Polyphenols from Red Wine Modulate Immune Responsiveness: Biological and Clinical Significance

T. Magrone[1], G. Candore[3], C. Caruso[3], E. Jirillo[1,2,*], V. Covelli[4]

[1]*Immunology, University of Bari, Bari, Italy;* [2]*National Institute of Gastroenterology, Castellana Grotte, (Bari), Italy;* [3]*Immunosenescence Group-Department of Pathobiology and Biomedical Methodologies, University of Palermo, Palermo, Italy and* [4]*Division of Neurology, Polyclinic Hospital, Bari, Italy*

Abstract: Many studies have been conducted on the effects of red wine polyphenols on certain diseases, primarily, coronary heart disease (CHD) and, in this respect, evidence has been demonstrated that intake of red wine is associated with a reduction of CHD symptomatology. In this framework, the purpose of this review is to illustrate the effects of polyphenols on immune cells from human healthy peripheral blood. Data will show that polyphenols are able to stimulate both innate and adaptive immune responses. In particular, the release of cytokines such as interleukin (IL)-12, interferon (IFN)-γ, and IL-10 as well as immunoglobulins may be important for host protection in different immune related disorders.

Another important aspect pointed out in this review is the release of nitric oxide (NO) from peripheral blood mononuclear cells (PBMC), stimulated by red wine polyphenols despite the fact that the majority of studies have reported NO production only by endothelial cells. Release of NO from PBMC may play an important role in cardiovascular disease, because it is known that this molecule acts as an inhibitor of platelet aggregation. On the other hand, NO exerts a protective role against infectious organisms.

Finally, some molecular cytoplasmatic pathways elicited by polyphenols able to regulate certain immune responses will also be discussed. In particular, it seems that p38, a molecule belonging to the MAPK family, is involved in the release of IFN-γ and, therefore, in NO production.

All these data confirm the beneficial effects of polyphenols in some chronic diseases.

Key Words: polyphenols, immune system, cytokines, immunoglobulins, nitric oxide, atherosclerosis, red wine.

POLYPHENOLS: DENOMINATION AND CHEMICAL STRUCTURE

Over the past ten years, researchers have focused their attention on the properties of natural substances such as polyphenols because there is a link between their assumption and prevention and/or treatment of some diseases, in particular cardiovascular disease [1-3].

Dietary polyphenols are the most abundant antioxidants in human diet. With over 8,000 structural variants, they are secondary metabolites of plants and denote many substances with aromatic ring(s) bearing one or more hydroxyl moieties [4]. They are subdivided into groups (Fig. **1**) by the number of phenolic rings and of the structural elements that link these rings [5]: (1) the phenolic acids with the subclasses derived from hydroxybenzoic acids such as gallic acid and from hydroxycinnamic acid, containing caffeic, ferulic, and coumaric acid; (2) the large flavonoid subclass, which includes the flavonols, flavones, isoflavones, flavanones, anthocyanidins, and flavanols; (3) tannins are a group of water-soluble polyphenols having a molecular weight from 500 to 3,000 which are subdivided into condensed and hydrolysable tannins, and commonly found complexed with alkaloids, polysaccharides and proteins, particularly the latter. On the basis of structural characteristics there are two groups, gallotannins and ellagitannins of hydrolysable tannins; (4) the stilbenes; and (5) the lignans and the polymeric lignins.

The flavonoids, which share a common structure (Fig. **2**) consisting of 2 aromatic rings (A and B) that are bound together by 3 carbon atoms that form an oxygenated heterocycle (ring C), may themselves be divided into 6 subclasses as a function of the type of heterocycle involved: flavanols, flavones, isoflavones, flavanones, antocyanidins, and flavonols (Fig. **3**). The most abundant flavonoids in the diet are flavanols (e.g. catechins, epicatechin, epigalocathechin, epigallocatechin-gallate) found in red wine, green tea, and chocholate; anthocyanins (pelargodin, cyanidin, malvidin) and the polymeric forms (proanthocyanidins), found in red wine and berry fruits. In addition to this diversity, polyphenols may be associated with various carbohydrates and organic acids and with one another.

Flavonols are mainly represented by myricetin, rutin, quercetin and kaempferol and are present in onions, broccoli and red wine. Flavone groups (e.g. apigenin, luteolin, wogonin) are present in cerely. Flavanone groups (e.g. naringenin, naringin, hesperitin, hesperidin) are present in citrus fruits, tomatoes and oranges. Isoflavones (e.g. genistein, daidzein, glycitein) are present in leguminous plants.

Resveratrol (3,4',5–trihidroxy-stilbene), a phenolic fitoalexin, is a derivative of stilbene (Fig. **4**) structurally characterized by the presence of a 1,2-diphenylethylene nucleus

