Polyphenols and Immunity

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28 Polyphenols and Immunity

Tirang R. Neyestani

Abstract

Polyphenols are the most abundant antioxidants in our diet. The pro-oxidant/antioxidant balance is believed to be an important determinant of the immune cell function as the rather high percentage of polyunsaturated fatty acids (PUFAs) in their cell membranes has made the immune cells particularly sensitive to oxidative stress. Polyphenols as dietary antioxidants may affect various aspects of both innate and adaptive wings of the immune system by shifting pro-oxidant/antioxidant balance toward antioxidant. Complement system, for instance, has been shown to be inhibited by polyphenols and this complement inhibitory effect may have some role in anti-inflammatory properties of polyphenols. The anti-inflammatory effects of polyphenols, which may be exerted at the molecular level, are likely to be dependent on the specific structure of polyphenolic compounds. Macrophage functions, including cytokine production, may also be affected by some flavonoids through modulation of inducible cyclooxygenase (COX-2) and inducible nitric oxide synthase (iNOS). Many experimental studies have reported immune modulating effects of polyphenolic compounds on both humoral and cell-mediated adaptive immunity. On the other hand, oxidative stress has been proposed as to play a role in many clinical conditions including allergy, cancer, and atherosclerosis, among others. Many experimental as well as human studies have supported the prophylactic effects of polyphenols against these pathologies. These data altogether suggest that the effects are exerted through antioxidant-mediated immune modulation mechanisms. On the other hand, polyphenolic compounds may act as a pro-oxidant under certain situations and this may in part explain the inconsistencies often seen in results of different studies. Finally, the results of most, notably in vitro, studies on polyphenols and immunity can be hardly interpreted as to long-term general health of human. Therefore, further laboratory and clinical studies are still needed to clarify the effects of polyphenolic compounds on the immune function as well as on health status.

Key Words: Lycopene; immunity; antioxidant.

1. INTRODUCTION

Polyphenols are plant molecules entering our bodies through diet. The relationship between polyphenol-rich food consumption and reduced possibility of being affected by some diseases has attracted increasing interest from consumers, food manufacturers and nutritional scientists. Fruit and vegetable consumption may prevent cancers *(1)* and stroke *(2)*, whereas wine consumption may have similar effect in preventing coronary heart disease (CHD) *(3,4)*, and prostate cancer *(5)*. Soy consumption may have protective effects against cancerous cells and osteoporosis *(6,7)* and tea polyphenols may prevent different cancers *(6,7)* and arthritis *(9)*.

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The possible therapeutic effects of polyphenols against some parasitic *(10)* bacterial *(11)*, viral, and fungal *(12)* agents have been proposed. The anti-histaminic effect of polyphenols is the other interesting field having evaluated by some investigators *(13–16)*.

Considering the fact that the immune system is critical to the establishment and maintenance of good health by providing a first line of defense against infection and neoplasia and by contributing to overall homeostasis *(17,18)*, the subject comes to mind that polyphenols may have some immune enhancing effects on one hand, and antipathogenic effects directly on pathogens, on the other. Looking at the roles of oxidative stress in a variety of human diseases *(19)*, the effects of antioxidants on immune function *(20)*, and antioxidant properties of Polyphenols *(21)* it is likely that polyphenols exert their immune enhancing effects mostly by acting against oxidative stress.

2. POLYPHENOLS: DEFINITION AND CLASSIFICATION

A phenolic molecule is of plant origin. It is therefore impossible to know precisely the nature of all of the polyphenols that we ingest. Polyphenols are reducing agents, and together with other dietary reducing agents, such as vitamin C, vitamin E, and carotenoids, they protect the body's tissues against oxidative stress. Commonly referred to as antioxidants, they may prevent various diseases associated with oxidative stress, such as cancers, cardiovascular disease (CVD), inflammation, and others. Indeed, polyphenols are the most abundant antioxidants in our diet *(21)*. The chemical structure of a polyphenol will affect its biological properties: bioavailability, antioxidant activity, specific interactions with cell receptors and enzymes among the others.

The main classes of polyphenols are defined according to the nature of their carbon skeleton: phenolic acids, flavonoids and the less common stilbenes, and lignans. Phenolic acids are abundant in foods. The most frequent encountered are caffeic acid and, to a lesser extent, ferulic acid. Flavonoids, the most abundant polyphenols in our diets, can be divided into several classes according to the degree of oxidation of the oxygen heterocyclic: flavones, flavonols, isoflavone, anthocyanins, flavonols, proanthocyanidins, and flavanones. The main source of isoflavones is soy, which contains about 1 mg of genistein and daidzein/g dry bean *(21,22)*. These two isoflavones have estrogenic properties so they may have a role in prevention of breast cancer and osteoporosis *(7)*.

The structural diversity of dietary polyphenols is not limited to differences in the structure of the carbon skeleton and in the oxidation state of the heterocyclic of flavonoids. It is further complicated by varying patterns of hydroxylation of the phenolic rings, by glycosylation of most flavonoids, by acylation with phenolic acids and by the existence of stereoisomers, among others *(21)*.

3. THE IMMUNE SYSTEM

To endure the hazards of existence, the individual needs to be defended. Evolution of the species has generated various physiological systems that defend but two systems bolster innate defense with individual experience: the nervous and the immune. The nervous system keeps us out of trouble by its organs that sense, see, smell, and hear, and by its brain that learns, anticipates, and plans. The immune system defends us against dangers that are beyond the knowledge of the nervous system: infectious agents, foreign

cells and molecules, and abnormal cell arising within our own bodies. Like the nervous system, the individual immune system learns and remembers *(23)*.

The immune system is a complex system of molecules, cells, and tissues widely dispersed throughout the body, which interact in a coordinated and orchestrated manner to control and eliminate infectious agents, malignant and transformed cells, and other unwanted antigens. At the cellular level, the immune system is composed of various types of cells. Macrophages and related cells engulf and degrade bacteria and other antigens and present degraded fragments of them to bone marrow-derived (B) and thymus-derived (T) cells, the two classes of lymphocytes are responsible for specific immune function. Natural killer (NK) cells nonspecifically recognize and destroy transformed and virus-infected cells *(18)*.

The immune system is quiescent until antigenically challenged. Once challenged, the natural immune response is initiated through the activation of macrophages and NK cells and the production of various associated soluble factors. With sufficient antigenic stimulation of lymphocytes, an acquired immune response is triggered, resulting in the clearance of the stimulating antigen and in the generation of memory cells (primary response) that will, upon subsequent challenge by the same antigen, lead to its more rapid and efficacious clearance (secondary response) *(17)*. The immune response can thus be divided into two types: (i) adaptive immunity, which develops through primary antigen encounter and sensitization and is enhanced through repetition of stimuli, and (ii) nonadaptive immunity, often called natural immunity, which does not require sensitization and is not enhanced by repetition. Physical barriers such as skin and mucous membranes that protect the internal environment of human body, are also considered as components of natural or innate immunity.

