Portal to the Interior: Viral Pathogenesis and Natural Compounds that Restore Mucosal Immunity and Modulate Inflammation

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Abstract

Most antigens, particularly viruses, enter the body through the mucosal epithelia where they are carried by afferent lymphatics to regional lymph nodes for presentation to the immune system. Although they share immunological similarities, immune processes that protect the mucosa are distinct from innate and acquired immunity. The barrier formed by the intestinal mucosa is the most studied, with its microenvironment having a marked influence on both local and systemic immune responses. A healthy microenvironment and resilient neighboring tissue provide protection against inflammation known to dampen mucosal immunity, promote carcinogenesis, contribute to systemic inflammatory processes, and result in autoimmune diseases. Numerous natural substances improve this microenvironment and thereby enhance immunity against microbial infections. Since mucosal immunity forms the first line of defense against many commonly transmitted pathogens, restoring and maintaining mucosal immunity is critical for disease prevention and intervention. This article discusses the nature of mucosal immunity and its relationship to viral infections and other conditions, and reviews natural compounds that help restore mucosal immunity.

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Introduction

The human gastrointestinal and respiratory tracts harbor the largest number and greatest diversity of microorganisms in the body. Proximity to the external environment makes these tissues especially vulnerable to pathogens and allergens, so it is not surprising mucosal tissue contains the greatest immunological activity and number of immune cells, cytokines, and secondary lymphoid tissues. Causes of mucosal immune dysfunction include microbial infections, dietary indiscretions such as excess refined sugars and lack of fiber, allergies and food intolerances, indiscriminant and overuse of oral antibiotics, disruption of lipid and fatty acid metabolism, and aging. All of these etiologies, most a product of modern lifestyle, can result in dysfunctional mucosal immunity and may even be responsible for a wide range of chronic diseases. The author intends to demonstrate the increasing importance of mucosal immunity in the clinical setting to stimulate further research in this age of emerging viruses.

The Mucosal Immune System

Primary lymphoid organs in the thymus and bone marrow constitute the major site of lymphocyte development. Secondary lymphoid organs are the spleen, lymph nodes, and a diffuse group

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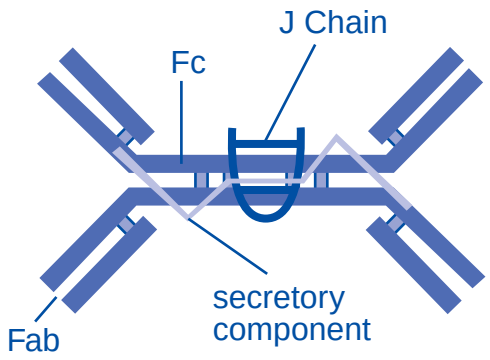
Figure 1. *Secretory IgA*



of tissues collectively known as the mucosa-associated lymphoid tissue (MALT), subclassified according to different organ and tissue groups.[1] The gut-associated lymphoid tissue (GALT)[2] includes the appendix and Peyer's patches in the small intestine. The tonsils and adenoids compose the nasal-associated lymphoreticular tissue (NALT),[3] while the bronchial-associated lymphoid tissue (BALT)[4] is made up of secondary lymphoid tissue of the respiratory epithelium. Referred to as the urogenital lymphoid tissue, the lining of the urogenital tract also contains mucosal immunological activity.[5]

The lining of these tracts is composed of a single column of epithelial cells continually bathed in mucus made of glycoproteins, proteoglycans, enzymes, and specialized immune cells and immunoglobulin – principally secretory IgA (sIgA),[6] a type of immunoglobulin that protects the ears, nose, throat, and gastrointestinal tract, and is also found in breast milk. Secretory IgA (Figure 1) plays an important role in local immune defense mechanisms, exhibiting antiviral, antibacterial, anti-inflammatory, and antiallergic activity. This viscous mucus is the major portal separating the interior of the body from pathogenic invasion from viruses and other antigens. It also has immunological effects by activating lymphocytes and preventing oncogenesis.[7,8]

Once thought of only as a passive barricade, it is now known that gastrointestinal epithelial cells are highly organized, regulated portals that open and close in response to messages within the cells, orchestrating the entrance of microbes and other substances. The gut mucosa also participates in immunological activity by allowing sIgA and lymphocytes, principally CD+4 T cells, to move into the mucosal epithelium. Between each epithelial cell are "tight junctions" or cell-to-cell connections that form a continuous column of cells. Unlike the epithelial cells of the skin that also form a continuous and formidable barrier against microbial infections, the epithelium comprising the lining of the respiratory, gastrointestinal, and urogenital tracts is more susceptible to infection because of the extent of exposure to antigens. In addition, the mucous membranes – unlike the skin that has no viable cells on the surface and is hostile because of dryness and acidity – are more exploitable by viruses through a variety of immunological mechanisms. Both soluble proteins and microorganisms cross the mucosal epithelial barrier. In the intestinal tract, the single cell layer that forms the intestinal lining also absorbs nutrients and fluids obtained from dietary sources.

Antigen Entry and Defense

Viruses, as well as other pathogenic microorganisms and allergens, are regularly ingested or inhaled. Many viruses cross the epithelial barrier using the oropharynx and gastrointestinal tract as the main portal of entry, with sites ranging from the tonsils to the colon and rectum (Table 1).[9] In temperate climates, the respiratory route is the most common portal of entry for common viral infections, while in the tropics, the most common route is through the gastrointestinal tract. Influenza virus, HIV, and hepatitis A and B enter the body through the mucosal portal.

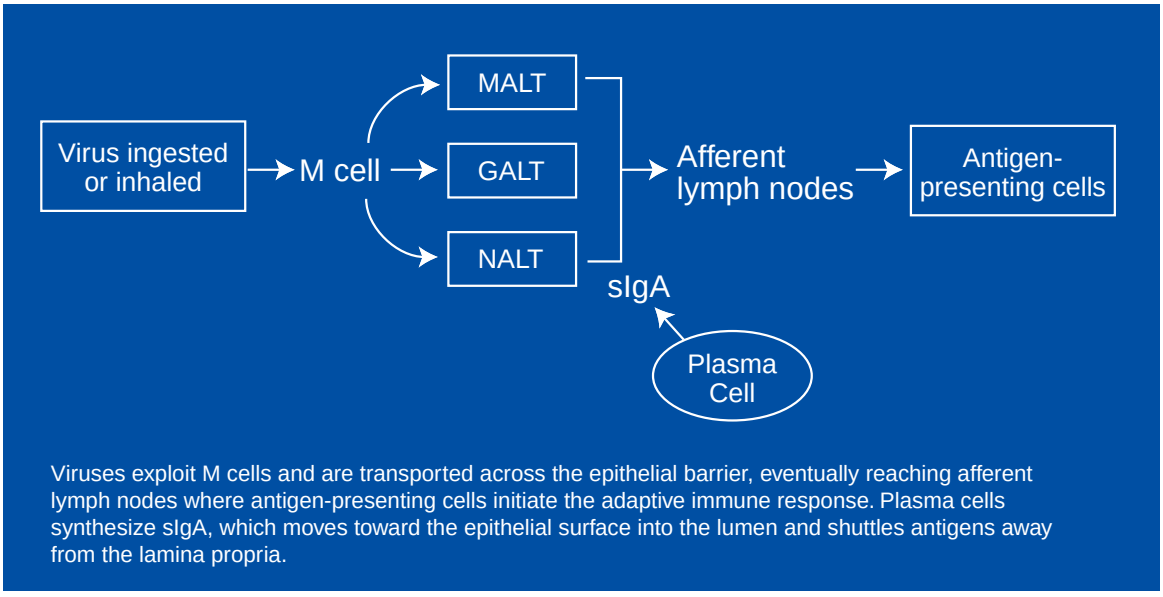
After passing through a formidable gauntlet of digestive juices and other physical and chemical barriers, viruses migrate toward the mucosal lining. On contact, the epithelial cells increase mucus secretion to adhere antigens to the viscous mucus and eliminate them via the stool, the urine, or through the respiratory tract by the