*Address correspondence to this author at the Immunology, Policlinico, University of Bari, Piazza G. Cesare 11, 70124, Bari, Italy; Tel: +39 080 5478492: Fax: +39 080 5478488; E-mail: jirillo@midim.uniba.it
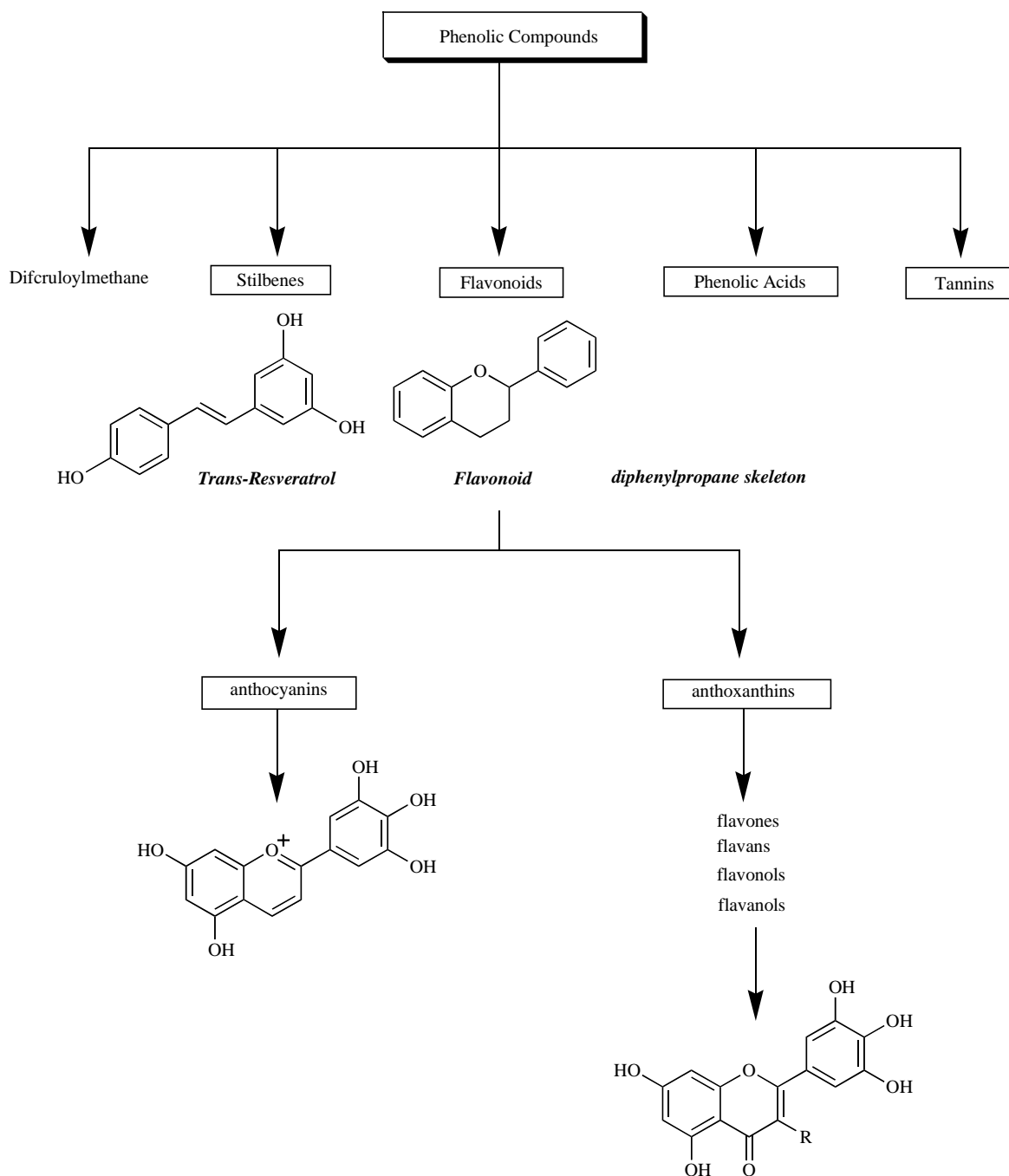
Fig. (1). Classification of dietary polyphenols.

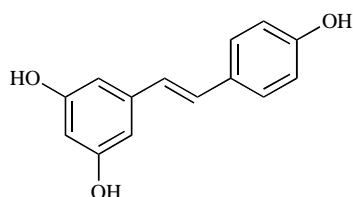with hydroxyls substituted on the aromatic rings, and present in the form of monomers or oligomers. The best known
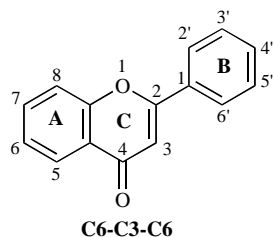
Fig. (2). General chemical structure of flavonoids.

