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Review

Natural Health Products, Modulation of Immune Function and Prevention of Chronic Diseases

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The immune system is increasingly found to be involved in the development of several chronic illnesses, for which allopathic medicine has provided limited tools for treatment and especially prevention. In that context, it appears worthwhile to target the immune system in order to modulate the risk of certain chronic illnesses. Meanwhile, natural health products (NHPs) are generating renewed interest, particularly in the prevention and treatment of several chronic diseases. Over 20 scientists from fields related to immune function and NHPs were thus convened to establish the state of knowledge on these subjects and to explore future research directions. This review summarizes the result of discussions held during the symposium. It thus seeks to be thought provoking rather than to comprehensively cover such broad areas of research. Notably, a brief overview of the immune system is presented, including potentially useful targets and strategies to keep it in an equilibrated state, in order to prevent certain disorders. The pertinence and limitations of targeting the immune system to prevent chronic diseases is also discussed. The paper then discusses the usefulness and limitations of current experimental tools available to study the immune modulating effects of NHPs. Finally, a concise review of some of the most studied NHPs showing promising immunomodulatory activity is given, and avenues for future research are described.

Keywords: NK cells – Cytokines – Microparticles – Probiotics – Natraprevention – Vitamins – n-3 fatty acids – Green tea – Echinacea – Ginseng

Introduction

This concise review presents an expanded summary of discussions held during a symposium entitled 'Maintenance of an efficient and equilibrated immune system through the novel use of natural health products' and that was the object of a recent meeting report (1). The symposium brought together between 20 and 30 scientists in disciplines ranging from herbalism, ethnobotany and phytochemistry, through to basic and clinical research in fields such as immunology, cancer and nutrition. It was held in Montreal, Canada, on September 23 and 24, 2004 under the auspices of the Lucie and André Chagnon Foundation, Canada's largest philanthropic organization. Participants were asked to discuss the relationship between the modulation of immune system functions and the prevention of chronic diseases, and to explore natural health products (NHPs) that may find novel and judicious use in this context. Other symposium details, including the list of participants, can be found in the published meeting report (1).

The complexity of the immune system is secondary only to that of the central nervous system although the immune system is older in evolutionary terms (2). Our immune system is often conceptualized as possessing two principal components: innate and adaptive immunity, which work in concert to defend the body against infection.

The innate system is ancestral and present in invertebrates as well as vertebrates. It is neither anticipatory (no memory) nor clonal and does not respond to environmental changes. The

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innate system reacts directly to a wide variety of microorganisms through receptors for highly conserved and essential microbial molecules, such as lipopolysaccharides present on the cell wall of Gram-negative bacteria and lipoteichoic acids present in Gram-positive bacteria. Indeed, the cells of the innate immune system represent the first line of defence in the immunosurveillance network. The primary cells involved in the identification and spontaneous lysis of offensive targets (virus-infected cells, tumor cells, bone marrow stem cells and embryonic cells) are natural killer (NK) cells, which have been the object of several ground breaking studies in the later part of the last century (3–8). These cells originate from the bone marrow, from which they travel in a unidirectional and almost exclusive way to the spleen; very few NK cells are found in lymph nodes or other secondary sites such as the gut-associated and bronchi-associated lymphatic tissue (3,9–15). Nonetheless, NK cells are lymphoid in morphology and need cells of the macrophage/monocyte lineage for the full expression of NK cell production or function. NK cells are numerically and functionally low in youth and old age, and maximal during the reproductive periods in mammals. Moreover, an inverse relationship exists between the rise and fall of NK cells and the incidence of tumor growth. This phenomenon is believed to be more than coincidental. The studies of Dussault and Miller (16–18) have elucidated some of the factors underlying this relationship. In elderly mice, bone marrow NK cell production and their capacity to bind onto offensive target cells are significantly reduced relative to that of the young adult mammal, thus attenuating any potential for tumor cell lysis. In contrast, very young mammals produce NK cells at a level comparable to that of young adults, but there exist NK-suppressive factors in the microenvironment of the spleen that prevent NK cells from lysing offensive targets. In humans, this corresponds to a time when pediatric leukemias and lymphomas are prevalent. This demonstrates, in part, the importance of NK cells and of the adaptive system in general for the maintenance of optimal immune function.