Both natural immunity and adaptive immunity consist of cellular and humoral components (*see* Fig. 1). It should be re-emphasized that all of these components work coordinately and intimately. Phogocytic cells and NK cells are among the cellular components of natural immunity while their cytokines such as interleukin (IL)-1, and tumor necrosis factor-α (TNF-α) play their roles in natural immunity as humoral factors *(24)*. Lymphocytes have undoubtedly the key role in adaptive immunity. Whereas B-cells may act as an antigen-presenting cell (APC), both T- and B-cells are highly differentiated upon repeated antigenic stimulation.

Based on cellular markers, T-cells can be divided into two main subsets: (i) helper/inducer cells, which are $CD4^+$, and (ii) suppressive/cytotoxic cells, which are $CD8^+$. It has been shown that T-helper (Th) cells can be further divided into Th-1 and Th-2 cells *(25)*. Some $CD4^+$ cells, which have another cellular marker, $CD25^+$, are referred to as regulatory T-(reg) cells. T-(reg) cells have important roles in the immunity against infections *(26)*. These cellular subsets have different cytokine profiles and hence various functional and physiological roles. Whereas Th-1 cells may secrete interferon γ (IFN-γ), Th-2 cells may release different cytokines such as IL-4, IL-5, and IL-13.

Th-1/Th-2 balance is very important as in some autoimmune disorders such as type 1 diabetes mellitus (DM), systemic lupus erythematous (SLE) and rheumatoid arthritis (RA) the balance has been demonstrated to be shifted toward Th-1, whereas in allergic reactions and parasitic infections, Th-2 arm predominates *(27)*. Therefore, the balance and occasionally the predominance of one of these two arms (i.e., Th-1 and Th-2, may

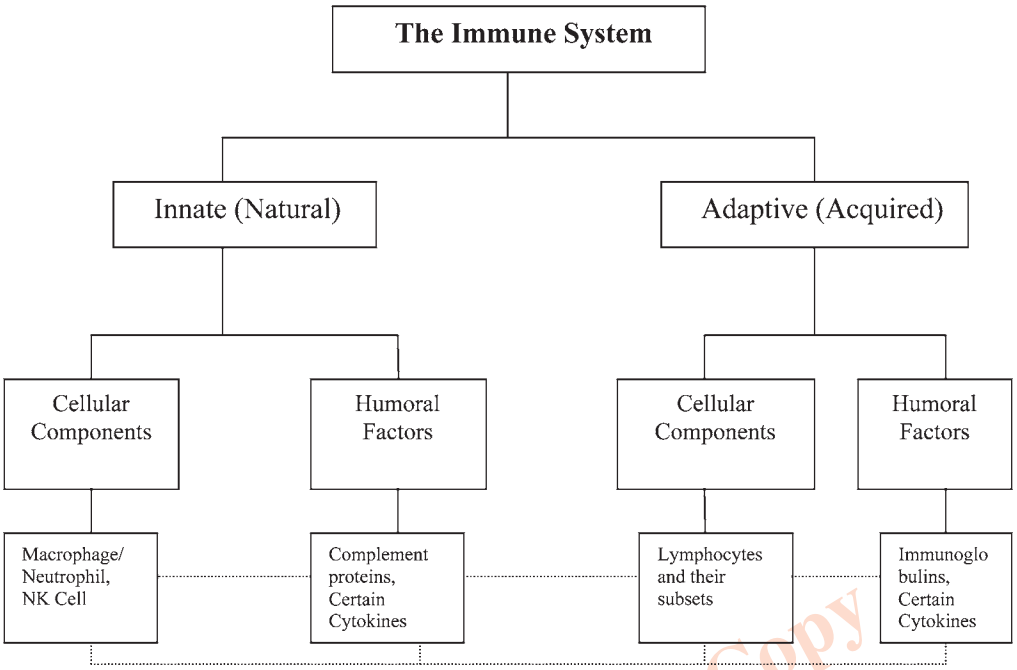

Fig. 1. Schematic representation of major cellular and humoral components of the immune system. All these components are interconnected (dotted). Physical barriers are also considered as parts of innate immunity (not shown).

have a crucial role in keeping health and determining the pathogenesis as well as the outcome of disease). Antigen-stimulated B-cells may also be differentiated into antibody-secreting plasma cells. Immunoglobulins may be divided to five isotypes including immunoglobulin (Ig) G, IgM, IgE, IgA, and IgD. A subgroup of both T- and B-cells will differentiate into memory cells, with highly specialized T-cell receptors (TCR) and surface immunoglobulins, respectively *(28,29)*.

4. OXIDATIVE STRESS AND IMMUNE FUNCTION

Chemical compounds contain two or more elements that are bound together by a chemical bond. In most instances, the bonding involves negatively charged electrons. The arrangement of the electrons determines the stability of the compound. A stable compound has electrons that are paired. If an electron is unpaired, the molecule becomes more reactive and unstable than it used to be. A compound or element with one or more unpaired electrons is referred to as a free radical. To stabilize itself, a free radical may attack other molecules and abstracts an electron from them and thus making them new free radicals. This chain reaction will continue until the free radical couples with another molecule containing unpaired electron or it is quenched by the action of other biologically active molecules called antioxidants *(20)*.

Oxygen is essential to aerobic life, but a part of the consumed oxygen turns into reactive oxygen species (ROS). For example, the superoxide anion (O_2^-) may be generated in many cell redox systems, such as those involving xanthine oxidase, aldehyde oxidase,

membrane-associated NADPH oxidases and the cytochrome P-450 system. It is estimated that about 1 to 4% of the total oxygen taken up by mitochondria may be used for O_2^- production, and about 20% of this may be ejected into the cell. Stimulated macrophages and monocytes also release large amounts of O_2^-. This radical is not very reactive and that is why it can diffuse rather large distance through the cell, where it is converted in a metal catalyzed reaction into the more reactive hydroxyl radical ($OH^\bullet$). Potentially injurious free radicals present in pollutants, halogenated anesthetics and cigarette smoke may have a role in disease pathogenesis not only in adulthood but in infancy and childhood as well *(30,31)*.

To scavenge or neutralize ROS, aerobic organisms have evolved a variety of systems including low molecular weight antioxidants like α-tocopherol, ascorbic acid, glutathione, and antioxidant enzymes such as superoxide dismutase (SOD), catalase and peroxidases. The imbalance in oxidant/antioxidant system shifted towards oxidant is referred to as oxidative stress. This is a potentially harmful phenomenon since ROS can interact with lipids, proteins and DNA, which may eventually result in mutation, neoplastic transformation, loss of cellular function, and, if progressed enough, cell death.

