THE EFFECT OF A COLOSTRUM EXTRACT OF PROLINE RICH POLYPEPTIDES (PRP) ON IMMUNE STATUS IN GUINEA PIGS AND ITS IMPLICATIONS ON THE POTENTIAL OF PRP IN AGING HUMANS

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ABSTRACT

Colostrum is the richest natural source of zoonutrients. Proline Rich Polypeptides (PRP) are active immune modulating zoonutrient peptides found in all mammalian colostrum. In particular PRP are thought to modulate thymus function, specifically the T helper 1 / T helper 2 (Th1 / Th2) balance. Th1, modulating cellular immunity, and Th2, modulating humeral immunity, are classified on the basis of the cytokines they produce.

To demonstrate the efficacy of PRP in down regulating Th2 dominance an experiment was performed on guinea pigs sensitized to egg protein. Exposure to egg protein resulted in development of acute phase bronchial spastic reaction in 100% of animals, 3 of 6 (50%) of whom died quickly from suffocation. The duration of acute phase and sub-acute phase in the three surviving animals was 14.3 minutes on average. Introduction of PRP prior to egg protein inhalation resulted in death of only 2 animals out 7 (28.6%). Development of acute bronchial spastic reaction on exposure to the antigen was present in 2 (40%) of the 5 surviving animals, but averaged only 16 seconds. None (0%) of the survivors experienced any sub-acute phase reactions. A similar model was employed using histamine as the initiator of bronchial spasm, with similar, though non-fatal symptoms. In this histamine model, pre-treatment with PRP had no effect. This supports the notion that any effectiveness of PRP pre-treatment was not related to an

inherent anti-histamine effect.

In humans the thymus is very sensitive to stress hormones (cortisol) which promote Th2 dominance, precipitating allergic and autoimmune reactions, while down regulating Th1 immunity, favoring infection and tumor growth proliferation. With aging, Cortisol / DHEA ratios favor cortisol over DHEA, leading to a similar immune imbalance. Furthermore, thymopoiesis is diminished with diminishing growth hormone (GH) secretion, which itself is diminished with aging.

This paper presents the ability of PRP from colostrum to modulate thymus / immune function in guinea pigs and the rationale for its use potential in humans as a nutraceutical for modulating immune function, especially in the aged and the *dis*tressed.

COLOSTRUM: NATURE'S ZOONUTRIENT SUPERFOOD!

Phytonutrients and Zoonutrients

Just as certain fruits and vegetables are naturally rich in special health promoting chemicals called *phyto-nutrients*, certain animal sourced foods are rich in special health promoting compounds called *zoo-nutrients* (pronounced zoo'-o-nutrients). Zoonutrients may be defined as compounds found in foods derived from animals beyond the usual macronutrients (fats, proteins, carbohydrates) and micronutrients (vitamins and minerals) that nonetheless have a salubrious effect on physiology. Examples include immunoglobulins, bio-active peptides, enzymes, glycol-conjugates, special amino acid bonds (double cystine).[1]

Colostrum

The richest of all zoonutrients is the "first meal" of all mammals, called colostrum. Colostrum provides a macro and micro nutrient dense "super food". This "first meal" for

all mammals is also rich in antibodies (Ig), cell signaling messengers (cytokines) that modulate the immune system, and growth factors stimulating stem cell activity.[2]

Colostrum is 50% or more protein, and rich in fat, vitamins and minerals. It is also the most zoonutrient rich of all foods providing immune protein sub-fractions and peptides, growth factors and other lesser known zoonutrients. The fats in colostrum provide essential fatty acids for cell development. Colostrum is also rich in sphingomyelin, cephalin, phosphatidyl choline and phosphatidyl serine that assist in development of the mucosal barrier, liver function, brain function, and/or immune function. The vitamins include thiamin, riboflavin, pantothenic acid, pyroxidine, folic acid, vitamins E, C, B12, beta carotene and retinoic acids. Riboflavin is by far in the richest concentration as pertains to percents of daily value (DV), followed by folic acid. The minerals include calcium, chromium, iron, magnesium, phosphorus, potassium, sodium and zinc, with calcium being the most abundant as pertains to percent of daily value (DV) provided.[3,4]

Growth factors in colostrum help activate stem cells. The potential for growth factors in the adult to help signal growth, repair and regeneration of tissue, especially when combined with stem cell therapy, has garnered much interest recently.[5] These growth factors include growth hormone (GH), insulin type growth factor (IGF-1), insulin type growth factor (IGF-2), transforming growth factor (TGF-alpha), transforming growth factor (TGF-beta), epidermal-GF, fibroblast-GF, and platelet-derived-GF.