In contrast, the adaptive system, also termed specific immunity, is acquired through interactions with the environment. It is subject to induction, anticipation (immune memory) and clonal expansion. Adaptive immune responses are central to the body's ability to eliminate bacterial, viral and parasitic infections. Understanding these responses is the key to understanding the phenomena of allergy, autoimmunity, vaccination, cancer and organ graft rejection/acceptance. Adaptive immunity has been known for over a century and an abundant literature exists, which is fully reviewed in classical works such as those of Roitt and Delves (19) and Paul (20). The cells involved in adaptive responses are thymus (T)-derived cytotoxic T lymphocytes and helper T lymphocytes (the latter existing in two subsets: Thl and Th2), bone (B) marrow-derived lymphocytes and assorted accessory cells (dendritic cells, macrophages and stromal cells). Lymphoid tissue is distributed all over the body. Lymphocytes (along with several of their accessory cells) circulate to and through the spleen, lymph nodes, specialized lymphoid tissue in the gut (Peyer's Patches and appendix), bronchi and oropharynx (adenoids and tonsils). These 'secondary' sites serve as clonal generating stations for T and B lymphocytes, once the appropriate antigen (from bacterial, viral or parasitic sources) has been encountered. Thus, an army of identical and antigen-specific lymphocytes is uniquely directed at the offensive antigen, ensuring its elimination in normal animals, including humans. Cytotoxic T lymphocytes destroy virus-infected cells by making direct contact with the latter in order to induce cytolysis. Th1 cells, on the other hand, 'activate' the appropriate accessory cell, and it is the latter that then delivers the cytolytic blow. Th1 cells are the target of the AIDS virus, and when the vital Th1 cells are crippled throughout the body (advanced AIDS), opportunist infections ultimately kill the infected host (human or animal). Th2 lymphocytes activate B lymphocytes whose function is to produce an appropriate antibody against the offensive antigen, leading to its destruction and elimination.

The innate and adaptive systems are highly integrated and interdependent (21). In addition to being a requisite for adaptive immunity, the innate system is responsible for the early killing and clearance of infectious pathogens and the resolution of the inflammatory response. The most important means by which the immune system communicates internally and externally is through the use of cytokines, small soluble signaling molecules. Cytokines play a crucial role in the selection, initiation and modulation of an appropriate immune response. The signaling network of cytokines is characterized by its complexity (synergy and antagonism) and by its redundancy (parallelism) that ensure the speed and flexibility required for an effective immune response.

The immune system can give rise to several disorders when it is weakened or overactivated (Table 1). For instance, a defective adaptive immune response can lead to recurrent infection despite previous encounters with antigens, to lowered tolerance to self-organs and tissues leading to organ-specific autoimmunity, and to faulty recognition and elimination of transformed cells leading to cancer. However, an overactivated innate immune response can cause chronic infection because of inefficient clearing of pathogen or chronic inflammation due to an inefficient regulation or resolution of the inflammatory response. Chronic inflammation can lead to extensive non-specific destruction of neighboring tissue. In fact, inflammation and the immune system are intimately tied. In addition,

Table 1. Health effects of imbalances of the immune system

Immune system status	Attributable pathologies
Weak	Prone to opportunistic infectious diseases
	Prone to cancer establishment and tumor escape
Overactivated	Chronic inflammation and autoimmunity (e.g. Type 1 diabetes)
	Heart disease, cancer (e.g. lymphoid)
	Skin disease, allergies and asthma
	Joint and tissue destruction

inflammation is increasingly found to be involved in the development of several chronic diseases such as arteriosclerosis, diabetes, neurodegenerative diseases and even cancer. Immune dysfunction has also been linked to conditions such as chronic pain, anxiety and depression, albeit sometimes as a consequence rather than a cause, and may be involved in other disease processes in a manner that is currently not fully understood (22). Indeed, its diverse influence upon health may be in part due to its evolution as a defence mechanism and in part due to its origins, possibly as a sensory organ (23).