The antioxidant systems work coordinately as "defense systems" against oxidant-induced tissue injury, but may also modulate ROS-sensitive signal transduction pathways *(30)*[3]. Oxidative stress may result in suppression of IL-2 production, protein tyrosine phosphorylation, and reduced intracellular calcium mobilization, low-DNA binding activity of nuclear transcription factors, NFAT, and nuclear factor-κB (NF-κB), and increased binding activity of activating protein-1 (AP-1). Treatment of oxidatively stressed cells with an antioxidant, *N*-acetylcysteine, can reverse these changes *(32)*.

Though free radicals may be potentially injurious to the cells, their production has a physiological philosophy behind. In fact, certain immune cells, mostly neutrophils and macrophages, use free radicals as weapons to destroy invading pathogens *(20)*. Free radicals are therefore like a two-edged sword that, if not subtly controlled by antioxidant defense mechanisms, may damage self-cells and tissues.

The pro-oxidant/antioxidant balance is an important determinant of the immune cell function, not only for maintaining of integrity and functionality of membrane lipids, cellular proteins, and nucleic acids of the immune cell, but for the control of signal transduction and gene expression as well. The immune cells are particularly sensitive to oxidative stress because of the rather high percentage of polyunsaturated fatty acids (PUFAs) in their cell membranes. On the other hand, these cells are frequently exposed to this stress because of the free radical production as a part of their normal function *(33)*. To overcome this problem, the immune cells usually contain higher amounts of antioxidants than do the other cells *(33,34)*.

Polyphenols as dietary antioxidants may affect various aspects of the immune system by shifting pro-oxidant/antioxidant balance towards antioxidant. This subject is reviewed briefly in this chapter.

5. INNATE IMMUNITY

5.1. Physical Barriers

Many tissues and cells, including skin and mucous membranes, obtain polyphenols through blood circulation. Actually the blood concentration of

polyphenols is relatively low. For example, plasma concentrations of flavonoids, even in the populations consuming large amounts of plant material, are around 1 μ*M* but it has been shown that some cell types accumulate certain flavonoids *(35)*. It is still unknown whether skin and mucosal cells have such a characteristic. The effects of systemic polyphenols on skin and mucous membranes have not been addressed yet but some of these compounds may be topically effective in treatment of skin and mucous membrane inflammation, especially in the form of hydrophilic cream *(36)*.

5.2. Humoral Factors: Complement System

The complement system is comprised of a number of serum and membrane-bound proteins that play an important role in the elimination of foreign microorganisms while protecting the host organism from complement-related damage. Complement system may be activated through classical, alternative or lectin pathway. Once activated, an orchestrated series of biochemical reactions will lead to cell lysis, opsonization, or chemotaxis. The activation of complement is therefore crucial in occurrence of inflammation *(37)*[1].

The possible effects of dietary polyphenols on complement system have not been fully investigated. In a study on allergenic plant pollen extracts, which are widely used in clinical practice for diagnostic as well as therapeutic purposes, it was demonstrated that such extracts are capable of consuming complement in every human serum, independent of the clinical condition. The capacity of distinct pollen extracts to inactivate hemolytic complement was dependent on the plant species and the most potent extracts were of the weeds and trees.

The ultraviolet (UV) spectroscopy analysis showed that flavonoids were firmly bound to the allergenic proteins. It was therefore speculated that complement inactivation by allergenic and nonallergenic pollen extracts was due to polyphenolic (flavonoid) structures bound to the pollen proteins *(38)*. In another experiment, complement-modulating properties as well as antimicrobial and superoxide scavenging effects of a series of dimeric procyanidins (1–9) were evaluated. Only the compounds with orthotrihydroxyl groups in the B-ring exhibited inhibitory effects on complement classical pathway *(39)*. The specific effect of individual polyphenolic compounds on complement was further demonstrated in a study on eight antioxidants from five different polyphenolic classes (cinnamic acids, benzoic acids, flavonoids, proanthocyanidins and stilbenes) for their antioxidant activities as well as complement modulating activities in vitro. Though procyanidin C1 was found to be a strong inhibitor of lipid peroxidation and the classical pathway of the complement system, genistein exhibited a very low antioxidant activity, a high cytotoxicity and a low complement-inhibiting activity *(40)*. The anti-complement activities of certain polyphenols have been shown in some other studies *(41–44)*. Complement inhibitory effects may have some role in anti-inflammatory properties of polyphenols. These effects are likely to be dependent on the specific structure of polyphenolic compounds.

[1]Boackle SA. Complement and autoimmunity. Biomed Pharmacother 2003;57:269–273.

5.3. Cellular Components: Phagocytes (Macrophages, Neutrophils), Natural Killer Cells

Various polyphenlic compounds may have different effects on macrophage functions, depending on their structure, dose and duration of consumption. Red grape juice and red wine are among the dietary sources of such polyphenols as resveratrol. However, consuming a single dose of 500 mL of red wine (12% ethanol), a 12% ethanol dilution, dealcoholized red wine, and red grape juice did not affect phagocytic activity and intensity of neutrophils and monocytes, production of TNF-α, IL-2, IL-4, and activity of NK cells *(45)*. When the effects of resveratrol and quercetin on intracellular killing of a pathogenic fungus, Candida albicans, in macrophage-like cells (U937 human promonocytic cell line) were compared, intracellular killing was decreased by both quercetin and resveratrol at 10 μ*M* concentration but was enhanced by 1 μ*M* resveratrol, after 20 h of treatment. Phagocytosis rate, expressed as phagocytosis frequency (i.e., percentage number of phagocytosing cells/total cells), at 20 h was highest with 10 μ*M* resveratrol and was higher with 10 μ*M* quercetin than with 1 μ*M* resveratrol.

Interestingly, both polyphenols showed cytostatic activity. Flow cytometric analysis demonstrated resveratrol-induced apoptosis after 4 h incubation and at concentrations as low as 1 μ*M* and 100 n*M*. Another interesting finding was that resveratrol- or quercetin-treated, but unstimulated, cells did not express TNF-α protein. These findings suggest that wine polyphenols, at the same concentrations as those found in plasma after moderate wine consumption, have immunomodulating effects on cellular components of natural nonspecific immunity and that these effects may be found of clinical applications in anti-infective, anti-inflammatory, and anti-cancer therapies *(46)*. Recently, it has been proposed that the anti-inflammatory effects of resveratrol may be exerted through both NF-κB-dependent *(47)* and NF-κB-independent pathways *(48)*.