Colostrum is rich in *passive* immunity factors, the antibodies named immunoglobulin types G1 &2, A, M, D, and E. These gamma globulin proteins are used by the immune system passively to identify and neutralize foreign objects, such as bacteria and viruses.

Some of the lesser known zoonutrients include the lacto-oligosaccharides which

promote the growth of good bacteria in the intestine; the sialyl-oligosaccharides and sialyl-glycoconjugates which also have an immune function in the intestine and are also involved in brain and nerve development, and in cell to cell recognition.[6]

Proline Rich Poly Peptides (PRP)

Colostrum is also rich in bio-active immune modulating peptides variously known as info-peptides, cytokine precursors, colostrinin, colostrinine, transfer factor and proline rich polypeptides (PRP).[7,8]

PRPs are short chains of amino acids, called polypeptides, with a molecular weight of 6000 Daltons, with a high concentration of the amino acid proline. They support the regulation of the thymus, the gland responsible for the normal development of immunologic function in the body. [9,10,11] The concentration of PRPs in whole colostrum powder is between 1-3% of the total powder weight. Specially fortified whole colostrum contains 5-6 % PRPs![12] It should be noted that many manufacturers of colostrum powders remove various amounts of the PRP fraction, lactose, minerals and water, using ultra-filtration technology, to elevate the protein / IGG content of the powder. In concentrating the *passive* Ig antibodies they sacrifice some of the *active* immune modulating cytokines of whole colostrum powder.

Proline rich polypeptides promote proper response to microbial invaders, toxins or allergens through cytokine modulation and natural killer cell (NK) activity.[13,14,15,16,17] NK cells, large lymphocytes that circulate in plasma, comprise of 10-15% of the lymphocytes in human blood. Of all the immune system's soldiers, NK cells are the most aggressive. NK Cells provide the front line of defense and as such are specially equipped to locate and kill diseased cells. NK cells attach to the surfaces of foreign substances, and

inject a chemical "grenade" (granule) into the interior. They are our first line of defense against mutant and virus infected cells like Severe Acute Respiratory Syndrome (SARS) or Bird Flu Virus.[18,19]

Th1/ Th2 Modulation

PRPs provide anti-viral, anti-allergy, and anti-inflammatory functions by stimulating T helper lymphocytes type 1 (Th1) responses to infections and tumors and lowering T helper lymphocytes type 2 in Th2 dominated hypersensitivity related inflammatory conditions. Th1 cells, which modulate *cell-mediated immunity*, produce the cytokines IL-2, IFN-gamma, and TNF-alpha. Th2 cells, which modulate *humoral immunity*, or antibody production, produce IL-4, IL-5, IL-6, IL-10, and IL-13. PRPs tend to stimulate Th1 and lower Th2 if these are in imbalance; i.e., PRP *modulates* Th1/Th2.
This is known as the "Th2 to Th1 shift to the left".[20,21]

Th1 helper responses are important in defense against viruses, bacteria, fungi, parasites, cancer and intracellular organisms. Cell-mediated immunity can be tested by delayed hypersensitivity skin testing, response to non-specific or specific mitogens (lymphocyte transformation) and allo-antigens (self-recognition).

If one has a Th2-dominated condition, with decreased cellular immunity and heightened humoral immunity, the conditions that tend to prevail are allergies, chronic sinusitis, atopic eczema, asthma; systemic autoimmune conditions such as SLE and mercury-induced autoimmunity, vaccination-induced reactions; malaria, parasite infestations, chronic giardiasis and candidiasis, viral infections, hepatitis C, AIDS and certain cases of autism, hyper-insulinism, hyper-cortisolism, cancer, and ulcerative colitis.[22,23]

Allergy is the result of a complex immune cascade leading to the dys-regulated production of Th2 cytokines, the generation of allergen-specific IgE-producing B cells and the subsequent activation and degranulation of mast cells upon allergen challenge.[24,25]

ASSESSMENT OF ANTI-ALLERGIC PROPERTIES OF PRP ON SYSTEMIC ANAPHYLAXIS IN GUINEA PIGS

Material and Methods

This study was commissioned to investigate the anti-allergic properties, if any, of PRP on systemic anaphylaxis.[26] It was conducted with both male and female guinea pigs at The Institute of General Pathology and Pathophysiology, Moscow, Russia. Egg Protein (ovalbumin) and histamine were used as antigens. Egg protein and histamine were injected into animals by using compressor nebulizer. The effect of pre-treatment PRP was measured upon repetition of both challenges.