compound is trans-resveratrol, possessing a trihydroxystilbene skeleton. Previous reports have demonstrated that resveratrol regulates many biological activities, mainly concentrating on tumor, oxidation, inflammation regulation, [6-9] neuroprotection, [10] and cardioprotection [11]. On the other hand, in recent experimental studies resveratrol seems to exert beneficial effects on the metabolic syndrome induced by excessive food intake, and characterized by central obesity, atherogenic dislipidemia, high blood pressure, and elevated blood glucose levels. Calorie restriction can antagonize the development of this syndrome, leading to improved glucose tolerance, decreased LDL-cholesterol, and increased HDL-cholesterol [12]. On the other hand, calorie restriction
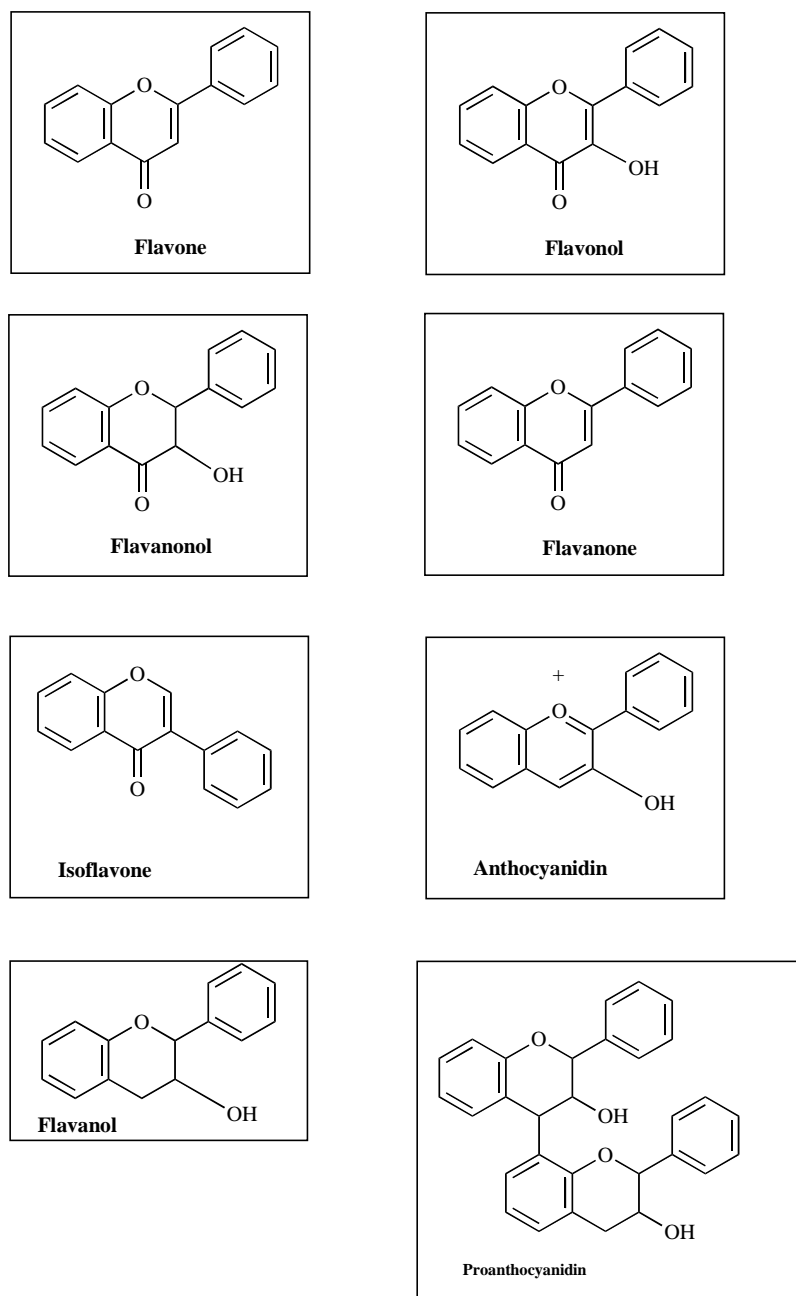
Fig. (3). Ring skeletons of flavonoids.

can prevent all diseases associated with metabolic syndrome such as insulin resistance, cardiovascular disease, and cancer [13,14]. Calorie restriction has also been shown to increase muscle mitochondrial biogenesis in healthy humans through SIRT1 and activation of Peroxisome Proliferator-Activated Receptor-γ Coactivator (PGC)-1α [15]. The SIRT1 activator, resveratrol, has been shown to improve insulin sensitivity, increase mitochondrial content, and prolong survival of mice fed a high fat, high calorie diet [16-18].

The effects of daily consumption of resveratrol have been observed in mice fed high calorie diet [16], and long-term treatment shifted the physiology of middle aged mice on a high-calorie diet towards that of mice on a standard diet, and markedly increased their survival. Mostly strikingly, resveratrol treatment prevented high calorie-induced insulin resistance and organ pathologies, particularly fatty liver diseases [16]. These changes were associated with increased mitochondrial numbers and improved motor functions. Furthermore, treatment of mice with resveratrol significantly in-

Fig. (4). Chemical structure of resveratrol.

creased their aerobic capacity, as evidenced by the increased running time and consumption of oxygen in muscle fibers [19].

Sirtuitins are an evolutionarily conserved class of proteins that regulate a variety of cellular functions such as genome maintenance, longevity, and metabolism [20-22]. There are seven human sirtuitins (SIRT1-7) that contain a conserved catalytic core domain composed of approximately 275 amino acids. SIRT1 has been implicated as a key mediator of the pathways downstream of calorie restriction, a dietary regimen that is known to delay the onset and reduce the incidence of age-related diseases.

In this context, it has been shown that resveratrol can counteract the actions of calorie excess improving health and lifespan in mammals as well as preventing the development of the metabolic syndrome [16-17].

It is well established that mitochondrial dysfunction is causally associated with reduced longevity. In addition, impaired mitochondrial function that directs fatty acids towards storage, as opposed to oxidation, contributes to intramyocellular and hepatic lipid accumulation, which has been proposed as a key etiological factor in the pathogenesis of insulin resistance and the metabolic syndrome [23].

In this context, it has been demonstrated that the beneficial effects of resveratrol on longevity and metabolic profiles are mediated by Sirt-1-induced PGC-1α activation, which leads to increased mitochondrial biogenesis and enhanced oxidative phosphorylation [16-17].

RED WINE AND EFFECTS ON HUMAN HEALTH: OBSERVATIONS MADE ON VARIOUS DISEASES

Many epidemiological studies have shown that regular intake of natural polyphenols in grape juice, red wine, and in some other beverages is associated with reduced risk of cardiovascular disease [24-26]. In general, more than two thirds of the polyphenols consumed in the diet are grapes which contain a wide variety of polyphenols including resveratrol, catechins, flavonoids, and its derivatives, flavons, flavonols, and anthocyanidins.