Table 1. *Representative Viruses that Invade Mucous Membranes*

Portal	Viral Family	Virus	Disease
Conjunctiva	Picornaviridae	Enterovirus type 70	Conjunctivitis
	Adenoviridae	Adenovirus type 8	Keratoconjunctivitis
Oropharynx	Herpesviridae	Herpes simples type 1	Oral herpes
Respiratory	Picornaviridae	Rhinoviruses	Common cold
	Orthomyxoviridae	Orthomyxoviruses	Influenza
	Herpesviridae	Varicella-zoster	Chickenpox
	Paramyxoviridae	Paramyxoviruses	Measles
Genitals	Retroviridae	Lentivirus (HIV)	AIDS
	Papillomaviridae	Papillomavirus	Genital warts
	Herpesviridae	Herpes simplex type 2	Genital herpes
	Hepadnaviridae	Hepadnavirus	Hepatitis B
Gastrointestinal	Picornaviridae	Poliovirus	Poliomyelitis
	Reoviridae	Rotavirus	Gastroenteritis
	Picornaviridae	Hepatitis A virus	Hepatitis A
Rectum	Retroviridae	Lentivirus (HIV)	AIDS

action of ciliated cells. Sneezing, coughing, vomiting, and diarrhea are dramatic methods of rapidly expelling pathogens; however, these methods are exploited by viruses by hastening the spread of infection to other individuals. If not effectively expelled, viruses undergo an initial replication cycle, multiplying at the mucosal lining. Enteric adenoviruses and Norwalk calicivirus are examples of viruses that replicate extensively in intestinal epithelial cells. Internally, the body's immune defenses are simultaneously alerted and, depending on the virulence of the pathogen, systemic reactions such as fever, malaise, and fatigue may occur. Not all microbes are defeated at the mucosal surface and, depending on type and virulence, as well as the integrity of the mucosal immunity, may cross the mucosal barrier and gain entry into the blood and lymph where they eventually reach target organs, such as the liver in the case of hepatitis C virus (Figure 2).

Figure 2. *Entry and Spread of Viruses*



Viruses exploit M cells and are transported across the epithelial barrier, eventually reaching afferent lymph nodes where antigen-presenting cells initiate the adaptive immune response. Plasma cells synthesize sIgA, which moves toward the epithelial surface into the lumen and shuttles antigens away from the lamina propria.

Damage to the mucosal barrier is significantly more sophisticated than merely the loosening of tight junctions, referred to as "leaky gut syndrome."[10] In fact, viruses utilize sophisticated chemical transport systems to gain entry into the interior of the body. Both invasive bacteria such as *Yersinia pseudotuberculosis* and *Salmonella typhimurium* and non-invasive organisms like *Escherichia coli* are also able to cross the epithelial barrier through a transport system involving microfold cells (M cells), specialized epithelial cells contained in the Peyer's patches, also known as GALT-inductive sites.[11,12] Peyer's patches are lymphoid follicles found in the distal ileum of the small intestine. Although still poorly understood, M cells are thought to act as gateways to the mucosal immune system, providing functional access to the epithelium for the transport of antigens, antibodies, proteins, and other substances from one side of the barrier to the other through complex molecular mechanisms.[13]

Cell-mediated immunity also operates at the mucosal surface with the production of CD4+ T helper-type cells and the release of numerous cytokines. Cytotoxic cells are also present, including CD8+ cytotoxic-type T cells and natural killer (NK) cells that function to decrease pathogenic load in the early stages of infection. They also produce cytokines such as interferons (IFN) and tumor necrosis factor (TNF), as well as interleukins (IL-1β, IL-6, IL-8), and a variety of chemokines such as macrophage-inflammatory proteins (MIP), for example MIP-1α.[12]

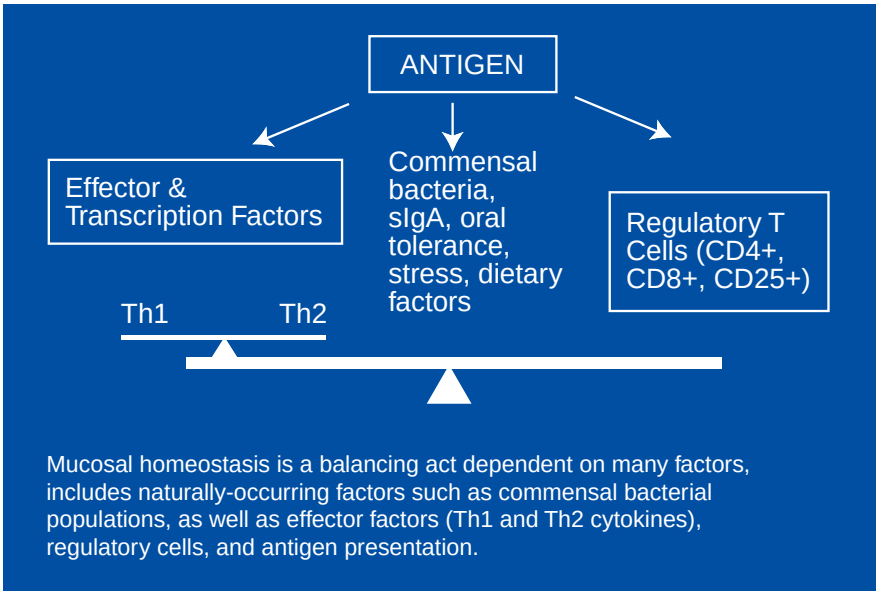
A hallmark of mucosal immunity is the synthesis of sIgA by mucosal plasma cells in the lamina propria, the connective tissue that lies beneath the epithelium. Secretory IgA is characterized by the ability to prevent infection and inflammation at the epithelial surface, thereby inhibiting viral attachment, entry, and replication.[14] Not only does sIgA play a key role in antiviral defenses at the mucosal surface by neutralizing viruses and preventing antigens from entering the epithelium, it also shuttles antigens out of the lamina propria and into the lumen where IgA-bound antigens or microorganisms are excreted. After transportation through the epithelium toward the interior of the body, antigens are met by additional antibody, a large number of lymphocytes including both T- and B-cells, and cytokines, all of which participate in the mucosal immune response. They are then delivered to antigen-presenting cells in the lymph nodes that initiate the adaptive immune response.

