THE ROLE OF PROLINE-RICH POLYPEPTIDES 
AND LACTOFERRIN IN HUMAN VIRAL DEFENSE

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EXECUTIVE SUMMARY

The lack of effective antiviral drugs has hampered modern medicine in the fight against infectious disease. Now a unique extract from colostrum, Lactopeptide™, is available that promises broad spectrum defense against viral infections. Proline-Rich Polypeptides and Lactoferrin, both present in large quantities in colostrum, provide both nonspecific and specific defense against a wide variety of viral pathogens and immunomodulatory abilities that turn up an underactive immune system or turn down an overactive one. Delivered in a spray form to expedite rapid uptake into the bloodstream, this product has the potential to fill a large gap in infectious disease treatment.

At long last, an antiviral product which is effective against a broad range of pathogenic viruses, a safe and natural product with no known side effects, a product which combines the potent antiviral effects of Proline-Rich Polypeptides and Lactoferrin which together are more effective than each separately.

Colostrum, the first milk produced by a mother after her child is born, is a rich source of beneficial components for not only newborns, but anyone of any age, including immunological factors, anti-inflammatory factors, gastrointestinal health factors, growth factors, antioxidant and anti-aging factors. Colostrum contains two substances, PRP, or Proline-Rich Polypeptides, and Lactoferrin, which have unique antiviral characteristics. Unlike other pathogens, such as bacteria, fungi and protozoan parasites, there are very few treatments against viral infections. This is particularly worrisome as viruses cause some of the most lethal infectious diseases, and new strains and types are being regularly discovered. The recent Asian flu pandemic scare is but one example. Estimates of deaths from an Asian flu outbreak in humans run into the millions. Concomitantly we live in an environment which is becoming increasingly polluted and which places great strain upon our immune systems. The end result is that we face greater threats from viruses with weakened natural defenses.

PRP, also known as colostrinin, colostrinine, transfer factor and other names, is actually a group of related polypeptides. A polypeptide is a simple string of amino acids with no secondary or tertiary structure like a true protein would have, such as folding, helices, sheets, and so forth. Its small size and uncomplicated structure make it easy for it to pass through mucous membranes in the body without being altered or digested. Thus it can be readily absorbed directly into the bloodstream, unlike most of the other components of colostrum which must pass through the digestive tract where they are exposed to powerful gastric acids and digestive enzymes. Proline-Rich Polypeptides
are so named because they contain a higher percentage of the amino acid proline than most other peptides and proteins.

PRP has the unique ability to modulate and stabilize many biologic processes in the body\(^2\), including cytokine\(^3\) and immune processes.

Lactoferrin belongs to the transferrin family of proteins which are marked by their ability to bind iron and other heavy metals such as zinc and manganese. Lactoferrin is a multifunctional molecule which plays an important role in the innate immune system, the first line of defense against pathogens (see below), particularly on mucosal surfaces\(^4\). It has anti-microbial activity against a broad range of pathogens, including bacteria, viruses, fungi and protozoan parasites. Other functions of lactoferrin in the organism include a role in iron homeostasis, anti-inflammatory, anti-tumor and analgesic activity, regulation of bone metabolism, participation in embryonic development, reproductive functions, and others. It is commonly found in nearly all body fluids, including milk, colostrum, tears, saliva, bile, pancreatic juice, genital and nasal secretions, as well as neutrophils. Highest concentrations are found in colostrum.
BRIEF REVIEW OF IMMUNE FUNCTION

The immune system is made up of two basic parts:

Innate immune system – nonspecific immunity, including barrier defenses (skin, intestinal lining, brain-blood barrier, macrophages, neutrophils, dendritic cells etc.), airway cilia (remove antigens and small particles that are breathed in), mucus, chemicals such as lactoferrin, lactoperoxidase and lysozyme which are potent killers of pathogens, strong acids in the stomach, and so forth.

Adaptive immune system – system which consists of immune and scavenger cells (lymphocytes, macrophages, NK [Natural Killer] cells, mast cells, monocytes and other blood cells) and which reacts to specific antigens.

The adaptive immune system furthermore has two response modes:

Humoral Immunity – production of specific antibodies in response to an antigen, mediated by B-cells.