Indeed, these compounds present in the red wine possess a number of biological effects that might participate in vascular protection, including anti-aggregatory, antioxidant, and free radical scavenging properties. Another therapeutically relevant effect of flavonoids may be their ability to interact with the generation of nitric oxide (NO) from vascular endothelium, which leads not only to vasodilatation, but also to the expression of genes that protect the cardiovascular system [27-29]. Due to their antioxidant properties, diets supplemented with foods containing flavonoids might also protect different tissues against ischemic damage. Flavonoids reduce oxidative and nitrosative stress leading to cellular death. All these effects of flavonoids might interfere with atherosclerotic plaque development and stability, vascular thrombosis, and occlusion, and they might, therefore, explain their vascular protective properties [28-30].

Epidemiological studies have suggested that light to moderate consumption of alcoholic beverages, particularly red wine, is associated with a reduction in overall mortality,

and this effect is attributable primarily to a reduced risk of coronary heart disease (CHD) [31].

Polyphenols have long been recognized to possess antihepatotoxic, antiinflammatory, antiatherogenic, antiallergic, antiosteoporotic, antioxidant and anticancer activities [32].

Atherosclerosis can be considered to be a form of chronic inflammation resulting from interaction between modified lipoproteins, monocyte-derived macrophages, T cells, and the normal cellular elements of the arterial wall. Once adherent to the endothelial cells, leukocytes enter the intima by diapedesis between endothelial cells at their junctions. Once resident in the arterial intima, monocytes acquire the morphological characteristics of macrophages, undergoing a series of changes that lead ultimately to foam cell formation characterized by the presence of modified forms of lipoprotein, such cholesterol esters accumulated in cytoplasmatic droplets [33]. The monocytes increase expression of scavenger receptor A (SRA) and CD36, and then internalize these oxidized Low Density Lipoprotein (oxLDL) [34,35]. Although the differentiation of monocytes into macrophages may initially serve a protective function by removing cytotoxic and proinflammatory oxLDL particles or apoptotic cells, progressive accumulation of macrophages and their uptake of oxLDL ultimately lead to macrophage necrotic death with subsequent release of cellular proteases, inflammatory cytokines, and prothrombotic molecules, which contribute to plaque instability, plaque rupture, and acute thrombotic vascular occlusion [36].

Among factors of endothelial dysfunction and atherosclerosis development elevated and modified LDL, hypertension, diabetes mellitus, herpes viruses or *Chlamydia pneumoniae,* lipopolysaccharides (LPS) [37,38] alone or in combinations have been included.

As above cited, polyphenols have generated a great amount of scientific research due to their *in vivo* and *in vitro* antioxidant capabilities with beneficial effects on cardiovascular health. This relation was clear in the "French Paradox" phenomenon as well as in the Mediterranean diet. The French Paradox is defined as low incidence of CHD [39,40] while consuming a diet rich in saturated fat. The Mediterranean diet, rich in fruits and wine, was shown to protect against the occurrence of coronary events [39,41] and may help reverse hyperlipidemia, alter the atherogenicity of LDL particle, and protect the cholesterol in LDL from oxidation [42]. The French Paradox hypothesizes that reduced incidence of cardiovascular manifestations of atherothrombotic disease in France and neighbouring Mediterranean countries is due, at least in part, to their cultural dietary features, mainly a regular and moderate consumption of wine, in comparison to Anglo-Saxon populations [43] whose incidence of cardiovascular events is much higher for the same level of "regular" cardiovascular risks (hypercholesterolemia, hypertension, diabetes).

Accordingly, a series of *in vitro* and experimental studies pointed out that the constituents of red wine other than alcohol are protective against cardio-metabolic disorders. In fact, administration of red wine could hamper *in vivo* platelet function, and thrombosis in coronary arteries [44]. However, white wine, whisky and beer did not exhibit same properties

on platelets. Furthermore, chronic consumption of alcohol free red wine prevented arterial thrombosis in hypercholesterolemic rats [45]. Dealcoholized red wine but not white wine reduced atherosclerosis in apolipoprotein E gene-deficient mice [46-48]. In human mononuclear cells, pre-treated with red wine but not vodka the activation of nuclear factor (NF)-κB induced by very LDL (VLDL) [49] was inhibited.

Polyphenols (procyanidin) absorbed at intestinal level are found in plasma with a maximum plasma concentration after 2 h ingestion and, then, decrease [50]. Administration of red wine or quercetin or catechin inhibited the development of aortic lesion on atherosclerotic Apolipoprotein E-knockout mice preventing LDL oxidation [51]. Ethanol facilitated the absorption of polyphenols from red wine, and their concentration is inversely correlated with CHD mortality and morbidity [51,52]. In hamsters, phenolic extracts from grape seeds and marc, at a dose equivalent to two glasses of red wine per meal, decreased plasma cholesterol and prevented aortic atherosclerosis occurrence [53].

Polyphenols can exert a direct antioxidant activity, displaying an array of antiatherogenic mechanisms such as: inhibition of platelet aggregation [54], downregulation of nuclear factor (κB) (NF-κB) activation, and subsequently inhibition of adhesion molecule expression [55], and endothelium vasorelaxation *via* increased synthesis of NO [56].

In particular, the enhanced generation of NO [57-59], a platelet inhibitor and a powerful vasodilator, caused by polyphenolic red wine components *in vitro* and *in vivo*, constitutes an important modulatory component of hemostatic function, and may represent a clinically relevant mechanism to explain the protective effect of moderate red wine consumption on cardiovascular disease in man [60,61].