Mucosal Inflammation of Non-Pathogenic Origin

In addition to microbial infection, local and widespread inflammation is a constant risk in the intestinal and respiratory mucosal environment. Regulation of inflammatory processes is dependent on a delicately balanced mucosal microenvironment. An estimated 400 different commensal species populate the gastrointestinal microenvironment, the most studied of the mucosal environments.[15] It is generally thought that adequate numbers of so-called "friendly" intestinal microorganisms help manage infection and mitigate inflammatory processes, but sub-populations of commensals can turn on, and then amplify, the inflammatory response, which may contribute to chronic inflammation such as that associated with Crohn's disease.[16,17] In addition, spontaneous mucosal inflammation can occur due to disrupted immunological communication, referred to as cross-talk, between naturally occurring friendly commensal bacterial populations and pro-inflammatory cytokines (IL-1, -2, -6, -15, and IFN-γ).[18]

Imbalanced microflora and dysfunctional regulatory cross-talk are only two of several principles by which researchers suggest mucosal inflammation occurs. Others include gene mutation,[19] barrier dysfunction,[20] increased effector-cell responses,[21] dysfunction in regulatory T cells (CD4+, CD25+, CD8+),[22] and defects in innate immunity. Another hypothesis is that mucosal inflammation is mediated by one of two immunological pathways: (1) excessive Th1 responses that are associated with increased secretion of IL-12, IFN-γ, and TNF; or (2) excess Th2 response that is associated with increases in IL-4, IL-5, and IL-13. These cytokines are typically associated with an inflammatory immune response. However, it is still unclear which, if any, of these different principles and pathways can solely explain gastrointestinal inflammation and what, if any, effect they have on other mucosal tissue.[23]

Figure 3. *Mucosal Homeostasis*



Mucosal homeostasis is a balancing act dependent on many factors, includes naturally-occurring factors such as commensal bacterial populations, as well as effector factors (Th1 and Th2 cytokines), regulatory cells, and antigen presentation.

MacPherson et al demonstrated that intestinal IgA is central to maintaining the immune balance among the numerous commensal microorganisms harbored in the gut, as well as against the endotoxins produced by commensals. This commensal-specific IgA, contrary to previous opinion that gastrointestinal IgA was a "natural antibody" of insignificant or at least unknown function, is now thought to play a central role in an "evolutionarily primitive form of specific immune defense."[24] Fiocchi of Case Western Reserve University suggests a complex interplay of immune and non-immune cell interactions takes place in the gastrointestinal environment and contributes to homeostasis (Figure 3).[25]

Transcription factors appear to play a key role in epithelial cell gene expression, particularly nuclear factor-kappa B (NF-κB), by regulating genes involved in the inflammatory response.[26] Phenotypic expression of genes involved in mucosal defense and inflammation also play a role in commensally induced inflammatory responses.[27] Genetic susceptibility also plays a role. In this case, an imbalanced microenvironment

coupled with cytokine dysregulation may cause negative gene expression, as seen in mutations on chromosome 16, NOD2 in a subset of Crohn's patients.[28]

Repair mechanisms that re-establish normal epithelial tissue and activity are also essential when injury occurs to the mucosa. Rapid migration of residual epithelium along the edge of damaged tissue, combined with normal cellular proliferative activity, allow reconstitution of the mucosa. This mucosal remodeling process is thought to be regulated by growth factors, peptides, and cytokines.[29]

Other than commensals and pathogenic microorganisms, proteins found in foods, principally those occurring in wheat and dairy products, are a primary source of antigenic stimulation to the gastrointestinal mucosa. Clinically, practitioners of complementary and alternative (CAM) therapies and environmental medicine have long suggested that food allergies play a pivotal role in inflammatory gastrointestinal disorders. Recent evidence suggests the link between inflammatory reactions caused by dietary proteins is one of intolerance.[30] Once oral intolerance to foods (perhaps as much caused by decades of overeating as to specific allergic reactions) is reached, immunological reactions take place. What is not known is how these occur and why systemic reactions such as fatigue, headache, mood changes, neurological imbalances, and allergic phenomena also develop. Animal models suggest spontaneous inflammation of the gastrointestinal mucosa is caused by a combination of factors, including disruption of the epithelial barrier; abnormal numbers and function of commensals; discommunication in the cross-talk between cytokines, immune cell activity, and other immunological molecules; and nutrient deficiencies – with an initiating event such as a viral infection or hypersensitivity to food.[31]

Another cause of chronic inflammation is disruption of arachidonic acid metabolism involving a family of lipid mediator substances, including prostaglandin E2 (PGE2), all important for the protection of gastrointestinal mucosa.[32] Oxidative damage by environmental factors and ingested toxic chemicals may also contribute to disruption of mucosal immunity and allow inflammatory changes to occur. In addition, mucosal immunity is influenced by aging, as demonstrated by Mbawuike, Shugars, and others. CD8+ cytotoxic T cells not only decline during aging, but also appear to be induced by dysregulation of cytokine expression.[33] Antimicrobial proteins, such as secretory leukocyte protease inhibitor (SLPI) and lactoferrin, play crucial roles in mucosal defenses and are reduced in older individuals.[34] In addition to mucosal immune deficiency during aging, proliferative changes to gastrointestinal epithelia occur, including age-related rise in abnormal mucosal proliferation, which may be one contributory factor in the increased incidence of gastrointestinal cancers in the elderly.[35] Actual levels of sIgA during aging remain controversial. In general, sIgA is nearly absent at birth, rises and reaches its peak at seven years, remains constant through mid-life, and then gradually declines with age. However, although lower sIgA levels in the elderly are considered a risk factor for the increased incidence of upper respiratory tract infections, particularly for influenza, findings by Percival et al indicate sIgA levels may remain constant even in advanced age, providing sufficient protection against upper respiratory viral infections.[36]

The Hygiene Hypothesis

First proposed by British evolutionary biologists and gaining respect among conventional immunologists, the "hygiene hypothesis" was introduced to the public in an editorial in the *Economist* in 1997,[37] then scientifically elaborated in *Nature Reviews*,[38] and further discussed by Sewell et al in *Immunology Letters*.[39] This theory suggests the emphasis by modern medicine on the eradication of all infectious diseases and aggressive intervention with drugs, primarily through the use of vaccines and antibiotics, may have wide ranging adverse effects on public health. As childhood infections and minor bacterial infections have decreased, chronic diseases have increased, especially autoimmune conditions, allergies and asthma, chronic viral diseases, and cancer – in effect, the replacement of one disease by another.

Investigators are building evidence that childhood infections reduce the probability of chronic diseases in adults. According to this hypothesis, exposure to infectious antigens during infancy and early childhood builds immunity and prevents autoimmune diseases.[38] Several environmental factors may affect susceptibility to infectious and allergenic agents and play a role in mucosal immunity. In developing countries where large family size is common and children of all ages mingle with each other, adults, livestock, and pets, the number and variety of endogenous gut flora increase, providing a wealth of stimuli for the developing immune system and the maintenance of balanced immunity. Such populations have much lower incidences of atopic allergies, asthma, and autoimmune diseases than their counterparts in the developed nations.[38] Paradoxical as it may seem, treatment based on this hypothesis, with colonization by commensal bacteria in early childhood combined with the avoidance of the overuse of antibiotics, may promote lifelong health.[40]

The elderly may also benefit from probiotic supplementation. Aging appears to alter gastric mucosal responses and there is increased proliferative activity of the gastrointestinal mucosa in older people, which may contribute to carcinogenesis and an increased incidence of inflammatory bowel disease.[36] Arunachalam demonstrated even short-term consumption of Bifidobacterium improved interferon production and increased phagocytic capacity.[41] These findings suggest a role for prebiotic, probiotic, and soil organisms in the treatment of allergic and autoimmune diseases. According to the hygiene hypothesis, the human immune system evolved elaborate mechanisms to accommodate a certain number of infectious agents, coevolute with microbes – both commensal and pathogenic – and allow for homeostasis within the organism.