Cell-Mediated Immunity – production of cytotoxic lymphocytes, activated macrophages and NK cells, and cytokines in response to an antigen, mediated by T-cells.

Lymphocytes come in several different varieties. B-lymphocytes (B-cells) are produced in the bone marrow, and T-lymphocytes (T-cells) are produced in the thymus gland. T-cells are further broken down into:

Cytotoxic or killer T-cells (CD8+) – the cells which actually kill invading pathogens

Helper T-cells (CD4) – cells which direct the immune response through the secretion of cytokines.

Suppressor T-cells – inhibit the production of cytotoxic T-cells when no longer needed to prevent excess tissue damage and turn down an adaptive immune response

Memory T-cells – retain memory of an encountered antigen so that if encountered again the response time will be much shorter

Helper T-cells secrete various cytokines to stimulate the production and differentiation of cytotoxic T-cells and B-cells, which produce antibodies. They also attract neutrophils (white blood cells) and stimulate macrophages to engulf and destroy pathogens.

Helper T-cells also have subsets.
**TH1 cells** – secrete the cytokines tumor necrosis factor-alpha (TNF-\(\alpha\)) and interleukin 12 (IL-12) which control cell-mediated immunity; TH1 activation can inhibit TH2 cell activation.

**TH2 cells** – secrete the cytokines IL-4, IL-5 which control humoral immunity; TH2 cell activation can inhibit TH1 cell activation.

Regulatory TH3 (Tr) cells\(^{10}\) – at least three different type of regulatory TH cells exist:

*Type 1 (Tr1)* – secrete large amounts of IL-10 and low-to-moderate amounts of transforming growth factor-beta (TGF-\(\beta\)), may help terminate TH1-related inflammatory responses.

*Type 3 (Tr3)* – primarily secrete TGF-\(\beta\), regulates multiple facets of immune response.

*CD4+CD25+* - inhibit immune responses through direct cell-to-cell contact.

The following diagram may help clarify how the immune system works as a coordinated system to protect the organism:
Pathogen

- engulfed by macrophage

Pieces of pathogen presented on surface of antigen-presenting cell (macrophage)

Helper and killer T-cells are activated by antigen-presenting macrophage, but only if T-cells recognize specific antigen presented by macrophage

Helper T-cell activates B-cell

Active helper and killer T-cells replicate, including formation of memory cells

- Killer T-cells require helper T-cells for activation

- Memory T-cells can respond to subsequent infection by that kind of pathogen

Immature, inactive helper and killer T-cells

- in thymus

Immature, inactive B-cells

- in bone marrow

Mature, inactive helper and killer T-cells

Mature, inactive B-cells

B-cell is activated by the antigen but only if B-cell recognizes specific antigen. Active helper T-cell is required for B-cell activation

Active B-cell replicates, and produces antibody molecules that can bind to specific antigens

- Antibody binds to antigen ("tagging")

- Memory B-cells can respond to subsequent infection by that kind of pathogen

Complement system destroys the antigen

Phagocytic cells engulf the tagged antigen

Free antigen in blood
ANTI-VIRAL PROPERTIES OF PRP AND LACTOFERRIN

PRPs are, like all cytokines, a molecular signaling device. Cytokines can act on the same cell that produced them (autocrine), on other cells near them (paracrine), and/or on distant cells (endocrine). The same cytokine may be produced by different types of cells, and often the activity of different cytokines is redundant. They often are produced in a cascade, such as is the case with TNF-α, which is the primary cytokine in the inflammatory response cascade. Cytokines interact with receptors on target cell membranes "telling" the target cell to start doing something or to stop doing something, such as synthesizing proteins.

The immunological properties of the PRP fraction of colostrum were first identified in Poland by Janusz and Lisowski, et al, in 197911,12,13,14. They noted that PRP from ovine (sheep) colostrum acted as a hormone in the thymus gland by stimulating thymocytes (lymphocytes which originate in the thymus gland) to differentiate and become activated as either helper or suppressor T-cells15,16,17, and furthermore that this effect was reversible18. PRP also induces the growth and differentiation of B-cells19. PRP increases the permeability of blood vessels in the skin, a typical pro-inflammatory action. PRP also induces leukocyte proliferation and mitogenic stimulation of peripheral blood cells to produce a variety of cytokines20,21. PRPs have also been shown to be potent stimulators of Natural Killer (NK) cell activity and induce the differentiation and maturation of monocytes and macrophages22.