Other studies provided evidence that oral administration of red wine polyphenolic compounds (ProvinolsTM) prevented the development of cardiovascular alterations in NO deficient hypertension [62] as well as it induced a faster and more profound decrease of blood pressure in developed NO deficient hypertension [63]. In this direction, there is evidence that a phenolic extract from red wine upregulates endothelial NO synthase expression and, thus, NO production by endothelium [60]. In turn, NO possesses other antiatherogenic activities such as inhibition of platelet aggregation, of adhesion to the vascular wall, of smooth muscle cell proliferation and of gene expression involved in atherogenesis, respectively [64,65], influx of atherogenic monocytes and LDL into the wall of arteries [66]. However, other investigations have failed to demonstrate any influence of red wine or alcohol on a rabbit model of atherosclerosis [67]. On the other hand, both red wine and non-alcoholic wine prevented rabbit atherosclerosis in a lipid-independent manner [68].

It is well known that NO is a simple diatomic molecule consisting of one atom of nitrogen and one atom of oxygen, whose physico-chemical and biological properties are determined by its small size (30 kDa), absence of charge and its single unpaired electron. NO is a gas under atmospheric conditions but a solute within cells and tissue [69]. It is generated by a group of cytosolic or membrane-bound isoenzymes, named nitric oxide synthase enzyme (NOS or NOS2)

and constituted by a sequence of 1,294 aminoacids. This enzyme converts the terminal guanidine-nitrogen atoms on the aminoacids L-arginine into NO and L-citruline either in mammalian or non-mammalian cells [70,71], and acts as a vascular relaxing agent, and inhibitor of platelet aggregation. NO is utilized throughout the animal kingdom as a signalling or toxic agent between cells [69]. NO plays several roles in immunity, as a toxic agent towards infectious organisms [72] either bacterial or viral, an inducer or suppressor of apoptosis [73] or an immunoregulator [74-77]. On the other hand, the formation of more toxic radicals occurs when NO combines with O_2^- radicals, leading to peroxinitrite formation which can degrade to hydroxyl radical. Regarding iNOS, it is known that its activation does not depend on calcium signal [69], but is continuously expressed once activated.

NO may regulate it own synthesis functioning as a negative feedback modulator for the same iNOS activity both at level of mRNA and at level of enzyme activity by interacting with the enzyme-bound heme of iNOS gene.

In our *in vitro* studies we have observed NO production from human mononuclear cells from healthy donors treated with red wine polyphenols. In particular in preliminary studies we have tested three different types of red wine (Primitivo, Lambrusco and Negroamaro) with different polyphenols contents. Negroamaro was the strongest inducer of NO production [78] and, therefore, in subsequent experiments this wine was consistently employed. An explanation for this different of behavior response among different wines may depend on the cultivar, on the type of grape, and the country of origin [79,80]. In our *in vitro* test system, we have challenged human healthy mononuclear cells with whole wine, polyphenols alone, and dealcoholized wine. All these samples were used at two different dilutions: 1:5 containing 3 μg/ml and 1:10 containing 1,5 μg/ml of polyphenols, respectively. Data obtained, clearly demonstrated a significant production of NO in presence of polyphenols and ethanol and polyphenols alone, whereas dealcoholized samples did not produce NO, thus excluding a possible contribution of alcohol in this function [78].

Despite the fact that the majority of studies [81-83] have observed NO production from endothelial cells stimulated with polyphenols, we were the first to observe NO production from PBMC, as also confirmed by further studies on the inducible nitric oxide enzyme (iNOS) expression in monocytes in the presence of polyphenols.

When mononuclear cells were stimulated in presence of whole wine and/or polyphenols plus LPS, respectively, no increase of NO production and, therefore, of iNOS expression was observed.

Of note, studies performed in western blotting demonstrated that TLR-4 expression was strongly expressed only in cells treated with E.coli LPS but not in presence of red wine polyphenols [84].

These results could be explained as an antagonism linkage for the same receptor (TLR-4) between polyphenols and LPS. These informations may be of clinical relevance because in the course of Gram-negative infections, intake of polyphenols could prevent noxious reactions triggered by LPS.

Also, NO is able to limit the flux of atherogenic plasma proteins into the artery wall.

In previous studies, it was reported that red wine polyphenol compounds (RWPCs) from different sources, including dry powder from red wine (Provinol), were able to produce *ex vivo* endothelium dependent relaxation in rat aortic rings [85,86] by enhanced NO synthesis rather than by enhancing the biological effectiveness of NO or by protecting it against breakdown by superoxide anions (O_2^{-2}). These effects of RWPC probably involved the NO pathway; in as much as enhanced *in vitro* endothelium-dependent relaxation was observed as a result of enhanced NO synthesis [87].

Polyphenols have been studied in other diseases, such as experimental colitis induced in mice, and it was observed that dietary rutin but not its aglycone quercetin ameliorated dextran sulphate sodium-induced colitis in mice, a model of inflammatory bowel disease [88].The presence or absence of a sugar chain in polyphenols is important for expressing activities. It is generally accepted that the type of sugar attached may represent, a major determinant of the extent of small intestinal absorption [89-91], although the aglycone is critical for the expression of biological activities.