The inhibitory effects of polyphenols on phagocytosis are seemingly dependent on their anti-oxidative properties. In an in vitro experiment on the effects of flavonoids on myelin phagocytosis by macrophages, luteolin, quercetin, and fisetin significantly decreased the amount of myelin phagocytosed by a macrophage cell line without affecting its viability. The inhibitory concentration [IC (50)] values for these compounds ranged from 20 to 80 μ*M*. Here again this inhibitory effect was found to be dependent on structure and antioxidant activity of the flavonoid. Those flavonoids with hydroxyl groups at the B-3 positions in combination with a C-2, three double bonds and with higher antioxidant activity were most effective. The fact that reactive oxygen species are required for phagocytosis by macrophages may partly explain the correlation between the capacity of various flavonoids to inhibit phagocytosis and their antioxidant activities. These polyphenolic compounds may therefore be found useful in limiting the demyelination process in such demyelinating disorders as multiple sclerosis *(49)*.

Though some flavonoids may have inhibitory effects on macrophage secretory function, certain polyphenols may act in a reverse direction. This concept was studied in lipopolysaccharide (LPS)/IFN-γ activated RAW 264.7 macrophages. While quercetin and genistein inhibited TNF-α production, kaempferol, myricetin, and daidzein induced TNF-α formation. Anthocyanidins/anthocyanins and anthocyanin-rich extracts also showed inhibitory effects on TNF-α secretion and acted as modulators of the immune response in activated macrophages. Chlorogenic acid had no effect on TNF-α production.

Of interest is that the effect of some polyphenols may be changed by glycosylation, as glycosylated genistein acted in contrast to genistein and inhibited TNF-α production. Glycosylation of daidzein had no effect on its activity *(50)*.

The biological effects on macrophage of some polyphenols, which may have different polymeric forms, may be influenced by degree of polymerization. For example, procyanidins, which are found in cocoa liquor and are potent antioxidants and may act as anti-inflammatory agents, may have different degree of polymerization. Though cocoa and isolated procyanidin fractions (monomer through decamer) may modulate mRNA expression of IL-1β, the pro-inflammatory cytokine, in phytohemagglutinin (PHA)-stimulated peripheral blood mononuclear cells (PBMCs), this effect is apparently influenced by the length of the molecule (i.e., the smaller fractions of cocoa [monomer–tetramer] reduce IL-1β expression in PHA-stimulated PBMCs by 1–15%, whereas the larger oligomers (pentamer–decamer) increase expression by 4-52%) *(51)*.

From the mechanistic point of view, the anti-inflammatory effects of some polyphenols such as theaflavin-3, 3′- digallate from black tea are exerted by suppressing the activation of NF-κ-B through down-regulation of Iκ-B kinase (IKK) activity in macrophage *(52)*. Polyphenols may also have some effects on arachidonic acid cascade and prostaglandin (PG) biosynthesis. As resveratrol was found to impair cyclooxygenase-2 (COX-2) induction stimulated by LPS and phorbol myrestate acetate (PMA) or by $O2^{-}$ or H2O2 exposure in murine resident peritoneal macrophages. These effects of resveratrol on arachidonic acid release and COX-2 over-expression were correlated with a marked reduction of PG biosynthesis *(53)*.

Macrophage functions may be affected by apigenin and related flavonoids through modulation of inducible cyclooxygenase (COX-2) and inducible nitric oxide synthase (iNOS). In LPS-activated RAW 264.7 macrophages, apigenin, genistein, and kaempferol were found to be active inhibitors of transcriptional activation of COX-2, with $IC_{50} < 15$ μ*M*. Apigenin and kaempferol showed inhibitory effects on transcriptional activation of iNOS, with $IC_{50} < 15$ μ*M*. Apigenin also blocked the LPS-induced activation of NF-κB *(54)*. Aarachidonate-derived PGs promote inflammatory reactions *(55)*. Cytokine production of macrophages may also be influenced by some polyphenols. Resveratrol, for instance, has been reported to enhance TNF-α, IL-12, and IL-1β production from LPS-activated phorbol myristate acetate (PMA)-differentiated THP-1 human macrophages. However, cytokine production may differ with IFN-γ-primed macrophages. As, in this situation, resveratrol suppressed the expression of HLA-ABC, HLA-DR, CD80, CD86 and inhibited production of TNF-α, IL-12, IL-6, and IL-1β induced by LPS. The differential effect of resveratrol on expression of CD14, LPS-binding protein, might be related to differential response of macrophages to LPS with or without IFN-γ priming *(56)*. Similar effects of resveratrol on expression of costimulatory molecules (e.g., CD80 and CD86) and HLA classes I and II in murine bone marrow-derived dendritic cells (BMDCs) has also been reported *(57)*. Down regulation of DC differentiation and maturation may be clinically useful in chronic inflammatory diseases. Some clinical trials, however, failed to show immunomodulating effects of acute resveratrol rich beverages intake in healthy men *(58)*.

Immune cell apoptosis is another possible mechanism of polyphenol immunomodulation. In an in vitro study, EGCG, and ECG were found to induce apoptosis in monocytes.

ECGC, in particular, activated caspases 3, 8, and 9, which play a central role in the apoptotic cascade, in a dose-dependent manner. Interestingly, the EGCG-induced apoptosis of monocytes was not affected by granulocyte-monocyte colony stimulating factor (GM-CSF) or LPS *(59)*.

Polyphenolic compounds may exert their anti-inflammatory effects by inhibiting neutrophils to produce reactive oxygen species *(60,61)*. Flavonoids have been found to modulate different aspects of neutrophil functions. Quercetin and some of the other polyphenols at a 10^{-5} *M* concentration inhibit neutrophil chemiluminescence (CL) response to opsonized zymosan particles by about 60%. On the other hand, quercetin and chalcone, only at concentrations of 1.5×10^{-4} to 2×10^{-4} *M*, inhibit lysosomal β-glucuronidase release from neutrophils stimulated with opsonized zymosan. Therefore, the effects of polyphenols on human neutrophil functions are likely to depend on such variables as the response measured, the activating stimulus, and specific polyphenol structural characteristics *(60)*. There are some reports suggesting that flavonoids exert their inhibitory effects on neutrophil superoxide production, at least in part, by suppressing tyrosine phosphorylation of neutrophil proteins in a dose dependent manner *(62–65)*. Tyrosine phosphorylation of neutrophil proteins is usually accompanied by respiratory burst and superoxide production *(66,67)*. Recently, the inhibitory effect of EGCG on neutrophil chemotaxis was evaluated in vivo using fluorescein isothiocyanate-labeled ovalbumin (FITC-OVA)-induced rat allergic inflammation model. In this model, EGCG directly suppressed neutrophil infiltration by suppression of chemokine production at the site of inflammation *(68)*.