Is the Immune System a Good Target to Aim at In Order to Prevent Chronic Disease? Yes, But. . .

A first question to address is whether the immune system is a good target to select in the fight to prevent chronic diseases. Consensus is generally positive with some important reservations being brought forward. In fact, it is accepted that the immune system represents our arsenal to combat infection and disease. As mentioned, an underactivated immune system increases risks of opportunistic infection and uncontrolled neoplastic tissue growth, while an overactivated immune system leads to many afflictions, including inflammation, allergy and autoimmunity. Hence, an unchecked and unbalanced immune system hampers good health and quality of life. This explains the consensus 'Yes' given as an answer by the experts regarding the pertinence of targeting the immune system to prevent chronic disease.

However, it must be noted that the immune system carries out its important defensive function in intimate and coordinated interaction with the nervous and endocrine systems (24,25). The innate complexity of the immune system and of its communication tool, the cytokines, is thus further complicated by these interactions with the nervous and endocrine systems. Some concern also arises when considering that targeting the immune system may be too reductionist an approach, particularly when dealing with complex pathologies like cancer. These considerations form the basis of the 'but. . .' part of the answer given in the title to this subsection.

Promising Research Avenues and Markers

It is important to consider that invertebrate species lack adaptive immunity but are clearly able to deal effectively with infections. It is also interesting to note that these organisms do not appear to develop cancer or autoimmune disease (at least not in any readily recognizable form), but they do demonstrate senescence (26–28). Therefore, the apparent absence of these age-related diseases in invertebrates could support an evolutionary argument to focus disease prevention upon maintenance of efficient and equilibrated innate immunity.

If this line of reasoning is correct, then the immune cells of greatest interest are ones of the NK cell lineage described above. These immune cells are present in invertebrate and vertebrate species and serve as a link between innate and adaptive immunity in vertebrates, as mentioned (25). Indeed, NK cell function has been identified as a reliable measure of immunotoxicity for use in pharmaceutical safety testing (29) and could perhaps provide a means to monitor immune system equilibrium.

Another important point to consider relates to the problem of markers for immune system function. It is difficult to reach a consensus on the appropriate markers to use in order to monitor the maintenance of a healthy immune system, primarily because biological heterogeneity precludes readily identifiable thresholds for concern in the prevention of chronic disease. However, there exist potential novel avenues to explore, including the use of NK cell activity, as mentioned above, or the use of a constellation of markers, of which cytokines and cytokine receptors would be a major part. Much has been written about the validity and validation of biomarkers for health monitoring (30). While it is beyond the scope of this paper to discuss these issues at length, it is worth highlighting some general considerations. Firstly, biomarkers must be relevant to the question being asked and practical to use in the desired setting since advances in analytical sensitivity can now provide detection limits at concentrations often below those that have biological importance (31). Secondly, it is generally easier to define thresholds for intervention (i.e. when a disease requires treatment) than for early detection or prevention (i.e. before onset of the disease). Indeed, thresholds for disease prevention require a good definition of what constitutes the 'normal' condition. However, biological heterogeneity and the redundancy present within the immune system makes the establishment of precise 'normal ranges' especially difficult. Therefore, although NK cell activity and cytokine assays can be used as reliable measures for treatment intervention (32,33) they have yet to be shown to be practical biomarkers for defining a threshold for the early detection of chronic illnesses.