Natural Compounds for Restoration of Mucosal Immunity

A large number of natural compounds are potentially useful in restoring mucosal immunity, including vitamins and minerals, amino acids, pre- and probiotics, soil-derived organisms, colostrum-derived nutrients, and herbal medications. Perhaps more fundamentally, the link between diet and immunity has been well demonstrated, confirming that nutrient deficiencies lead to an increased incidence of infection. It is well-known that infection and malnutrition aggravate each other, but even moderate deficiencies of individual nutrients such as zinc, iron, selenium, and vitamins A, B6, B12, C, and E lead to weakened immunity and increased susceptibility to infections.[42-44] Viruses can also mutate and alter their virulence, depending on the nutritional status of the host at the time of infection.[45] Generally, improving antiviral immunity and restoring mucosal immune integrity begins with a diet composed of adequate protein, sufficient fiber, high nutrient density foods like fruits and vegetables, moderate amounts of complex carbohydrates, avoidance of refined carbohydrates, appropriate amounts of essential fatty acids, and the addition of nutritional supplements.[46] Dietary intervention for the treatment of inflammatory bowel disease has been well-addressed by other authors.[47]

Functional foods (a food for which a health claim has been authorized), like yogurt, inulin, oligofructose, and soluble dietary fiber (often termed "prebiotics"), have shown considerable positive influence on restoring healthy commensal populations.[48-50] Supplementing the substrate with functional foods and prebiotics promotes recolonization of the microenvironment and helps maintain normal inflammatory responses in the gastrointestinal mucosa.

Yogurt, derived from the fermentation of lactic acid in milk by *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, and other bacteria, exerts a nonspecific immunostimulatory effect on host defenses due to changes in the gastrointestinal microenvironment. The therapeutic benefits of yogurt and lactic acid bacteria are well-documented.[51] *In vitro* and *in vivo* studies provide

evidence that yogurt stimulates phagocytosis,[52] mobilizes antibody including sIgA, increases cytokine levels involved in the inflammatory response and antiviral immunity (IL-1β, IL-6, IL-10, TNF-α,[53] and IFN-γ), exerts an antitumor effect,[54,55] mitigates against IgE-mediated hypersensitivity, improves gastrointestinal disorders, and reduces asthma.[56] In order to accomplish these immunological effects, dosages of whole organic milk yogurt in patients who are not dairy intolerant should be at least 200 grams daily.[57]

Inulin (a heterogeneous blend of fructose polymers) and oligofructose (a subgroup of inulin) are natural substances widely distributed in nature and commonly found in the diet.[58] The average U.S. diet contains insufficient amounts of prebiotic substances, with only an estimated 2.6 grams of inulin and 2.5 grams of oligofructose consumed daily. However, since the average U.S. diet contains large amounts of refined and processed foods, and because the amount of prebiotics consumed may be too low for the maintenance of gastrointestinal homeostasis, several authors propose supplementing inulin and oligofructose to improve gastrointestinal function, modulate gut microflora, and stimulate mucosal immunity.[59,60] Studies suggest daily prebiotic dosages of 8-40 grams per day are necessary to stimulate growth of Bifidobacteria strains.[61]

Although probiotics do not appear to have specific antiviral properties, taken orally they demonstrate enhanced nonspecific host immunity, increased sIgA response, increased TNF-α production, enhanced IL-2, -5, and –6, and prevention of pathogenic transmission at the epithelia.[62-64] All of these actions improve mucosal integrity and indirectly protect against viral infection. Commercial probiotic supplements include *Lactobacillus acidophilus, Bifidobacterium bifidum, L. sporogenes, L. casei, L. brevi, Streptococcus thermophilus, Saccharomyces boulardii,* and others. Daily dosing of probiotics range from 1-10 billion viable units of live organisms.[65]

In addition to the indirect immune benefits from restoring friendly intestinal bacterial species, several species of microflora enhance sIgA, which is important in the prevention of viral attachment. *Saccharomyces boulardii*, a nonpathogenic yeast useful to protect against antibiotic-induced colitis,[66] has been shown in experimental models to stimulate sIgA production and enhance phagocytosis.[67] These and similar findings suggest Saccharomyces probiotic supplementation may modulate host immune responses and therefore have indirect antiviral benefits.

Medicinal soils such as Luvos Heilerde from Germany, a finely ground dried soil that protects the gastrointestinal mucosa, and microorganisms derived from soil like *Bacillus subtilis*, a common gram-positive bacteria with antifungal properties, hold promise in restoring normal gastrointestinal function and enhancing mucosal immunity.[68] An experimental recombinant form of *Bacillus subtilis* (Subalin 2335 strain) has been shown to increase interferon synthesis and exert antiviral activity.[69] In addition, laboratory grown, commercially prepared, homeostatic soil organism (HSO) blends have been used for the treatment of inflammatory bowel conditions and autoimmune diseases.[70] Preliminary research suggests HSO blends have antifungal, antimicrobial, and immunomodulating properties.[71] There are no clinical standards for dosing these compounds and recommended amounts are dependent on manufacturer guidelines.

Although some dietary proteins can activate mucosal inflammation, the individual amino acids glutamine and arginine act to moderate inflammation and promote repair mechanisms in the gastrointestinal tract. Glutamine is considered to be a non-essential amino acid; nevertheless, the body synthesizes large amounts, accounting for 30-35 percent of the amino acid-bound nitrogen in plasma, with the gastrointestinal tract using the majority of glutamine to promote growth, metabolism, structure, and function of the intestinal mucosa.[772,73] L-arginine has immunostimulatory properties and serves as a protective nutrient for the gastrointestinal tract.[74,75] Absorbed in the gut and transported into the circulation by intestinal cells, it is involved in immune function and nitric oxide (NO) synthesis. Uptake of L-arginine by the small intestine plays an important role in regulating NO synthesis and thereby immune

activity.[76] Daily dosages of L-glutamine range from 1-8 g and L-arginine from 1-6 g, although some sources suggest considerably higher amounts.

Colostrum-derived supplements, known for general immune-modulating activity, are usually not thought of as mucosal immune stimulants; however, recent research suggests they may help protect the mucosal barrier against pathogenic organisms. Research has shown that lactoferrin, a protein found in tears, saliva, mucus, and human milk, exerts immunoregulatory activity through cytokine modulation and bacteriostatic effects by reducing iron levels in the mucosa, thereby depriving bacteria of an essential proliferative nutrient.[77-79] Research also suggests lactoferrin has antiviral properties, appearing to disrupt viral replication and inhibit viral entry through the mucosal portal.[80-83] Typical dosages for lactoferrin range from 250-750 mg.