ROLE AND SIGNIFICANCE OF TOLL-LIKE RECEPTORS AND THEIR MODULATION BY POLYPHENOLS

LPS is an integral component of the outer membrane of Gram-negative bacteria, inducing cellular responses by its complexing with circulating LPS-binding protein and subsequently, binding to CD14. This, in turn, facilitates the interaction of LPS with signaling molecules belonging to the Toll-like receptor (TLR) family [92]. TLRs induce innate immune responses by recognizing invading microbial pathogens leading to the activation of adaptive immune responses [93]. TLRs are evolutionarily conserved Pattern recognition receptors (PRRs) that recognize conserved Pathogen Associated Molecular Patterns (PAMPs) present on various microbes [94-96]. These receptors have varied tissue distribution and recognize many different PAMPs. TLR signaling is initiated by the recruitment of cytosolic adapters that all share the TIR domain [97]. Currently, at least 13 TLRs in mammalian cells are identified with different types of agonists [98]. TLR agonists include LPS for TLR4, bacterial lipopeptides, and peptidoglycan for TLR2, dsRNA for TLR3, flagellin for TLR5, and ssRNA and bacterial unmethylated CpG DNA for TLR7 and TLR9, respectively [99-106]. It was reported that TLR4 signaling pathways can be activated by nonbacterial agonists such as heat shock protein 60, fibronectin, Taxol, respiratory syncytial virus fusion protein and saturated fatty acids [107-112]. This fact points to the possibility that TLRs are involved in inflammatory responses induced by molecules with non-infectious origin.

Broadly, the stimulation of TLRs by agonists can trigger the activation of two downstream signaling pathways: MyD88-dependent and -independent pathways [96]. MyD88 is the immediate adaptor molecule that is common to all TLRs, with the exception of TLR3.

MyD88 was the first TIR domain containing adapter protein characterized and was shown to interact with the TIR domain on TLR/IL-1R cytoplasmic tails by homotypic inter-

action. MyD88 is crucial for normal NF-κB induction in response to Interleukin (IL)-1, IL-18, and LPS [113,114]. MyD88 recruitment to TLR4 following receptor aggregation leads to recruitment of another TIR-domain containing adapter, TIRAP or Mal. TIRAP mediates NF-κB activation downstream of TLR2 and TLR4, but not IL-1R or other TLRs [115,116].

Currently it is not known how TIRAP selectively acts only in a subset of MyD88-mediated signaling pathways [117].

MyD88 also recruits IL-1R-associated kinase and TNFR-associated factor 6 (TRAF6) [118], leading to activation of the canonical IKB kinase (IKK) αβγ complex that phosphorylates IκBα on serine residues 32 and 36, causing its ubiquination and the subsequent degradation of IκBα, leading to the nuclear translocation and DNA binding of NF-κB [119-123]. Deregulated activation of TLRs can lead to the development of severe systemic inflammation including septic shock with high mortality. Moreover, chronic inflammation is known to be an important etiological condition for various chronic diseases including atherosclerosis, diabetes, and cancer. Recent evidence suggests the involvement of TLRs in these chronic diseases [124-126]. Identifying molecular targets by which pharmacological or dietary factors modulate TLR-mediated signaling pathways and target gene expression would provide new opportunity to manage the deregulation of TLR-mediated inflammatory responses, leading to acute and chronic inflammatory diseases.

The intestinal mucosa is constantly exposed to a myriad of antigens, including bacteria and bacterial products (LPS, peptidoglycan), viruses, parasites and dietary antigens. The host has evolved sophisticated mechanisms to maintain homeostasis in the face of such a hostile environment [127-129].

With respect to innate signaling polyphenols, inhibit LPS-induced TNF-α secretion by macrophages *in vitro* also displaying anti-inflammatory activity in mice [130,131].

In a study performed using luteolin, it was found that this substance failed to block LPS-induced RelA phosphorylation [132]. This suggests that luteolin mainly modulates NF-κB activity through RelA shuttling and likely not by interfering with the transactivating ability of the subunit. In addition, the lack of inhibitory effect on p38 phosphorylation indicates that luteolin exerts some level of specificity. Thus, the blockade of LPS-induced IKK activity and IκBα phosphorylation in the absence of impaired RelA is a surprising finding.

Dysregulated innate responses to the endogenous microflora are a hallmark of intestinal inflammation such as that observed in Inflammatory Bowel Disease (IBD) and blockade of innate signal transduction may help to restore host homeostasis and alleviate inflammation [133,134]. Therefore, TLR modulation by polyphenols may play a beneficial role in IBD characterized by secretion of proinflammatory cytokines, such as TNF-α.

NF-κB

The basic scheme of NF-κB signaling consists of a series of positive and negative regulatory elements. Inducing stim-

uli triggers IKK activation leading to phosphorylation, ubiquitination, and degradation of IκB proteins. Released NF-κB dimers are further activated through various posttranslational modifications and translocate to the nucleus where they bind to specific DNA sequences and promote transcription of target genes. In its most basic form, therefore, the pathway consists of receptor and receptor proximal signaling adaptor molecules: the IKK complexes; IκB proteins and NF-κB dimers.

The NF-κB family of transcription factors consists of five members of the mammalian NF-kB family, p50/p105 (NF-KB1), p52/p100 (NF-KB2), p65 (RelA), c-Rel, and RelB, encoded by NFKB1, NFKB2, RELA, REL, and RELB, respectively, which share an N-terminal Rel homology domain (RHD) responsible for DNA binding and homo-and heterodimerization. NF-κB dimers bind to κB sites within the promoters/enhancers of target genes and regulate transcription through the recruitment of coactivators and corepressors. The transcription activation domain (TAD) necessary for the positive regulation of gene expression is present only in p65, c-Rel, and RelB. As they lack TADs, p50 and p52 may repress transcription unless associated with a TAD-containing NF-κB family member or other proteins capable of coactivator recruitment. Constitutive binding of p50 or p52 homodimers to κB sites on NF-κB-responsive promoters may, thus, act to check NF-κB transactivation until displaced by transcriptionally competent NF-κB dimers. There is considerable structural information about NF-κB dimers in both its inactive IκB-bound form and active DNA bound state. Crystal structures of NF-κB dimers bound to κB sites reveal how the immunoglobulin-like domains that comprise the RHD contact DNA. The NH2-terminal Ig-like domain confers selectivity for certain types of κB sites, whereas the hydrophobic residues within the C-terminal domain provide the dimerization interface between NF-κB subunits [135].