Guinea pigs (250-300 g) were actively sensitized by a one time injection of 10 g Ovalbumin (OA) and 100 mg aluminum hydroxide (Al(OH)3) in 1ml total volume: 0.2 ml intramuscularly in thigh, on two sides, and 0.6ml intra-abdominally. (P. Anderson Method, 1980)

The experiment began 5 weeks after sensitization, when IgE antibodies were formed in high enough quantities. Bronchial spasms in the animals were initiated quickly after the injection of either antigen (histamine and ovalbumin).

Experimental Design

Histamine Model

Measurements of bronchial spastic duration time after histamine introduction was

used as an internal control. A 0.2% histamine solution containing 0.9% of NaCl was inhaled by the guinea pigs, until first signs of bronchial spastic reaction. Duration times of these reactions were measured. Acute phase reactions, with the guinea pigs lying on their side exhibiting deep breathing at a frequency of 10-15 breaths per minute, were experienced by all the animals and averaged 225 ± 8.4 seconds. Sub-acute phase reactions, with the guinea pigs sitting up while exhibiting increased chest muscular activity with a breathing at a frequency of 40-50 breaths per minute, were experienced by all the animals and averaged 495 ± 51,0 seconds.

Then, 24 hours later, an introduction of PRP thru 5-10 sprays into the mouth was administered 30 minutes before repeated histamine inhalation and evaluation. Acute phase reactions were experienced by all the animals and averaged 207.5 ± 50.4 seconds. Sub-acute phase reactions were experienced by all the animals and averaged 430.0 ± 53.1 seconds. These initial tests demonstrated no anti-histamine action of PRPs.

Egg Protein Model

The egg protein sensitization experiment was conducted on guinea pigs actively sensitized by egg protein. Exposure to the antigen induced a bronchial spastic reaction. The animals were dived in two groups, one pre-treated with PRP and one not. PRP was delivered by 10-12 sprays into mouth-throat 30 minutes prior to the antigen challenge. The egg protein in dose of 2.5 g/kg was given to animals of each group. This egg albumin, dissolved in 1 ml of 0.9 % NaCl solution, was inhaled during 3 min with nebulizer.

In the animals not pre-treated with PRP, the results were as follows. Half (3 of 6) of the animals died within 140 to 260 seconds. In the survivors, the acute phase reaction

lasted 313.3±37.1 seconds. The sub-acute phase lasted 546.7±208.0 seconds.

In the animals pre-treated with PRP, 2 of 7 animals died (29%). In the 5 survivors, 3 (60%) had no acute or sub-acute phase reactions. Two (40%) of the 5 surviving animals had acute phase reaction that lasted only 16. 0 ± 11.7 seconds. None (0)% of the 5 surviving animals had sub acute reactions.

Conclusions

PRP didn't affect on the development of systemic anaphylaxis induced in sensitized guinea pigs by exogenous histamine. PRP showed clear anti-allergic activity and inhibited the development of systemic anaphylaxis induced in sensitized guinea pigs by egg albumin.

Discussion

As explained previously, when our immune systems do not react strongly enough to infectious agents or neoplastic cells, or are overwhelmed by them, it may have developed in part because of a down regulation of Th-1. When our immune systems are over-reactive we may have Th-2 up regulation that manifests as heightened allergic and chemical sensitivities and even auto-immune disease.

It is proposed that egg protein sensitization lead to an over-activity of Th2 as seen in asthma, for example. It is clear that PRP do not act as anti-histamines. The dramatic increase in survival rates and decrease in acute hypersensitivity reactions, and complete prevention of sub-acute hypersensitivity reactions, supports the purported ability of PRP to modulate immune function, specifically the a "shifting" down of Th2.

With this understanding, in this experiment we would not conclude that proline rich

polypeptides (PRP) treat any allergic symptoms or related diseases. Rather, these studies support the hypothesis that PRPs that can send signals to the thymus to help the immune system regain its proper balance (modulation), in this case apparently down regulating Th2, thereby lessening the generation of allergen-specific IgE-producing B cells and the subsequent activation and degranulation of mast cells upon allergen challenge (ie., allergy).