A number of natural compounds derived from plants have demonstrated usefulness in regulating mucosal immunity. These include lectins, arabinogalactan, Croton alkaloids, and tannins. Plant lectins, principally mistletoe (*Viscum album*) lectins type I and II (ML-1, ML-II), have shown strong affinity for mucosal tissue and exert immunostimulating effects.[84-86] Immune modulation with mistletoe lectins can cause transient elevations in pro-inflammatory cytokines (TNF, IL-6); however, low-dose therapy as generally used in clinical practice has not been shown to over stimulate the inflammatory response.[87] Mistletoe is most commonly provided in injectable forms manufactured in Germany and Switzerland, with dosages ranging from 0.05 mg-25.0 mg, containing up to 375 ng/mL of total lectin.

Arabinogalactan, a plant fiber with immune-enhancing properties, is found in carrots, radishes, pears, corn, wheat, and tomatoes, as well as in herbs such as *Echinacea spp.* and *Curcuma longa*. Larch arabinogalactan, derived from *Larix occidentalis*, is an excellent source of dietary fiber and can serve as a prebiotic in the restoration of gut microflora by promoting an increase in Bifidobacteria.[88,89] The dosage of larch arabinogalactan is 1-4 g in two divided dosages daily.

Table 2. *Natural Compounds that Restore Mucosal Immunity*

Vitamins A, B6, B12, C, E
Yogurt
Inulin and Oligofructose
Lactobacillus acidophilus
Lactobacillus sporogenes
Lactobacillus casie, L. brevi, others
Streptococcus thermophilus
Saccharomyces boulardii
Bacillus subtilis
Luvos soil
Homeostatic soil organisms
L-glutamine
L-arginine
Lactoferrin
Mistletoe lectins
Larch arabinogalactan
Croton lechleri extract
Plant tannins

Sangre de grado (*Croton lechleri*), an Amazonian botanical, exhibits antiviral, antibacterial, antifungal, anti-inflammatory, and antidiarrheal effects and, because of its affinity for the gastrointestinal tract, may exert a positive influence on mucosal immunity.[90] Sangre de grado is available as a standardized extract (SP-303) and 250-500 mg is generally prescribed 2-4 times daily.

Plant tannins are naturally occurring, water-soluble polyphenols found in a variety of foods, herbs, and red wine. They have antioxidant properties,[91] antidiarrheal effects,[92] and are toxic to a wide range of microbial organisms including parasites[93] and some common viruses. Dietary tannins and polyphenols in red wine may offer protective effects against oxidative damage to the gastrointestinal mucosa.[94] Antiviral research using plant tannins has shown inhibitory effects on *Herpes*

simplex virus (HSV-1, HSV-2),[95,96] influenza A virus,[97] Epstein-Barr virus,[98] and HIV.[99,100] Although clinical application of these findings requires more investigation, dietary tannins from red wine, tea, and herbs may play a role in the management of viral conditions and mucosal restoration.

Table 2 summarizes natural compounds that may restore mucosal immunity.

Future Directions

Since the mucosal portal is a common route for many infectious diseases, including HIV, cholera, rotavirus, and influenza, medical researchers are investigating new oral and nasal vaccines that affect a broad range of tissues. Many of these novel vaccines use live, attenuated pathogens from bacterial toxins that prevent gene expression and increase mucosal immune efficiency.[12] Novel, genetically-altered bacteria that block a pathogen's ability to penetrate mucosal barriers are also under investigation as anti-infective agents. These include attenuated *Salmonella enterica*[101,102] and *Neisseria gonorrhoea*.[103] These new-generation vaccines are not without risk and at least one, a tetravalent rotavirus vaccine, has been discontinued due to its association with bowel intussusception. Lethal effects have also been observed in some of these vaccines, such as the Hong Kong H5N1 virus that killed chicken eggs in laboratory tests.[104]

Also under investigation as a vaccine adjuvant is a highly purified saponin (QS-21) derived from *Quillaja saponaria Molina* bark, a South American tree in the Rosaceae family with anti-inflammatory, antimicrobial, and immunostimulatory properties.[105,106] QS-21 is an immune adjuvant, designed to enhance the immune response to an antigen contained in a vaccine, stimulating an increase in total T-cell response. Since vaccines remain the main intervention in preventing infectious diseases, it is critical that immunological performance is optimal, necessitating research into methods for increasing their activity. Adjuvants are divided into two groups: vaccine delivery systems and immunostimulants.[107,108] Current research using QS-21 is directed at malaria,[109] HIV, and hepatitis C virus.[110]

Gene cloning is yet another area being investigated for the restoration of immunity in elderly and immune-deficient individuals. In this scenario, novel vaccines using influenza or other viruses capable of stimulating CD8+ cytotoxic T-cells are taken orally to improve mucosal immunity rather than targeting immune response to a specific virus. Modulation of immune cells and cytokines is theoretically appealing from the molecular point of view and may hold promise in the future.

While unsanitary conditions contribute extensively to the spread of viral infections, an overemphasis on sterile living conditions as suggested by the "hygiene hypothesis" may be yet another cause of dysfunction of mucosal immunity. Further research is needed to understand the environmental and ecological relationships between mucosal health and disease, suggesting a new role for public health.

Pharmacological and immunological strategies, including antihistamines, steroids, epinephrine, desensitization injections, and vaccines, and newer investigational methods such as selective blockade of the NF-κB system and a variety of mucosal antiviral factors,[111] may be effective in managing symptoms of illnesses that originate at the mucosal barrier. Although these methods will continue to find a place, a more ecological approach using natural compounds as preventive and primary medications may be the better approach for restoration of mucosal immunity, reserving pharmaceutical agents as a second line of defense. Additional research is necessary in the area of integrative medicine for the treatment of infectious disease.

As infectious diseases become increasingly more common, the clinical challenge for the practitioner of CAM therapies is to develop clearer diagnostic criteria and more specific therapeutic approaches to immunologically mediated diseases. An even greater challenge is the role CAM therapies will play in the changing medical model from empiricism to medicine-by-design, one ruled by molecular biology and genomics. Biologists are still working toward a greater understanding of the molecular model; meanwhile, empiricism works even if it cannot always be explained why.

Ironically, this may be particularly true in the case of mucosal immunity and vaccine development where we do not yet have a clear molecular model for newly emerging viruses.

Although CAM practitioners more commonly see chronic inflammatory disorders than serious infectious diseases, chronic inflammation of any of the mucosal tracts may be complicated by subclinical bacterial and viral infections, microenvironment disruption such as yeast and mold overgrowth, allergic reactions, parasitic infection, and abnormal proliferation of normally benign commensals. All of these conditions work to weaken normal mucosal defenses, predisposing the epithelium to easier entry and more rapid viral pathogenesis than is the case for intact healthy mucosa surrounded by an ecologically balanced internal microenvironment.

Although much has been learned in recent years, much remains to be understood of deeper questions of mucosal immunity. How does infection and antigen presentation govern tolerance and regulate systemic immunity? What are the specific roles of sub-populations of commensal organisms to health and disease? How does the immune system recognize and differentiate naturally occurring symbiotic microorganisms from infectious ones? How is gene expression, as related to chronic inflammation, influenced by the gastrointestinal microenvironment? What role does empiricism play in the new molecular model, if any? In the process of answering these and other questions, scientists must gain insights into the portal to the interior, mucosal immunity, which may help in the development of safe, novel vaccines and natural immune-modulating compounds for the treatment of infectious diseases, inflammatory conditions, and allergic disorders. Further research may also lead to a better understanding of the role of natural medicinal alternatives such as probiotics, colostrum-derived supplements, soil organisms, and herbal extracts that offer attractive clinical benefits, not only for treatment of disease, but also for immune modulation and prevention of chronic diseases.