Though polyphenols may modulate human neutrophil functions, there are some documents showing that neutrophils may modify polyphenolic compounds biochemically. During inflammatory response, certain cytokines and proinflammatory oxidants such as hypochlorous acid (HOCl) and peroxynitrite ($ONO2^-$) are produced by neutrophils and macrophages, respectively. The aromatic nature of polyphenols makes them potential targets of HOCl and $ONO2^-$ so the oxidants react with phenolic tyrosine residues on proteins to form chloro- and nitrotyrosine. These reactions may therefore create novel pharmacophores at the site of inflammation. Differentiated HL-60 cells, a neutrophil-like cell line, were shown to form chlorinated and nitrated isoflavones *(69)*. It has also been demonstrated that chlorinated and nitrated genistein are both formed by isolated human neutrophils after induction of respiratory burst with phorbol ester. Interestingly, the extent of chlorination of genistein was markedly increased by the phorbol ester whereas the low level of nitration of genistein was constitutive and unaffected. It is therefore hypothesized that inflammatory cell-specific metabolism of polyphenolics can modify the properties of these compounds at the local site of inflammation *(69,70)*.

Polyphenolic compounds may also influence natural killer (NK) cell functions. In human peripheral blood NK cells, genistein at concentrations below 0.5 μ*M*/L and daidzein and genistein glucuronides (DG and GG) at 0.1 to 10 μ*M*/L were demonstrated to enhance NK cell-mediated K562 cancer cell killing significantly. This effect was, however, dose-dependent for genistein, as at concentrations above 0.5 μ*M*/L of genistein significantly inhibited NK cell cytotoxicity. Isoflavones, especially the isoflavone glucuronides, additively enhanced activation of NK cells by interleukin-2 (IL-2). Showing weak estrogenic properties, DG and GG activated human NK cells in

nutritionally relevant concentrations in vitro, probably at a site different from IL-2 action *(71)*. The effects of genistein on NK cells may be partly mediated through down-regulation of certain matrix metalloproteinases *(72)*.

In rat experimental model, it has been demonstrated that some polyphenols, notably isoflavones genistein and methoxychlor, may affect immune system and its cellular components, including NK cells, differentially in two sexes with greater effects observed in developing rats. In F0 females, genistein did not affect the activity of NK cells but in F1 males increased spleen NK cell activity. In contrast, in F1 females, genistein decreased the activity of spleen NK cells but methoxychlor increased the percentages of spleen NK cells and $CD8^+$ T-cells *(73)*.

6. ADAPTIVE IMMUNITY

6.1. Humoral Immunity: Serum Immunoglobulins

Immunoglobulin (Ig) production may be enhanced in Sprague-Dawley rats fed on quercetin *(74)*. The effect on Ig production of polyphenols can be complex and class specific. In a study on the effects of culture medium and serum components on Ig production by mouse splenocytes, daidzein enhanced IgM and IgE levels at concentrations above 10 μ*M*, and genistein induced a decrease in IgM level and an increase in IgE level at concentrations above 10 μ*M*. Moreover, quercetin and luteolin enhanced medium IgE level at all concentrations tested, whereas IgA, IgG, and IgM levels were not affected *(75)*. These effects, however, may be different in vivo. Though these studies mostly indicate polyphenol-induced general increase in immunoglobulin concentrations, some investigators have reported the immune-enhancing effects of certain polyphenols on antigen-specific antibody production through selective augmentation of IL-2 generation both in vitro and in vivo *(76)*. There are some documents showing the inhibitory effects of some polyphenols found in cacao liquor on polyclonal Ig production by B cells in a dose-dependent manner *(77)*. Green tea polyphenols (GTP) may also have such inhibitory effects as in an experimental study; GTP-fed mice had lower levels of total and chicken type II collagen (CII)-specific IgG2a antibody in the arthritic joints. Green tea and its polyphenolic compounds may therefore be useful as an adjunct therapy for the treatment of arthritis and other autoimmune disorders with similar pathologies *(9)*. However, when female mice were fed on polymethylated flavones (PMFs) by gavage at 5, 50, 150, and 500 mg/kg/d for 28 d, a mild suppression of NK-cell activity resulting from long-term, high-dose exposure to PMFs was observed but humoral immunity was found insensitive to this immune suppressive effect as judged by determination of antibody-forming cell (AFC) four days after sensitization of mice with sheep red blood cells (SRBCs) through tail vein injection. The PMFs containing nobiletin (30.7%), 3, 3′, 4′, 5, 6, 7, 8-heptamethoxyflavone (27.9%), trimethylscutellarein (14.5%), tangeretin (10.4%), sinensetin (5.8%), 5-demethyl-nobiletin (2.0%), hexa-*O*-methylquercetagetin (1.3%) 5-methyl-tetramethylscutellarein (0.6%), and other flavonoids (2.7%), was extracted from orange peel oil *(78)*. The effects of polyphenols on humoral immunity seem to depend on the specific polyphenolic compound. The effects of polyphenols on aquired humoral immunity need to be more elucidated by further studies.

6.2. Cell-Mediated Immunity: T-Cells and Their Subsets

The huge bulk of our knowledge on immunomodulating effects of polyphenols come from the experiments performed on cellular components of the immune system. In this context, though some studies suggest that acute consumption of polyphenol-rich beverages like red grape juice and dealcoholized red wine has no effect on lymphocyte proliferation, and IL-2 and IL-4 production *(45)*, there is some evidence of T-cell-modulating effects of polyphenols. In a study on 30 patients with end-stage diabetic nephropathy (ESDN) on hemodialysis and healthy controls, a significantly decreased cellular thiol observed in patients correlated directly to a significant diminished T-cell activation to pokeweed mitogen (PWM) and an elevated synthesis of TNF-α. Interestingly, treatment with flavonoids resulted in restoration of the thiol status within 72 h in vitro and in vivo. Also in parallel, T-cell activation was improved substantially along with a significant decrease in TNF-α release *(79)*. TNF-α is a critical negative regulator of type-1 immune activation during intracellular bacterial infection whose primary role, different from those of other type 1 cytokines, is to keep an otherwise detrimental type 1 immune response in check *(80)*.

The effect of polyphenols on TNF-α may in turn influence other inflammatory cytokines, like IL-8 that are normally induced by TNF-α. For instance, theaflavin, a black tea-derived polyphenol, has been shown to inhibit TNF-α-mediated IL-8 gene expression in vitro *(81)*. IL-8 is a key mediator in neutrophil-mediated acute inflammation *(82)*.