Finally, one interesting alternative to conventional immune system biomarkers is the presence and quality of microparticles present in the blood. Such microparticles, including exosomes, originate from several cells, including some prominent immune cells, and may represent a novel type of intercellular signaling mechanism (34,35). Hence, research into the development of novel and pertinent biomarkers will certainly contribute to finding ways to modulate the immune system and prevent certain chronic diseases.

Tools to Study the Immune System and NHPs

In this section we briefly review several experimental models and approaches that have been used to study immunomodulatory NHPs. Table 2 summarizes the advantages and limitations of each modality. Human clinical studies remain the gold standard upon which scientists and health care professionals can rely to determine the usefulness and efficacy of a given NHP. Nonetheless, animal models, *in vitro* bioassays, and

Table 2. Tools to study NHPs as related to the immune system

Experimental models/tools	Pros	Cons
In vitro measurement on cell culture	Can target specific effects of the studied product on specific cell types	Simplistic model, systemic effects not taken into consideration
	Very helpful for investigating cellular and molecular mechanisms	NHP administration generally not physiological
	Large number of very well characterized systems with efficient markers of activation/inhibition	
Genomics/proteomics	New analytical tools	Cost of utilization
	Very precise results	Availability of source material
	Can be used in fundamental research as well as clinical trials	
Animal models	Mouse has a very well characterized genome	Despite high similarities with man, uncertainty whether mice and men react the same way
	Complete living organism	Difficulty in measuring environmental components on a mouse
	Genetically modifiable (transgenic and knock out mice)	
	Large number of well characterized models for several human pathologies	
Clinical trials	Gold standard for safety and efficacy	Weakness or lack of proper markers to evaluate success of intervention
	Direct and systemic effects measured on health	Improper administration/standardization of NHPs
Epidemiological studies	Large-scale studies on the population	Challenge in scientifically measuring dietary exposure in the population
	Can include both the environmental and germ line components of disease	Large cohorts needed for significant conclusions

modern proteomic and genomic technologies can be most helpful in delineating the mode of action of NHPs. Finally, epidemiological studies bring a wealth of information on the influence of genetic (germ line) and environmental factors on health outcomes in the general population and pertinent subgroups.

In addition, it is generally recognized that a great effort should be placed on the standardization of methods used to study immune modulation by NHPs. In terms of herbals, this also includes a crucial component of standardization of the herbal preparations themselves. As mentioned in Table 2 above, the greatest inconsistencies of clinical research related to immunomodulatory NHPs are caused in major part by the lack of quality control of the source materials used (36). The US agency NCCAM came to a similar conclusion after its first 5 years of functioning and is now focusing on more preclinical studies to prepare better clinical studies, notably with herbal products (http://nccam.nih.gov/about/plans/2005/index.htm).

NHPs having Immunomodulatory Activity

During the last century, modern medicine has contributed major advancements to sanitation and public health initiatives and discoveries like antibiotics and vaccination. These efficiently hindered the progression of infectious diseases through the population. In fact, modern medicine has succeeded best in treating acute illnesses. It is in the realm of chronic diseases that modern medicine and drugs have been more disappointing and that NHPs have elicited resurgent interest. In this section, we review the evidence for the immunomodulatory action of several NHPs, including probiotics, products of animal origin (principally milk and thymic extracts), nutritional factors (especially vitamins, minerals and fatty acids) and herbal products. Table 3 summarizes the strongest evidence brought forward for some of the most promising NHPs. Readers are also oriented to recent reviews (37,38) for complementary information.