Conclusions

Although dominated by sIgA, mucosal immunity involves a complex interplay of immune cells, cytokines, and chemokines, as well as interaction from the microenvironment on the epithelial surface and within the respective tracts that serve to prevent pathogenic infiltration and inflammation. With the increasing incidence of newly emerging viruses, increasing prevalence of antibiotic-resistant bacterial super-strains, and the growing incidence of allergic and inflammatory disorders, the role of the mucosal immune system in clinical medicine requires scrutiny. Once viral infection has occurred, it is generally considered a systemic condition; however, the primary portal of entry is at the mucosal epithelium, necessitating an understanding of viral pathogenesis and treatment from both the systemic and cellular perspectives, with an emphasis on mucosal immunity and its role in disease transmission and progression.

References

1. Benjamini E, Coico R, Sunshine G. *Immunology, A Short Course.* New York, NY: John Wiley & Sons, Inc.; 2000.
2. Shields JW. The functional evolution of GALT: a review. *Lymphology* 2000;33:47-57.
3. Zuercher AW, Coffin SE, Thurnheer MC, et al. Nasal-associated lymphoid tissue is a mucosal inductive site for virus-specific humoral and cellular immune responses. *J Immunol* 2002;168:1796-1803.
4. Sato A. Basic and clinical aspects of bronchus-associated lymphoid tissue. *Nihon Kokyuki Gakkai Zasshi* 2000;38:3-11. [Article in Japanese]
5. Roitt I, Brostoff J, Male D. *Immunology.* London, England: Mosby; 1998.
6. Parham P. *The Immune System.* New York, NY: Garland Publishing; 2000.
7. Kettunen HL, Kettunen AS, Rautonen NE. Intestinal immune responses in wild-type and Apcmin/+ mouse, a model for colon cancer. *Cancer Res* 2003;63:5136-5142.
8. Glushkov AN. Induction of immunological tolerance to the chemical carcinogens in early ontogenesis. *Med Hypotheses* 2001;57:123-124.

9. Nathanson N. *Viral Pathogenesis and Immunity.* Philadelphia, PA: Lippincott, Williams & Wilkins; 2002.
10. Liska DJ, Lukaczer D. Gut restoration and chronic disease. *J Am Nutraceutical Assoc* 2002;5:20-33.
11. Nagler-Anderson C. Man the barrier! Strategic defences in the intestinal mucosa. *Nat Rev Immunol* 2001;1:59-67.
12. van Ginkel FW, Nguyen HH, McGhee JR. Vaccines for mucosal immunity to combat emerging infectious deseases. *Emerg Infect Dis* 2000;6:123-132.
13. Neutra MR. Current concepts in mucosal immunity V. Role of M cells in transepithelial transport of antigens and pathogens to the mucosal immune system. *Am J Physiol* 1998;274:G785-G791.
14. Lamm ME. Current concepts in mucosal immunity. IV. How epithelial transport of IgA antibodies relates to host defense. *Am J Physiol* 1998;274:G614-G617.
15. McCracken VJ , Lorenz RG. The gastrointestinal ecosystem: a precarious alliance among epithelium, immunity and microbiota. *Cell Microbiol* 2001;3:1-11.
16. Gionchetti P, Rizzello F, Venturi A, Campieri M. Probiotics in infective diarrhoea and inflammatory bowel diseases. *J Gastroenterol Hepatol* 2000;15:489-493.
17. Guslandi M, Mezzi G, Sorghi M, Testoni PA. *Saccharomyces boulardii* in maintenance treatment of Crohn's disease. *Dig Dis Sci* 2000;45:1462-1464.
18. Bouma G, Strober W. The immunological and genetic basis of inflammatory bowel disease. *Nat Rev Immunol* 2003;3:521-533.
19. van Deventer SJ. Taming the mucosal immune response in Crohn's disease. *Best Pract Res Clin Gastroenterol* 2002;16:1035-1043.
20. Suenaert P, Bulteel V, Lemmens L, et al. Anti-tumor necrosis factor treatment restores the gut barrier in Crohn's disease. *Am J Gastroenterol* 2002;97:2000-2004.
21. Kiyono H, Kweon MN, Hiroi T, Takahashi I. The mucosal immune system: from specialized immune defense to inflammation and allergy. *Acta Odontol Scand* 2001;59:145-153.
22. Das G, Augustine MM, Das J, et al. An important regulatory role for CD4+CD8 alpha alpha T cells in the intestinal epithelial layer in the prevention of inflammatory bowel disease. *Proc Natl Acad Sci U S A* 2003;100:5324-5329.
23. Kidd P. Th1/Th2 balance: The hypothesis, its limitations, and implications for health and disease. *Altern Med Rev* 2003;8:223-246.
24. Macpherson AJ, Gatto D, Sainsbury E, et al. A primitive T cell-independent mechanism of intestinal mucosal IgA responses to commensal bacteria. *Science* 2000;288:2222-2226.
25. Fiocchi C. Intestinal inflammation: a complex interplay of immune and nonimmune cell interactions. *Am J Physiol* 1997;273:G769-G775.
26. Jobin C, Sartor RB. The I kappa B/NF-kappa B system: a key determinant of mucosal inflammation and protection. *Am J Physiol Cell Physiol* 2000;278:C451-C462.
27. Ogawa H, Fukushima K, Sasaki I, Matsuno S. Identification of genes involved in mucosal defense and inflammation associated with normal enteric bacteria. *Am J Physiol Gastrointest Liver Physiol* 2000;279:G492-G499.
28. Ogura Y, Bonen DK, Inohara N, et al. A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. *Nature* 2001;411:603-606.
29. Podolsky DK. Mucosal immunity and inflammation. V. Innate mechanisms of mucosal defense and repair: the best offense is a good defense. *Am J Physiol* 1999;277:G495-G499.
30. Kato H, Fujihashi K, Kato R, et al. Oral tolerance revisited: prior oral tolerization abrogates cholera toxin-induced mucosal IgA responses. *J Immunol* 2001;166:3114-3121.
31. Perdue MH. Mucosal immunity and inflammation. III. The mucosal antigen barrier: cross talk with mucosal cytokines. *Am J Physiol* 1999;277:G1-G5.
32. Wallace JM. Modulation of the inflammatory cascade: an essential target in cancer therapy. *Int J Integr Med* 2002;4:6-24.
33. Mbawuike IN, Acuna CL, Walz KC, et al. Cytokines and impaired CD8+ CTL activity among elderly persons and the enhancing effect of IL-12. *Mech Ageing Dev* 1997;94:25-39.
34. Shugars DC, Watkins CA, Cowen HJ. Salivary concentration of secretory leukocyte protease inhibitor, an antimicrobial protein, is decreased with advanced age. *Gerontology* 2001;47:246-253.