All T-cell subsets may not be activated to the same extent as a result of polyphenolic treatment. Indeed, when peripheral blood mononuclear cells (PBMCs) from normal subjects were cultured with different concentrations of quercetin (0.5–50 μ*M*) for 24 to 72 h, the gene expression as well as production of Th1-derived IFN-γ was enhanced whereas Th2-derived IL-4 was suppressed. Therefore, the beneficial immunomodulatory effects of quercetin may result from cytokine-mediated shifting of Th1/Th2 balance toward Th1 *(83)*. These findings are in contrast with the effects found in propolis, the resinous product collected by honeybee from plants, on T-cells. When the effects of different propolis extracts and of its main flavonoids including hesperidin and quercetin as well as caffeic acid phenethyl ester (CAPE) on basic human immune cell functions were evaluated, it was found that phytohemagglutinin (PHA)-induced DNA synthesis of PBMCs and T-cells was suppressed by propolis and its constituents in a dose-dependent manner. Also, the production of cytokines IL-1β, IL-12 (monocyte/macrophage-derived), IL-2 (Th1-derived), and IL-4 (Th2-derived) were found all suppressed whereas the production of TGF-β1 by T-(reg) cells was ascertained elevated[2]. Recently, catechin and especially EGCG have been found to bind to CD11b on $CD8^+$ T-cells and hence inhibit infiltration of them to the sites of inflammation. This effect may have clinical application in chronic inflammatory disease *(85)*.

6.3. Immune Response to the Pathogens

The immunomodulatory effects of polyphenols may help host to overcome the infection. It has been shown that 0.5 g green tea polyphenols (containing

[2]Ansorge S, Reinhold D, Lendeckel U. Propolis and some of its constituents down-regulate DNA synthesis and inflammatory cytokine production but induce TGF-beta1 production of human immune cells. Z Naturforsch [C]. 2003;58:580–589.

(–)-epigallocatechin-3-gallate)/kg body weight completely inhibited LPS-induced lethality in male BALB/C mice. In the macrophage cell line, RAW264.7, (–)-epigallocatechin-3-gallate (EGCG) decreased LPS-induced TNF-α production in a dose dependent manner (50% inhibition at 100 m*M*). EGCG also inhibited LPS-induced TNF-α mRNA expression and NF-κB binding activity in RAW264.7 cells (30–40% inhibition at 100 m*M*). Similarly, EGCG inhibited LPS-induced TNF-α production in elicited murine peritoneal macrophages. The inhibitory effects of oral green tea polyphenols on LPS-induced TNF-α in serum was also demonstrated in male BALB/C mice *(86)*. Altogether, these observations suggest that TNF-α inhibitory effect of polyphenols mediated by NF-κ-B inhibition may enhance the resistance against endotoxin-induced TNF-α lethality.

This hypothesis was further confirmed by this observation that pretreatment with a series of flavonoids protected mice injected with LPS and D-galactosamine (D-GalN) from two types of endotoxin lethality. This protection against TNF-α-mediated lethal shock was also observed in mice sensitized with just D-GalN but not in mice injected with high dose of LPS *(87)*.

However, the in vitro TNF-inducing potential and IFN-like activities of some polyphenols have been proposed as possible mechanism for their anti-leishmanial effect *(88)*.

7. POLYPHENOLS AND MICROBIAL AGENTS

Polyphenols may have protective effects against pathogens, not only by modulating the host immune system but also by acting against the pathogen itself. The inhibitory effects of water-alcohol extract and of four fractions from the polyphenolic mixture of *Epilobium hirsutum* on the reproduction of influenza viruses in vitro, in ovo, and in vivo were demonstrated over a decade ago. Some geranium polyphenolic extracts also caused an increase of the survival rate in an infection with *Klebsiella pneumoniae* in mice. Epilobium and geranium are Bulgarian medicinal plants *(12)*. The inhibitory effects of polyphenols on certain pathogens have been reported in several studies *(89–92)*. Recently, the inhibitory effect of tea polyphenols on the proliferation of *Chlamydia trachomatis* and *C. pneumoniae* was studied *in vitro*. In this study, a product of tea polyphenols, Polyphenon 70S, containing (–)-epigallocatechin gallate (35.9%), (–)-epigallocatechin (18.3%), (–)-epicatechin gallte (11.2%), and other polyphenolic compounds, was used. *Chlamydia trachomatis* and *C. pneumoniae* were cultured in HeLa229 cells and HL cells, respectively. Two methods, preinoculation and postinoculation, were used to test the susceptibility of bacteria to Polyphenon 70S in vitro. Complete inhibition of *C. trachomatis* D and L2 strains at concentrations of 1.6 and 0.4 mg/mL, respectively, was observed. With *C. pneumoniae* strains, the end points were 0.8 and 1.6 mg/mL for AR-39 and AC-43, respectively. Whereas in the preinoculation method, Polyphenon 70S had no toxicity to HeLa229 and HL cells, it showed toxic effects in the postinoculation method at 0.25 mg/mL *(11)*.

Antimicrobial activities of some polyphenols may not be confined just to the bacteria. It has been shown that proanthocyanidins and structurally related compounds may inhibit the intracellular survival of the intracellular parasite *Leishmania donovani* amastigotes (EC_{50} 0.8-10.6 n*M*) compared to the antileishmanial drug, Pentostam (EC_{50} 10.6 n*M*). They were, however, all ineffective against the extra cellular form of the parasite

(EC_{50} 7.8 to over 86 n*M*. Of interest is that all polyphenolic compounds tested showed only moderate or no toxicity to the murine host cells at 7.8 to over 56 n*M*[10]!

8. POLYPHENOLS AND ALLERGY

Many studies indicate that oxidative stress may play an important role in pathogenesis of allergic diseases *(93–96)* . The fact that polyphenols are potent antioxidants gave rise to this hypothesis that they might be useful in treatment of such atopic diseases as asthma, allergic rhinitis, and allergic urticaria. Interestingly, it was found that polyphenols might also modulate the release of allergic mediators. In a study on the effects of tea polyphenols on the release of histamine and leukotriene B4 from rat peritoneal exudates cells (PEC), EGCG, (–)- epicatechin gallate (ECG) and (–)- epigallocatechin (EGC) were found to have inhibitory effects on histamine and LTB4 release from the cells stimulated with a calcium ionophore, A23187 or compound 48/80. Of the other tea polyphenols, (+)- catechin (C) and (–)- epicatechin (EC) had no effect. The inhibitory potency of the polyphenols was in order of EGCG > ECG > EGC *(16)*. These effects of tea polyphenols may be exerted through the metabolic events occurring after the elevation of intracellular $Ca2^+$ concentration *(15)*. The same anti-histaminic effect has been reported for polyphenols extracted from immature apple fruits *(14)*, tannins and related polyphenols such as agrimoniin and euphorbin C[3]. The antioxidant activity, membrane permeability and C4-carbonyl group of phenolic compounds seem to be essential for the inhibition of LTB4 release *(98)*.