In its inactive state, NF-κB dimers are associated with one of three typical IκB proteins, IκBα (*NFKBIA*), IκBβ (*NFKBIB*), or IκBε (*NFKBIE*), or the precursor proteins p100 (*NFKB2*) and p105 (*NFKB1*). These IκBs maintain NF-κB dimers in the cytoplasm, and are crucial to signal responsiveness. All IκB proteins are characterized by the presence of multiple ankyrin repeat domains**.** The prototypical and most extensively studied member of the family is IκBα. IκBα is rapidly degraded during activation of canonical NF-κB signaling pathways, leading to the release of multiple NF-κB dimers, although the p65:p50 heterodimer is likely the primary target of IκBα.

Binding to IκB prevents the NF-κB:IkB complex from translocating to the nucleus, thereby maintaining NF-κB in an inactive state. NF-κB signalling is generally considered to occur through either the classical or alternative pathway [136].

Stimuli, such as proinflammatory cytokine and, PAMPS can activate the classical NF-κB pathway, leading to activation of the IKK complex. This complex is composed of two catalytic subunits, IKKα (also known as IKK1) and IKKβ (also known IKK2), and a regulator subunit, IKKγ (also called NEMO). The activation of the classical pathway mainly acts through the phosphorylation of IκBs catalyzed by IKKβ in an IKKγ-dependent manner.

The degradation of IκB exposes the nuclear localization signal of the NF-κB family protein, leading to its nuclear translocation and binding to enhancers or promoters of target genes. Instead the alternative pathway is strictly dependent on and independent of IKKβ and IKKγ. Therefore, the IKKα is an essential component of the alternative NF-κB activation pathway based on regulated NF-κB2 processing rather than IκB degradation. In this pathway, IKKα phosphorylates NF-κB2 at two C-terminal sites, and this activity requires its phosphorylation by upstream kinases, one of which may be NF-κB-inducing kinase (NIK). Phosphorylation of these sites is essential for p100 processing to p52, while polyubiquitination and proteasomal degradation are also indispensable. The phosphorylation-dependent ubiquitination of p100 results in degradation of its inhibitory C-terminal half, which is different from the complete degradation of p100, as seen with IκBs. The activation of this alternative pathway then brings about nuclear translocation of p52-RelB dimers [136]. There are seven IκB family members IκBα, IκBβ, BCL-3, IκBε, IκBγ, and the precursor proteins p100 and p105, which are characterized by the presence of five to seven ankyrin repeats that assemble into elongated cylinders that bind the dimerization domain of NF-κB dimers. The crystallographic structures of IκBα and IκBβ bound to p65/p50 or p65/c-Rel dimers revealed that the IκB proteins mask only the nuclear localization sequence (NLS) of p65, whereas the NLS of p50 remains accessible [137,138]. The presence of this accessible NLS on p50, coupled with nuclear export sequences (NES), that are present on IκBα and p65, results in constant shuttling of IκBα/NF-κB complexes between the nucleus and the cytoplasm, although the steady-state localization is in the cytosol [139]. The dynamic balance between cytosolic and nuclear localization is altered upon IκBα degradation, because it removes the contribution of the IκB NES and exposes the masked NLS of p65, resulting in predominantly nuclear localization of NF-κB.

Inhibitory IκB proteins tightly control the biological activity of Rel/NF-κB transcription factors through their association with homo- or heterodimers of this family. Members of the family share a highly conserved NH2-terminal sequence termed the Rel homology domain, which is required for DNA binding, dimerization, nuclear localization, and interaction with the IκB molecules. In response to an inflammatory stimulus, cytokines, or viral infections, IκB proteins are rapidly degraded by the 26 S multicatalytic proteasome. Degradation of IκBα, the most intensively characterized inhibitor, requires phosphorylation on serine residues 32-36 [140] by the activated IκB kinase complex.

As a consequence of IκBα degradation, the freed NF-κB accumulates in the nucleus, where it activates gene transcription [141]. NF-κB acts on genes codifying for cytokines, chemokines, immune receptors, and adhesion molecules, and its activation leads to a coordinated increase in the expression of inflammatory and immune response mediators. Apart from the well characterized inhibitory function on NF-κB in the cytoplasm [141], IκB also participates in the inhibition of NF-κB-dependent transcription in the cell nucleus. Once the stimulus is withdrawn, NF-κB activity is rapidly shut down, ensuring that the IκB-dependent transcriptional activity is only transient.

This is accounted for by two mechanisms. First, free, non-NF-κB-associated IκBα has the capacity to enter the nucleus when the protein is overexpressed from a heterologous promoter. Such a property seems to rely on an active process mediated by a non-canonical nuclear import sequence, located within the second ankyrin domain of IκBα protein [142]. Secondly, IκBα has the ability to both prevent NF-κB binding to and to dissociate NF-κB from specific DNA consensus sequences. Nuclear localization of IκBα is induced by stimuli activating NF-κB and can be considered as part of a physiological mechanism regulating NF-κB-dependent transcription. This assumption is supported by the fact that a massive accumulation of IκBα, which becomes detectable in the nucleus upon extinction of the cell signaling, occurs concomitantly with loss of NF-κB-DNA binding activity and extinction of NF-κB-dependent transcription. The main observation in this study is that nuclear IκBα, known to be detectable transiently upon extinction of the activation stimulus, is in fact entering the nucleus steadily in cells continuously exposed to stimulation, but is constantly degraded in the nuclear compartment as long as stimulation persists.

