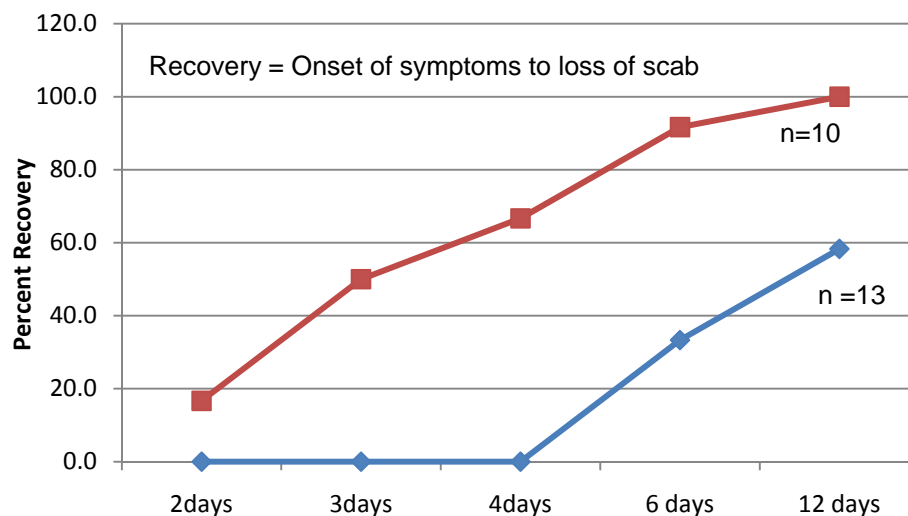
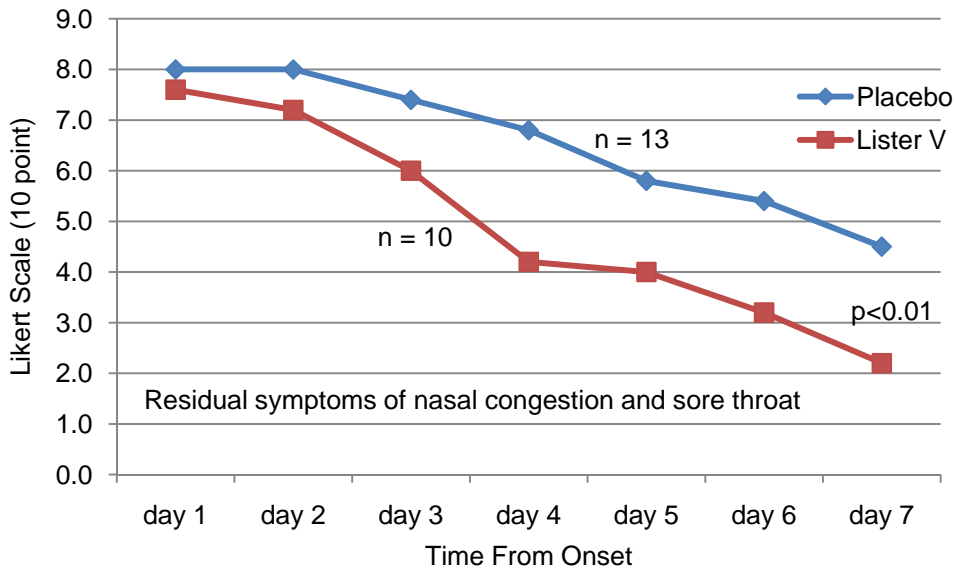


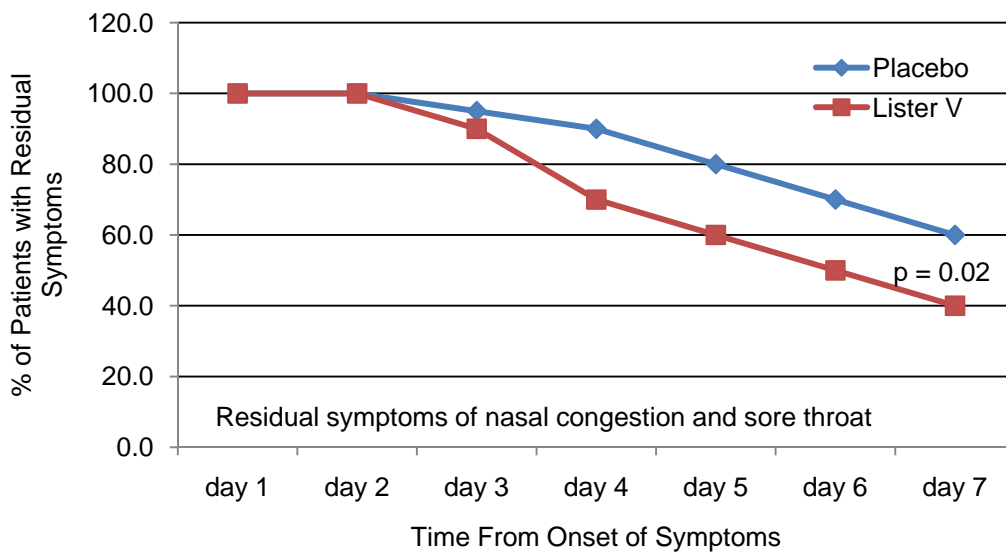
Figure 8 presents the differences in changes in severity of cold symptoms between patients receiving **Lister V** (n=13) and controls who received placebo (n=10). After 7 days, a statistically significant difference in severity was noted between groups (p<0.01). The differences in severity of symptoms between the **Lister V** group and placebo appeared on the second day of treatment.

Figure 8. Severity of Cold Symptoms



The prevalence of symptoms following onset of a common cold assessed as residual symptoms of nasal congestion and sore throat was also compared in this study (Figure 9). At Day 7, the difference in the percentages of patients with these symptoms between treatment and placebo groups was statistically significant ($p=0.02$). At this time, 40% of subjects in the **Lister V** group reported residual symptoms compared with 60% in the Placebo group. Differences between groups were first noted between the third and fourth day after start of treatment with **Lister V**.

Figure 9. Prevalence of Symptoms Following Onset of Common Cold



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