Further studies have elucidated the actual molecular mechanisms by which PRP is able to modulate immunity. A study by Inglot, et al, [1996] showed that PRP could stimulate the production of two major cytokines, tumor necrosis factor-alpha (TNF-α) and interferon-gamma (INF-γ) in white blood cells23, peritoneal cells24, and the placenta and amniotic membrane25. Later it was discovered that PRP stimulates other cytokines as well, such as IL-6 and IL-10. PRP is non-species specific, meaning that PRP from bovine colostrum works on humans just as well as human PRP26.

PRP acts on specific surface membrane receptors on target cells9. These receptors then release intracellular signaling peptides which act to either stimulate or inhibit actions of the cell, such as stimulating a thymocyte to differentiate into a mature lymphocyte or a lymphocyte to produce TNF-α.

PRPs obtained from colostrum can be classified into five sub-classes; PRP1 (inactive), PRP2a & 2b (very active interferon modulator with antiviral activity31), PRP3a & 3b (very active pro-inflammatory cytokine modulator), PRP4 (less active, lower concentration, unstudied), and PRP5 (again less active, lower concentration, unstudied)27. Chemically PRPs show amino acid sequence homology to three protein precursors: annexin, beta casein, and a hypothetical beta-casein homolog14. Further study may make it possible to selectively utilize PRP fractions to produce certain effects, such as selectively stimulating T-cells or selectively turning on or off the production of certain cytokines or cytokine cascades. Some efforts to that end have already been attempted using active fragments of PRPs28,29,30,31.
The use of PRP against viral infections has resulted in some success\textsuperscript{32}. In experimental systems, PRP has shown to induce immunity to vesicular stomatitis virus (VSV)\textsuperscript{33} (a close relative of the rabies virus used in experimental systems to study the properties of Rhabdoviruses), herpes viruses\textsuperscript{34,35,36}, Epstein-Barr virus\textsuperscript{37}, HIV\textsuperscript{38}, measles\textsuperscript{39} and others\textsuperscript{40,41,42}. PRP has also been shown to target viruses associated with autoimmune conditions, such as Epstein-Barr virus and Human Herpes Virus-6 (HHV-6), a possible causative agent in chronic fatigue syndrome\textsuperscript{43}.

PRP may have the potential to help the immune system regain its balance in the dangerous and often fatal “cytokine storm” produced by H5N1 influenza virus (bird flu) in which the inflammatory response of the immune system is stimulated out of control\textsuperscript{44}. In one small outbreak of bird flu in Thailand, four patients were diagnosed with the disease. Only one survived, a patient who had been regularly taking colostrum powder (which contains PRP). While hardly conclusive, this supports the idea that PRP could be used to turn off the “cytokine storm” associated with bird flu.

In clinical studies conducted in the nations of Nigeria, Kenya and Zambia in Africa, where AIDS is a particularly devastating disease, PRP oral spray products were shown to boost T-cell (CD4+) levels to normal or near-normal levels (median 502, none less than 300) in AIDS patients whose T-cell levels prior to treatment were well below normal (median 275). Along with the increase in T-cells came a remission of AIDS symptoms within two days of start of treatment, including nausea, vomiting and diarrhea. In the Nigerian study, weight gains of 5% were recorded. Patients taking the PRP spray fared much better in terms of quality of life than did patients on anti-retroviral drugs\textsuperscript{45}. Thus the ability of PRP to stimulate the immune response when it is insufficient by inducing the production of new helper T-cells appears to enable the immune system of AIDS patients to recover sufficiently so that it is able to fight the HIV on its own.