35. Turner JR, Liu L, Fligiel SE, et al. Aging alters gastric mucosal responses to epidermal growth factor and transforming growth factor-alpha. *Am J Physiol Gastrointest Liver Physiol* 2000;278:G805-G810.
36. Percival RS, Marsh PD, Challacombe SJ. Age-related changes in salivary antibodies to commensal oral and gut biota. *Oral Microbiol Immunol* 1997;12:57-63.
37. No author listed. Plagued by cures. *The Economist* 1997;95-97.
38. Wills-Karp M, Santeliz J, Karp CL. The germless theory of allergic disease: revisiting the hygiene hypothesis. *Nat Rev Immunol* 2001;1:69-75.
39. Sewell DL, Reinke EK, Hogan LH, et al. Immunoregulation of CNS autoimmunity by helminth and mycobacterial infections. *Immunol Lett* 2002;82:101-110.
40. Rajan T. Remembrance of pathogens past. *Nat Hist* 2002;Feb:228-233.
41. Arunachalam K, Gill HS, Chandra RK. Enhancement of natural immune function by dietary consumption of *Bifidobacterium lactis* (HN019). *Eur J Clin Nutr* 2000;54:263-267.
42. Chandra RK. Nutrition and the immune system: an introduction. *Am J Clin Nutr* 1997;66:460S-463S.
43. Chandra RK. Nutrition and immunology: from the clinic to cellular biology and back again. *Proc Nutr Soc* 1999;58:681-683.
44. Watson RR. *Nutrients and Foods in AIDS.* Boca Raton, FL: CRC Press; 1998.
45. Chandra RK. Nutrition, immunity, and infection: from basic knowledge of dietary manipulation of immune responses to practical application of ameliorating suffering and improving survival. *Proc Natl Acad Sci U S A* 1996;93:14304-14307.
46. Williams JE. *Viral Immunity: A 10 Step Plan to Enhance Your Immunity Against Viral Disease Using Natural Medicines.* Charlottesville, VA: Hampton Roads Publishing Co; 2002.
47. Head KA, Jurenka JS. Inflammatory bowel disease part 1: ulcerative colitis – pathophysiology and conventional and alternative treatment options. *Altern Med Rev* 2003;8:247-283.
48. Roberfroid MB. Global view on functional foods: European perspectives. *Br J Nutr* 2002;88:S133-S138.
49. Roberfroid MB. Functional foods: concepts and application to inulin and oligofructose. *Br J Nutr* 2002;87:S139-S143.
50. Roberfroid MB. Concepts in functional foods: the case of inulin and oligofructose. *J Nutr* 1999;129:1398S-1401S.
51. Perdigon G, Locascio M, Medici M, et al. Interaction of bifidobacteria with the gut and their influence in the immune function. *Biocell* 2003;27:1-9.
52. Schiffrin EJ, Rochat F, Link-Amster H, et al. Immunomodulation of human blood cells following the ingestion of lactic acid bacteria. *J Dairy Sci* 1995;78:491-497.
53. Perdigon G, de Moreno de LeBlanc A, Valdez J, Rachid M. Role of yoghurt in the prevention of colon cancer. *Eur J Clin Nutr* 2002;56:S65-S68.
54. Lidbeck A, Nord CE, Gustafsson JA, Rafter J. Lactobacilli, anticarcinogenic activities and human intestinal microflora. *Eur J Cancer Prev* 1992;1:341-353.
55. Rachid MM, Gobbato NM, Valdez JC, et al. Effect of yogurt on the inhibition of an intestinal carcinoma by increasing cellular apoptosis. *Int J Immunopathol Pharmacol* 2002;15:209-216.
56. Meydani SN , Ha WK. Immunologic effects of yogurt. *Am J Clin Nutr* 2000;71:861-872.
57. Van de Water J, Keen CL, Gershwin ME. The influence of chronic yogurt consumption on immunity. *J Nutr* 1999;129:1492S-1495S.
58. Niness KR. Inulin and oligofructose: what are they? *J Nutr* 1999;129:1402S-1406S.
59. Jenkins DJ, Kendall CW, Vuksan V. Inulin, oligofructose and intestinal function. *J Nutr* 1999;129:1431S-1433S.
60. Gibson GR. Dietary modulation of the human gut microflora using the prebotics oligofructose and inulin. *J Nutr* 1999;129:1438S-1441S.
61. Rao AV. Dose-response effects of inulin and oligofructose on intestinal bifidogenesis effects. *J Nutr* 1999;129:1442S-1445S.
62. Isolauri E, Sutas Y, Kankaanpaa P, et al. Probiotics: effects on immunity. *Am J Clin Nutr* 2001;73:444S-450S.
63. Erickson KL, Hubbard NE. Probiotic immunomodulation in health and disease. *J Nutr* 2000;130:403S-409S.
64. Drisko JA, Giles CK, Bischoff BJ. Probiotics in health maintenance and disease prevention. *Altern Med Rev* 2003;8:143-155.
65. Catanzaro JA, Green L. Microbial ecology and probiotics in human medicine (part II). *Altern Med Rev* 1997;2:296-305.

66. Qamar A, Aboudola S, Warny M, et al. *Saccharomyces boulardii* stimulates intestinal immunoglobulin A immune response to Clostridium difficile toxin A in mice. *Infect Immun* 2001;69:2762-2765.

67. Rodrigues AC, Cara DC, Fretez SH, et al. *Saccharomyces boulardii* stimulates sIgA production and the phagocytic system of gnotobiotic mice. *J Appl Microbiol* 2000;89:404-414.

68. Chitarra GS, Breeuwer P, Nout MJ, et al. An antifungal compound produced by *Bacillus subtilis* YM 10-20 inhibits germination of *Penicillium roqueforti* conidiospores. *J Appl Microbiol* 2003;94:159-166.

69. Sorokulova IB, Beliavskaia VA, Masycheva VA, Smirnov VV. Recombinant probiotics: problems and prospects of their use for medicine and veterinary practice. *Vestn Ross Akad Med Nauk* 1997;3:46-49. [Article in Russian]

70. Rothschild PR, Huertas JG. Ambulatory treatment of chronic digestive disorder with malabsorption syndrome with Primal Defense. *Prog Nutr* 2002;4:97-108.

71. Goldberg P. Primal Defense homeostatic soil organisms as applied to medically unresponsive chronic disease conditions in adults. *Prog Nutr* 2002;4:109-115.

72. Miller AL. Therapeutic considerations of L-glutamine: a review of the literature. *Altern Med Rev* 1999;4:239-248.

73. Bouteloup-Demange C, Claeyssens S, Maillot C, et al. Effects of enteral glutamine on gut mucosal protein synthesis in healthy humans receiving glucocorticoids. *Am J Physiol Gastrointest Liver Physiol* 2000;278:G677-G681.

74. Potenza MA, Nacci C, Mitolo-Chieppa D. Immunoregulatory effects of L-arginine and therapeutical implications. *Curr Drug Targets Immune Endocr Metabol Disord* 2001;1:67-77.

75. Duggan C, Gannon J, Walker WA. Protective nutrients and functional foods for the gastrointestinal tract. *Am J Clin Nutr* 2002;75:789-808.

76. Pan M, Meng QH, Wolfgang CL, et al. Activation of intestinal arginine transport by protein kinase C is mediated by mitogen-activated protein kinases. *J Gastrointest Surg* 2002;6:876-882.