The gastrointestinal (GI) tract and the skin epidermis are the major barriers humans possess to confront the external environment. Both are responsible for maintaining systemic integrity and use lymph nodes as efficient immune checkpoints. The GI tract distinguishes itself by holding the greatest number of lymph nodes, and also by being the most intricate and complex immune organ. Probiotics are live bacteria that colonize the GI tract. They have received a lot of research attention, particularly in clinical studies in children and the elderly (39–42). Indeed, they positively affect general health going from the development of the immune system in infants right after birth to the enhancement of general immunity in the elderly (critically exposed to immunosenescence). Probiotics offer much promise because they appear not only to help with the maturation of the visceral immune system but also to equilibrate the body's defence mechanisms. In that sense, they act as true immunomodulating agents, enhancing immunity when it is weakened or developing, and buffering exaggerated reactions (e.g. allergies and inflammatory bowel disease). They also represent the only NHP category that research has shown to have well established curing

Table 3. Promising NHPs exhibiting modulatory effects on the immune system

NHP source	Prevention	Treatment
Probiotics	Helps immune system maturation (96)	Helps manage atopic eczema/dermatitis (97)
	Helps prevent allergies (96), atopic disease and hypersensitivities in infants (98,99)	Promise in treating inflammatory bowel disease (IBD) through immune modulation (100,101)
	Enhances immunity in the elderly (102,96)	
Green Tea (EGCG, flavonoids . . .)	Daily intake could prevent tumor growth (65,103,104)	Potent antiangiogenic effect (66–68)
		Potent anti-inflammatory effect (105,106)
Vitamins C and E, n-3 fatty acids	Antioxidant: decrease oxidative stress in several chronic diseases (73)	Helps manage arthritis, asthma and IBD (76,75)
	Anti-inflammatory: n-3 fatty acids (74)	
Echinacea	Enhance antitumor immunity (mouse NK cells) (107)	May help treat upper respiratory tract infection (108)
	Enhance NK cell immunity in aged mice (107)	Putative antifungal properties (109) and antiviral properties (110)
Ginseng (ginsenosides)	Immunosuppressant properties (111)	May have benefit as an adjunct to cancer therapy (112)
	Anti-inflammatory properties (113)	
Ginseng (polysaccharides)	Immune stimulating properties (NK, macrophages and antibody production) (114)	Helps clear infections agents like *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans* in mice (115)

as well as preventive properties. Probiotics exert their actions principally by

1. Improving the intestinal immunologic barrier function through spatial exclusion of other pathogenic microbial that could otherwise colonize the gut (43,44).
2. Restoring normal intestinal permeability (45) in the face of inflammatory or allergic diseases by still ill-defined mechanisms that include cell wall and DNA components playing the role of immunomodulatory products (46) and by lowering the production of pro-inflammatory cytokines (47,48).
3. Synthesizing vitamins (folic acid, biotin and other group B vitamins, vitamin K and so on) (116).

Suggestions have been made that probiotics may absorb heavy metals (49–51) and thus help their elimination through the feces. Probiotics may also promote the production of enzymes that help decrease the severity of certain food intolerances/allergies (52). However, these actions have not been fully documented.

Data on milk peptides, bovine colostrum and thymic extracts are less convincing in terms of immune modulation, most of the research having been done in cellular models *in vitro* and showing contradicting results (53–64). Several peptides released from enzymatic hydrolysis of milk proteins were reported to modulate immune functions such as phagocytosis, lymphocyte proliferation and cytokine production. The type of enzymatic treatment has an influence on the immunomodulatory functions of peptides generated from milk proteins and, without standardized procedures, it is difficult to drawn conclusions from those studies. Further research, especially clinical trials, will be required to identify the impact of those products on the immune system in humans.