The inhibitory effects on KO2-induced histamine release from rat peritoneal mast cells of procyanidins, flavonoids and related polyphenols with small molecular weights, except for EGCG, have been found negligible *(87)*. In contrast to these findings, some investigators have reported that the flavonoids, luteolin, baicalein and quercetin inhibited the release of histamine, leukotrienes, prostaglandin D2 (PGD2), and granulocyte macrophage-colony stimulating factor (GM-CSF) from human cultured mast cells (HCMCs) in a dose-dependent fashion. Of interest is that luteolin and quercetin strongly and baicalein slightly inhibited $Ca2^+$ influx. On the other hand, protein kinase C (PKC) translocation and activity were inhibited by luteolin, quercetin and, to a lesser extent, baicalein. The luteolin, a flavone, may therefore be a potent inhibitor of human mast cell activation through the suppression of $Ca2^+$ influx and PKC activation *(99)*. It seems that the conditions of experiment, including the cell line used and the stimulatory agents for histamine as well as other mediators release may all affect the results. The effects of polyphenols on hypersensitivity reactions deserve further studies.

9. POLYPHENOLS, IMMUNITY, AND CANCER

Polyphenols have been known as potent nutritional anticancer substances *(5,6,8)*. Among the proposed mechanisms for anticarcinogenic effects of resveratrol is inhibition of various stages of carcinogenesis, scavenging incipient populations of androgen-dependent prostate cancer cells through androgen receptor antagonism, and scavenging incipient

[3]Kanoh R, Hatano T, Ito H, Yoshida T, Akagi M. Effects of tannins and related polyphenols on superoxide-induced histamine release from rat peritoneal mast cells. Phytomedicine 2000;7:297–302.

populations of androgen-independent prostate cancer cells by short-circuiting epidermal growth factor-receptor (EGFR)-dependent autocrine loops in the cancer cells *(5)*.

EGCG, a major tea polyphenol, inhibits mitogen-activated protein kinases, cyclin-dependent kinases, growth factor-related cell signaling, activation of activator protein 1 (AP-1), NF-κB, topoisomerase 1, matrix metalloproteinases, and some other potential targets. In some studies, the inhibition correlated with an increase in tumor cell apoptosis and a decrease in cell proliferation *(100)*. Though the targets of anti-cancer activities of polyphenols have been detected at the molecular level *(8,101–103)* there is strong evidence suggesting that anticancer effects of polyphenols may be exerted through immune-mediated mechanisms, as well. In an experimental study on the protective effects of various doses of oral isoflavone genistein (4,7,4′-trihydroxyisoflavone) against B16F10 tumor in adult female B6C3F1 mice, increased host resistance to the tumor was observed. This resistance was reflected by a decrease in the number of lung tumor nodules after injection of middle and high doses of tumor cells. Interestingly, inhibition of B16F10 tumor formation was not due to a direct effect of serum genistein and/or its metabolites on the proliferation of B16F10 tumor cells. On evaluation of innate and acquired immune responses, there was a dose-dependent increase of cytotoxic T-cell activity in genistein-treated mice with significant changes observed at the middle and high dose levels. Meanwhile, in vitro IL-2-stimulated NK cell activity was significantly enhanced in the high genistein dose group (20 mg/kg $\cdot$ BW^{-1}), although the basal NK cell activity was not affected. Basal splenocyte proliferation was also significantly increased due to exposure to genistein. The chemoprevetive effects of polyphenols may therefore be related to the increases in the activities of cytotoxic T-cells and NK-cells *(104)*.

The anticancer effects of flavonoids resulting from IL-2-mediated NK cell activation have been reported in several studies *(70,105)*, though some studies failed to show such a relationship between anticancer activities of polyphenols and immune enhancement *(106)*. Simultaneous consumption of cancer therapeutic drugs with some polyphenolic compounds may not only have no stimulatory effect on NK-cells, but in some cases it may lead to the reduced efficacy of adjuvant therapy. As it was shown that excessive consumption of citrus flavonoid tangeretin together with antineoplastic drug tamoxifen in female nude mice inoculated subcutaneously with human MCF-7/6 mammary adenocarcinoma cells, not only did not inhibit tumor growth, but in some cases completely neutralized tamoxifen's inhibitory effect. This might be, at least in part, result from the inhibitory effects of tangeretin at 1×10^{-6} *M* or higher concentrations on the cytolytic effect of murine NK-cells, which was shown on MCF-7/6 cells in vitro *(107)*. Decreased NK-cell cytotoxicity in rat splenic NK-cells exposed to 100 m*M* quercetin has been reported by other investigators *(106)*.

Nonylphenol and genistein have been found to be potential promoters of rat lung carcinogenesis, possibly via a mechanism involving stimulation of cell proliferation and DNA damage caused by oxygen radicals *(108)*. While using a multiorgan carcinogenesis model in male F344 rats, it was shown that cocoa liquor proanthocyanidins (CLPr) exert chemopreventive effects in the lung without any promoting influence in other major organs *(109)*. On the other hand, tea polyphenols and EGCG, in particular, have been reported to enhance the cytotoxicity of doxorubicin on KB-A-1 cells by 5.2 and 2.5 times, respectively, without modulating effects on KB-3-1 cells. Both tea polyphenols

and EGCG showed reversal effects on the multi-drug resistance phenotype, which is a common problem in cancer treatment *(110)*.

Briefly, the anticancer effects of polyphenols seem to depend not only on the specific structure of each polyphenol but on the dose and the way it is used (with or without antineoplastic drugs). These effects are exerted by antioxidative immune-mediated mechanisms.

10. POLYPHENOLS, OXIDATIVE STRESS, IMMUNITY AND ATHEROSCLEROSIS

Flavonoids, probably the most important class of antioxidant phenolic compounds, may play a much wider role than acting just as antioxidants *(111)*. These effects of polyphenols and, in particular, flavonoids may be proved to be beneficial in prevention of such diseases as atherosclerosis, which is an immune-mediated disorder *(112)*. Polyphenolic flavonoids inhibit macrophage-mediated oxidation of low-density lipoprotein (LDL) and attenuate atherogenesis *(113,114)*. A significant anti-atherosclerotic activity has been also shown for tannin-fraction isolated from pomegranate juice *(115)*. It has been demonstrated that the capacity of the various flavonoids to inhibit phagocytosis correlates well with their potency as antioxidant, which is in accord with the requirement of ROS for the phagocytosis *(49)*. On the other hand, polyphenols may inhibit macrophage cholesterol accumulation, foam cell formation and hence atherosclerosis via inhibition of lipid peroxidation *(115)*. Therefore, the antiatherogenic effects of flavonoids seem to act both on macrophage and LDL.