77. Dohler JR, Nebermann L. Bovine colostrum in oral treatment of enterogenic endotoxaemia in rats. *Crit Care* 2002;6:536-539.

78. Caccavo D, Pellegrino NM, Altamura M, et al. Antimicrobial and immunoregulatory functions of lactoferrin and its potential therapeutic application. *J Endotoxin Res* 2002;8:403-417.

79. Singh PK, Parsek MR, Greenberg EP, Welsh MJ. A component of innate immunity prevents bacterial biofilm development. *Nature* 2002;417:552-555.

80. Beljaars L, Bakker HI, van der Strate BW, et al. The antiviral protein human lactoferrin is distributed in the body to cytomegalovirus (CMV) infection-prone cells and tissues. *Pharm Res* 2002;19:54-62.

81. Giansanti F, Rossi P, Massucci MT, et al. Antiviral activity of ovotransferrin discloses an evolutionary stratetgy for the defensive activities of lactoferrin. *Biochem Cell Biol* 2002;80:125-130.

82. Berkhout B, van Wamel JL, Beljaars L, et al. Characterization of the anti-HIV effects of native lactoferrin and other milk proteins and protein-derived peptides. *Antiviral Res* 2002;55:341-355.

83. Challacombe SJ, Sweet SP. Oral mucosal immunity and HIV infection: current status. *Oral Dis* 2002;8:55-62.

84. Lavelle EC, Grant G, Pusztai A, et al. The indentification of plant lectins with mucosal adjuvant activity. *Immunology* 2001;102:77-86.

85. Pae HO, Seo WG, Oh GS, et al. Potentiation of tumor necrosis factor-alpha-induced apoptosis by mistletoe lectin. *Immunopharmacol Immunotoxicol* 2000;22:697-709.

86. Elsasser-Beile U, Voss M, Schuhle R, Wetterauer U. Biological effects of natural and recombinant mistletoe lectin and an aqueous mistletoe extract on human monocytes and lymphocytes *in vitro*. *J Clin Lab Anal* 2000;14:255-259.

87. Braun JM, Blackwell CC, Weir DM, Beuth J. Cytokine release of whole blood from adult female donors challenged with mistletoe lectin-1 standardised mistletoe extract and *E. coli* endotoxin or phytohaemagglutinin (PHA). *Anticancer Res* 2003;23:1349-1352.

88. Kelly GS. Larch arabinogalactan: clinical relevance of a novel immune-enhancing polysaccharide. *Altern Med Rev* 1999;4:96-103.

89. Kim LS, Waters RF, Burkholder PM. Immunological activity of larch arabinogalactan and Echinacea: a preliminary, randomized, double-blind placebo-controlled trial. *Altern Med Rev* 2002;7:138-149.
90. Williams JE. Review of antiviral and immunomodulating properties of plants of the Peruvian rainforest with a particular emphasis on Una de Gato and Sangre de Grado. *Altern Med Rev* 2001;6:567-579.
91. Lin CC, Hsu YF, Lin TC. Antioxidant and free radical scavenging effects of the tannins of *Terminalia catappa* L. *Anticancer Res* 2001;21:237-243.
92. Abdullahi AL, Agho MO, Amos S, et al. Antidiarrhoeal activity of the aqueous extract of *Terminalia avicennoides* roots. *Phytother Res* 2001;15:431-434.
93. Molan AL, Waghorn GC, Min BR, McNabb WC. The effect of condensed tannins from seven herbages on *Trichostrongylus colubriformis* larval migration *in vitro*. *Folia Parasitol (Praha)* 2000;47:39-44.
94. Giovannelli L, Testa G, De Filippo C, et al. Effect of complex polyphenols and tannins from red wine on DNA oxidative damage of rat colon mucosa *in vivo*. *Eur J Nutr* 2000;39:207-212.
95. Fukuchi K, Sakagami H, Okuda T, et al. Inhibition of herpes simplex virus infection by tannins and related compounds. *Antiviral Res* 1989;11:285-297.
96. Cheng HY, Lin CC, Lin TC. Antiherpes simplex virus type 2 activity of casuarinin from the bark of *Terminalia arjuna* Linn. *Antiviral Res* 2002;55:447-455.
97. Rajbhandari M, Wegner U, Schopke T, et al. Inhibitory effect of *Bergenia ligulata* on influenza virus A. *Pharmazie* 2003;58:268-271.
98. Liu KC, Lin MT, Lee SS, et al. Antiviral tannins from two Phyllanthus species. *Planta Med* 1999;65:43-46.
99. Liu S, Jiang S, Wu Z, et al. Identification of inhibitors of the HIV-1 gp41 six-helix bundle formation from extracts of Chinese medicinal herbs *Prunella vulgaris* and *Rhizoma cibotte*. *Life Sci* 2002;71:1779-1791.
100. Yoshida T, Ito H, Hatano T, et al. New hydrolyzable tannins, shephagenins A and B, from *Shepherdia argentea* as HIV-1 reverse transcriptase inhibitors. *Chem Pharm Bull (Tokyo)* 1996;44:1436-1439.
101. Chen H, Schifferli DM. Mucosal and systemic immune responses to chimeric fimbriae expressed by *Salmonella enterica serovar typhimurium* vaccine strains. *Infect Immun* 2000;68:3129-3139.
102. Chen H, Schifferli DM. Construction, characterization, and immunogenicity of an attenuated *Salmonella enterica serovar typhimurium* pgtE vaccine expressing fimbriae with integrated viral epitopes from the spiC promoter. *Infect Immun* 2003;71:4664-4673.
103. Veiga E, de Lorenzo V, Fernandez LA. Autotransporters as scaffolds for novel bacterial adhesins: surface properties of *Escherichia coli* cells displaying Jun/Fos dimerization domains. *J Bacteriol* 2003;185:5585-5590.
104. Wizemann TM, Adamou JE, Langermann S. Adhesins as targets for vaccine development. *Emerg Infect Dis* 1999;5:395-403
105. Boyaka PN, Marinaro M, Jackson RJ, et al. Oral QS-21 requires early IL-4 help for induction of mucosal and systemic immunity. *J Immunol* 2001;166:2283-2290.
106. Kensil CR. Saponins as vaccine adjuvants. *Crit Rev Ther Drug Carrier Syst* 1996;13:1-55.
107. O'Hagan DT, Valiante NM. Recent advances in the discovery and delivery of vaccine adjuvants. *Nat Rev Drug Discov* 2003;2:727-735.
108. O'Hagan DT, Singh M. Microparticles as vaccine adjuvants and delivery systems. *Expert Rev Vaccines* 2003;2:269-283.
109. Rafi-Janajreh A, Tongren JE, Kensil C, et al. Influence of adjuvants in inducing immune responses to different epitopes included in a multiepitope, multivalent, multistage *Plasmodium falciparum* candidate vaccine (FALVAC-1) in outbred mice. *Exp Parasitol* 2002;101:3-12.
110. Qiao M, Murata K, Davis AR, et al. Hepatitis C virus-like particles combined with novel adjuvant systems enhance virus-specific immune responses. *Hepatology* 2003;37:52-59.
111. Shugars DC. Endogenous mucosal antiviral factors of the oral cavity. *J Infect Dis* 1999;179:S431-S435.