However, stronger evidence was presented to support the usefulness of a number of micronutrients to prevent certain chronic illnesses. A new term coined 'nutraprevention' is applied to the use of empirical combinations of foods, spices and other food-grade herbals in the fight against cancer (65). So far, this approach is based on epidemiological studies clearly showing relationships between cancer prevalence and food consumption in different populations around the world. Moreover, research is now showing potent antiangiogenic activity for polyphenolic components of green tea (66–68); angiogenesis being the process by which growing tumors promote the development of new blood vessels to ensure their survival. Several plant constituents, including soy isoflavones (69,70) and compounds in cruciferous vegetables (71,72), are also known to modulate enzymes involved in xenobiotic biotransformation, namely, the activation or detoxification of 'foreign', pro-carcinogenic chemicals. Vitamin C has been clearly linked to the modulation of immune function, particularly in the context of novel research showing improved cognitive status in patients with neurodegenerative disorders (73), which are increasingly shown to involve an immune dysfunction component. Similarly, polyunsaturated fatty acids, in particular the now famous ω-3 fatty acids, have been used successfully in the management and prevention of several inflammatory and allergic diseases (74–76). However, clinical research on lipids has yielded inconsistent results concerning their potential to modulate immune system function. This state of affairs is related to poor study design and to the great variability in immune cells selected and methods used to assess immune function.

Intake of green tea, *Brassica* vegetables (e.g. broccoli, cabbage and others) and soy foods has been shown to be associated with lower risk of several cancers in epidemiologic studies [reviewed in (77–82)]. Furthermore, data from experimental studies suggest that green tea and its constituents, such as isoflavones, may modulate markers of inflammation and immune function (83–87). A limited number of herbal products also show promise in the realm of immune modulation.

It is notably the case with *Echinacea* that has received significant scientific attention in the past two decades, particularly in studies on upper respiratory tract infections (36,88,89). Among its many immune modulating activities reported, activation of macrophages has been demonstrated most convincingly. However, novel mechanisms of action include effects on B and T lymphocytes as well as NK cells (90,91). In particular, Currier and Miller (90) observed that upon incorporating *Echinacea* in the daily diet of elderly mice for only 14 days NK cell production in the bone marrow, as well as the number of mature functional NK cells reaching the spleen, increased to levels found in young adults (92). Brousseau and Miller (93) have also revealed that daily dietary intake of *Echinacea* throughout life, beginning in youth, increased the number of individuals reaching old age to 74% as opposed to 46% in control animals. Thus, any herbal treatment that would augment NK cells would clearly be worthy of investigation for its therapeutic/prophylactic potential, especially in the realm of immune modulation.

Ginseng is the other herbal that shows much promise as an immune modulating NHP. Indeed, it appears to have both immunostimulant and immunosuppressant activities, which would lend credence to the view that ginseng is an 'adaptogenic' NHP. Immunostimulant activities have been attributed to polysaccharide fractions, notably acting on both macrophages and B lymphocytes (94,95). However, ginsenosides display immunosuppressant activity *in vitro* by inhibiting cytokine release in activated macrophages (I. Lemaire, unpublished results). Hence, further research is warranted on ginseng and other adaptogenic herbals to define their potential use in preventing chronic diseases related to immune dysfunction.

Summary and Conclusion

Dysfunction of the immune system is clearly implicated in the development of several chronic diseases. Hence, attempting to maintain an efficient and equilibrated immune system is a valid approach to prevent certain chronic illnesses. However, this approach is limited by the great complexity of the immune system and its close interactions with the nervous and endocrine functions. This is further hampered by the difficulty in identifying reliable and convenient analytical tools to assess immune function and its modulation. In that context, interesting areas for future research include NK cells, constellation of cytokine markers and receptors and assessment of blood-borne microparticles. Despite these limitations, research on NHPs has shown that certain products offer novel avenues to modulate the immune system and help prevent chronic diseases. The most promising ones are probiotics, nutraprevention (combinations of biologically-active fruits, vegetables and spices) and so-called adaptogenic plants (e.g. echinacea and ginseng). Notwithstanding these encouraging results, future research efforts with NHPs will need to address the important issues of standardization in both product quality and research methodology. Among areas to favor in the research agenda on NHPs and immune modulation, this review highlights the following needs: (i) studies on the interaction between NHPs and immune cells, particularly the influence of NHPs on the innate immune system; (ii) epidemiological studies and clinical studies with NHPs in chronic illnesses where immune dysfunction may play a role. Finally, social/economic impact of NHPs in health care must also be considered.

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