11. POLYPHENOLS AS PRO-OXIDANTS

Though polyphenols are potent dietary antioxidants, they may be metabolized by peroxidase to form pro-oxidant phenoxyl radicals, which may be reactive enough to oxidize GSH or NADH accompanied by extensive oxygen uptake and ROS formation. Polyphenolic compounds with phenol ring are generally more pro-oxidant than those with catechol ring *(116)*[4]. In an in vitro study, it has been shown that green tea polyphenols at very high concentrations (200–500 μ*M*) enhance sodium nitroprusside-induced neurotoxicity in human neuroblastoma SH-SY5Y cells. Indeed, coincubation of green tea polyphenols and sodium nitroprusside caused loss of mitochondrial membrane potential, depletion of intracellular GSH, and accumulation of ROS, and exacerbated NO-induced neural apoptosis via a Bcl-2 sensitive pathway *(117)*. However, again the dose and structure of the specific polyphenolic and the cell line used seem to determine whether the polyphenol act as a pro-oxidant or antioxidant.

It has been shown that EGCG, the most abundant polyphenol of green tea, may act as a pro-oxidant at low concentrations (1–5 μ*M*) but as a scavenger of superoxide anion at high concentrations (above 10 μ*M*) *(118)*. This dose-dependent induced oxidative stress may act selectively against tumor cells while keeping safe normal cells. The activation of certain pathways that create different oxidative environments, favoring either normal cell survival or tumor cell destruction, may also have some role *(119)*. This

Fig. 2.

[4]Galati G, Sabzevari O, Wilson JX, O'Brien PJ. Prooxidant activity and cellular effects of the phenoxyl radicals of dietary flavonoids and other polyphenolics. Toxicology 2002;177:91–104.

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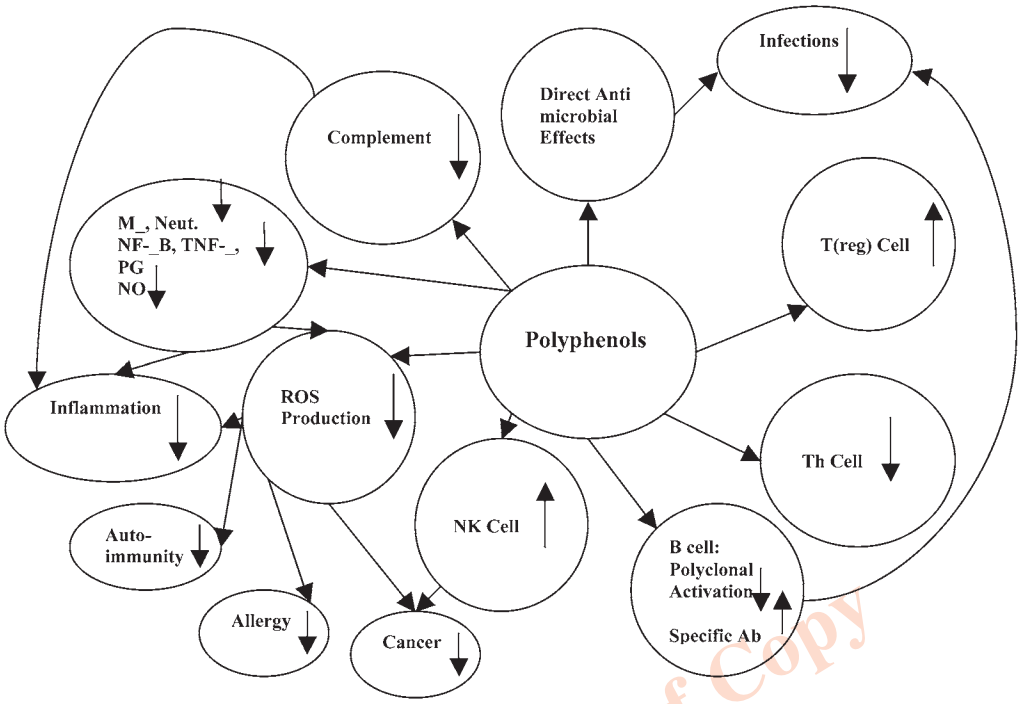

Fig. 2. Schematic representation of some immunomodulating effects of polyphenolic compounds.

pro-oxidant activity of polyphenols, which may involve mobilization of endogenous copper, may in part explain their anti-cancer properties. Green tea extract shows a high rate of Cu(II) reduction and consequent hydroxyl radical formation. Cu(II) reduction may be accompanied by the formation of 'oxidized species' of tea polyphenols, which in turn may catalyze the reduction of Cu(II) leading to redox cycling of copper ions *(120)*. It is still not fully clear how the pro-oxidant activity of polyphenols may affect the immune function in favor of normal cells.

12. CONCLUSIONS

Polyphenols are organic plant compounds with a wide range of biological activities including antioxidative, free radical scavenging, anti-carcinogenic, and anti-inflammatory. This class of compounds, which are abundant in our diet, may affect different aspects of the immune function, as well (*see* Fig. 2). Considering the inter-relationship of oxidative stress and immunity at the molecular level, there is no wonder that polyphenols, as potent antioxidants, may affect different aspects of immunity. The modulating effects of polyphenols on cellular and humoral components of natural immunity (e.g., macrophage/neutrophil, NK-cell, the related cytokines and complement system), and of adaptive immunity (e.g., lymphocytes, the related cytokines and immunoglobuli) seem to be dependent on individual structure, dose and duration of usage of polyphenolic compound, among the other factors. Some flavonoids, for instance, may have inhibitory effects on macrophage secretory function, however, certain polyphenols

may act in a reverse direction. The biological effects on macrophage of some polyphenols, which may have different polymeric forms, may be influenced by degree of polymerization.

Some polyphenols have been found to induce general increase in immunoglobulin concentrations and even enhance antigen-specific antibody production through selective augmentation of IL-2 generation. On the other hand, some polyphenols may directly be harmful to the microbial agents. These biological effects may make polyphenols a potential good candidate for adjunct therapy in many pathological conditions, such as inflammatory and autoimmune disorders, allergies, cancers, cardiovascular disease, and infectious disease and so on. Polyphenolic compounds may exert their anti-inflammatory effects by inhibiting complement and also neutrophils to produce ROS. Immune enhancement and health promotion via polyphenol-rich diet consumption is also of great concern. However, under some conditions certain polyphenols may act as pro-oxidants and besides not all above-mentioned properties are shared equally by polyphenolic compounds. Finally, the results of most, notably in vitro, studies on polyphenols and immunity can be hardly interpreted as to long-term general health of human. Therefore, further laboratory and clinical studies are still needed to clarify the effects of polyphenolic compounds on the immune function as well as on health status.

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