ENDOCRINE-IMMUNOLOGY NETWORK

Distress physiology expresses itself by a release of thalamic cortico-tropin releasing hormone factors (CRF) leading to a cascade of corticotrophin releasing hormone (ACTH), vasopressin and oxytocin via the pituitary and cortisol secretion via the adrenal glands. Cortisol cause a Th1/ Th2 "shift to the right", up regulating Th2 *humeral* immunity and inhibiting Th1 *cellular* immunity, known as the "Th2 Dominant Profile". [27]

This is the same profile registered during acute and chronic distress situations. These cortisol effects on the immune system are mediated by the thymus, mainly. Specifically, cortisol induces thymic apoptosis of the immature T lymphocytes but not of immature B cells. Increased corticosteroids thereby cause a decrease in macrophage progenitors and differentiation of the monocyte cell line to macrophage cell line while increasing granulocyte (polymorphonuclear leukocytes or PMN) progenitors.

The Th2 dominant profile favors anti-inflammatory activity of Th2 and decreased Th1 surveillance of infectious and tumor-producing agents. This explains why medicinal corticosteroids favor humoral (Th2) immunity, and interfere with cell-mediated (Th1) immunity.[28]

Distress physiology is also associated with a decrease in thalamic growth hormone

releasing hormone (GHRH) with resultant decreases in pituitary growth hormone (GH) release. Progressive reduction of GH secretion by the pituitary hypophysis is also observed with normal aging. As GH is a thymopoetic factor, this results in decreased thymopoeisis. Decreased thymopoeisis results in decreased naïve T cells while not adversely affecting memory T cells. Indeed, both the thymus and GH fade progressively with aging, resulting in a typical aging pattern of fewer naive T cells while memory T cells remain normal.[29] Exogenous GH is able to trigger thymopoeisis in adults and elder people.[30] This favors reappearance of naive T cells. GHRH and GH have been used in the treatment of many stress-associated (Th2) diseases.[31] Various thymotropic pharmaceuticals and nutraceuticals endeavor to accomplish similar goals.[32,33]

Colostrum active peptides (PRPs) also promote thymopoeisis of naïve T Cells and modulate Th2, generally promoting a "shift to the left", down regulating Th2 and up regulating Th1, if these are out of balance.[34]

Hormonal and Immune Functional Relations in Aging

In summation, neuro-hormonal disorders (cortisol, adrenaline, free serotonin, etc.) registered during uncontrollable distress are important factors which cause a Th1 / Th2 shift to the right, favoring lowered resistance, especially to new infectious agents, tumor progression and metastasis, and heightened allergic and autoimmune responses.

It is also amply accepted that past forty GH secretion diminishes and cortisol rises. With increasing age and / or *dys*-stress we usually observe increasing cortisol (with decreasing DHEA) and decreasing GH combined with thymic atrophy resulting in decreased thymopoeisis and a Th2 dominant profile.[35] This results in decreases in native

T cells and NK cells with lowered resistance to infection and lowered tumor surveillance (Th1↓) and a greater sensitivity to or worsening of allergic and auto immune disorders (Th2↑).[36]

Anti-aging health professionals may endeavor to effect immune function through modulating the hormones of youth (GH) and the hormones of aging (Cortisol/DHEA) to a more optimal and youthful balance. They may use various extracts of thymus tissue. The objective of the paper has been to present that PRP have the potential to be used toward the same ends of modulating immune status, namely Th1/ Th2 balance, and the benefits obtained thereby, as a coincident therapy to hormone and /or glandular therapy, or as a more conservative therapeutic or immune modulating stand alone nutraceutical support.

PROLINE RICH POLYPEPTIDES (PRP) AS NUTRACEUTICALS

PRP's can be extracted from colostrum using ion exchange and nano-membranes. PRP's are small polypeptides that when separated from natural milk liposomes, glycol-macro peptides (GMP) and protective anti–enzymes found in whole colostrum and whey may not survive the alimentary tract. PRPs, being less than 10,000 Daltons, should be absorbable sublingually.[37]

PRPs may be protected by liposomal delivery and allow better absorption orally. Presuming efficacy, whole colostrum and whey isolates/ concentrates can be fortified by PRP nano-encapsulated in liposomes for use pills and functional foods.[38,39,40,41,42]

Conclusion

Colostrum is rich in active immune peptides called Proline Rich Polypeptides (PRP).

PRP demonstrate immune modulating potential in the animal hypersensitivity model.

PRP may well have potential as a conservative immune modulator nutraceutical in those patients who experience neuro-endocrine / immune imbalance related to high cortisol and low GH levels associated with stress and aging.

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