Lactoferrin, like PRP, has been shown to be an immune system modulator. Lactoferrin increases the number and the activity levels of T and B lymphocytes and Natural Killer (NK) cells. It also stimulates the release of a number of cytokines, including the pro-inflammatory cytokines IL-1 (T, B cell activation), IL-6 (B cell stimulation), IL-8 (macrophage attractant), IL-18 (stimulates naïve CD4+ T cells to become Th1 cells, induces INF-γ production), interferon-gamma (INF-γ) (activates macrophages), and tumor necrosis factor-alpha (TNF-α) (initiates inflammatory cytokine cascade). It accelerates the maturation of T and B lymphocytes, increases the phagocytic activity and cytotoxicity of monocytes and macrophages, and increases the production of several types of cell receptors, important components of the immune response, including CD4 receptors, the zeta chain of the CD3 complex, LFA-1, CD11, ICAM-1 and selectin P\textsuperscript{46}.

Lactoferrin has also been identified as a potent antiviral protein. It blocks virus replication mainly by competing for binding sites on target cells, thus preventing viruses from penetrating the cells. Viruses known to be affected by competitive binding of Lactoferrin to cell receptors include echovirus 6\textsuperscript{47}, herpes simplex virus 1 and 2\textsuperscript{48}, canine herpes virus\textsuperscript{49}, feline herpes virus\textsuperscript{50}, enterovirus 71\textsuperscript{51}, human immunodeficiency virus (HIV-1)\textsuperscript{52}, cytomegalovirus\textsuperscript{53}, human papilloma virus\textsuperscript{54}, rotavirus\textsuperscript{55}, polio virus\textsuperscript{39}, adenovirus\textsuperscript{39}, feline calicivirus\textsuperscript{56}, hantavirus\textsuperscript{57}, respiratory syncytial virus\textsuperscript{58}, and Sindbis
and Semliki Forest viruses. It is also known to bind to viral structural proteins in some viruses, including enterovirus 71, adenovirus, and hepatitis C virus. Lactoferrin is also known to interfere with the action of viral enzymes required for capsid assembly, thus blocking viral replication. Lactoferrin inhibits influenza virus hemagglutination, the clumping of red blood cells, a characteristic of influenza virus infection. Lactoferrin strongly inhibits HIV-1 reverse transcriptase as well as cysteine protease, an enzyme used by both bacteria and viruses. Lactoferrin exhibits synergy with some antiviral drugs as well, such as zidovudine, used in the treatment of HIV-1 infection, ribavirin, used against hantavirus, cidofovir, used against cytomegalovirus, and acyclovir, used in the treatment of herpes virus.

It is notable that this list of viruses includes some of the most lethal known. HIV-1, the virus which causes AIDS, is well-known, as are polio, herpes and hepatitis C viruses. The enteroviruses (echovirus and enterovirus 71) cause aseptic meningitis, nonspecific febrile illness in newborns, encephalitis, paralysis, respiratory diseases, rashes, diarrhea, pericarditis, myocarditis, liver disorders and muscle pain. Enterovirus 71 can also cause Guillain-Barre syndrome (an autoimmune condition in which the body’s immune system attacks portions of the peripheral nervous system) and rapidly fatal pulmonary edema and hemorrhage. It has been responsible for several large outbreaks, including one in Taiwan in 1998 which affected close to a million and a half people and caused some fatalities. Human papilloma virus causes warts, but is also a prerequisite for cervical and other cancers. Cytomegalovirus, a common congenital (present at birth) virus, can cause birth defects and is the principal cause of mononucleosis. It is particularly dangerous in those with compromised immune systems, such as in AIDS. Rotavirus is the principal cause of severe diarrhea in infants. It is responsible for approximately 600,000 infant deaths annually. Alphaviruses (Semliki Forest virus and Sindbis virus) cause tropical fevers in Africa. Adenoviruses cause infections of respiratory tract, eyes, intestines and urinary tract and are commonly picked up while swimming in polluted water. Hantavirus, carried by rodent vectors, causes an often fatal pulmonary disease and hemorrhagic fever with renal syndrome. Respiratory syncytial virus is the most common cause of bronchitis and pneumonia in infants under one year of age. It is particularly dangerous in the elderly and those who are immunocompromised.

When taken together, the combined immunomodulatory actions of PRP and Lactoferrin and their activity against viruses of many different varieties, including both DNA and RNA viruses, demonstrate the value of a product which can deliver these potent compounds in a direct, rapid and timely manner to help our bodies protect against viral attack.


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