Current findings reveal the existence of two dynamically related mechanisms finely tuning the transcriptional activity of NF-κB into the nucleus of mammalian cells. The first one permits nuclear NF-κB to remain transcriptionally active as long as stimulation is ongoing, and it results from proteasome-mediated degradation of nuclear IκBα, thus suppressing the termination properties of this inhibitor. The second one, intervening later when NF-κB activity is no longer needed, results from retrograde transport of NF-κB proteins to the cytoplasm by nuclear IκBα molecules whose destruction is stopped when stimulation is finished, thus liberating the nucleus from then unwanted NF-κB molecules. These two mechanisms would thus successively act to optimize the efficiency and the timing of NF-κB-dependent gene transcription, adapting the latter to cell activation or rest, death, or survival. Inducible transcription factors regulate immediate and long-lived cellular responses necessary for organism adaptation to environmental plasticity. Such responses are mediated to a large degree through changes in gene expression [143]. One transcription factor that serves as a key responder to changes in the environment is NF-κB, an evolutionarily conserved signaling module that plays a critical role in many biological processes.

The biological system in which NF-κB plays the most important role is the immune system [123,136]. In particular, recent results suggest that the classical pathway is mostly involved in innate immunity while the alternative pathways may be involved in adaptive immunity.

NF-κB regulates the expression of cytokines, growth factors, and effector enzymes in response to ligation of many receptors involved in immunity including T-cell receptors (TCRs), B-cell receptors (BCRs) and Toll/IL-1R family [144,136].

NF-κB also regulates the expression of genes outside the immune system and, hence, can influence multiple aspects of normal physiology and disease. Recognition of IκBα leads to polyubiquitination at conserved residues, Lys 21 and Lys 22 on IκBα, and NF-κB plays an essential role in early

events of innate immune responses through TLR signalling pathways.

NF-κB is a redox-sensitive transcription factor that is involved in the transmission of various signals from the cytoplasm to the nucleus of numerous cell types [145]. It is found as a trimer consisting of p50, p65, and IκB subunits in the cytosol. The release of IκB from the trimer results in the migration of the p50/p65 heterodimer to the nucleus and the subsequent DNA binding [146]. This process activates genes involved in the immune, inflammatory, or acute-phase response, such as cytokines [monocyte chemoattractant protein-1 (MCP-)1, IL-8], adhesion molecules, and procoagulant proteins (tissue factor, plasminogen activator inhibitor 1). Recent data strongly suggest that NF-κB could be involved in the pathogenesis of atherosclerosis [147]. NF-κB is present in the human atherosclerotic lesions in the nuclei of macrophages and endothelial cells [148] and participates in dysregulation of vascular smooth muscle cells in human atherosclerosis [149]. Conversely, accumulating evidence suggests that postprandial lipemia is strongly associated with the risk of developing atherosclerotic lesions [150]. In fact, in this study, it has been shown that a fat-enriched breakfast increases triglycerides and chylomicrons, whereas the simultaneous consumption of red wine was associated with an increment in total triglycerides, chylomicrons, and VLDL triglycerides. Postprandial lipemia was correlated with an increment of NF-κB activation in PBMCs that was prevented by red wine intake. An intake of another form of alcohol, vodka, did not prevent the activation of this transcription factor provided by postprandial lipemia.

Because VLDLs were the only lipoproteins augmented following red wine intake but not after the fat ingestion alone, the effects of VLDLs on NF-κB activation were tested. VLDLs elicited an increase in NF-κB activation in human mononuclear THP-1 cells that was prevented by co-incubation with quercetin and α-tocopherol succinate, two antioxidants contained in red wine [151]. Because NF-κB regulates many genes involved in the pathogenesis of coronary artery disease, these results provided a new explanation concerning the potential beneficial effects of moderate consumption of red wine in human beings. Therefore, it has been suggested that NF-κB activation could be involved in the pathogenesis of atherosclerosis, because numerous proinflammatory genes are regulated by this transcription factor [152]. Furthermore, an increased expression of numerous genes known to be regulated by NF-κB has been found in the atherosclerotic lesions, [149] and NF-κB is selectively and markedly activated in humans with unstable angina pectoris [147]. Furthermore, monocytes are involved in the progression of atherosclerosis and are potent activators of blood coagulation through their ability to synthesize procoagulant factors (plasminogen activator inhibitor-1, tissue factor) that are regulated by NF-κB.

Conversely, it is known that oxidants increase NF-κB activation, whereas such antioxidants as pyrrolidine dithiocarbamate and *N*-acetyl cysteine inhibit NF-κB activation [153]. Because of the redox regulation of NF-κB, it is possible that the antioxidants contained in red wine were the cause of the inhibition of NF-κB activation. In this sense, Feng *et al.*, [154] demonstrated that red wine intake inhibited

MCP-1 expression in cholesterol-fed rabbits, a protein regulated by NF-κB, and this effect might be partly attributed to its antioxidant effects. In addition, catechin and vitamin E prevented the development of fatty streak in hypercholesterolemic hamsters [155] and attenuated early lesion development in rabbits [156]. Moreover, red wine and non alcoholic wine products can prevent plaque formation in hypercholesterolemic rabbits despite significant increases in LDL [68]. Also, red wine polyphenols inhibited proliferation of vascular smooth muscle cells [157] and reduced the susceptibility of LDL to cause oxidation *in vitro* [158] and *in vivo* [159]. In conclusion, red wine intake, but not another form of alcohol beverage intake (vodka), prevented NF-κB activation in PBMCs elicited in healthy volunteers by postprandial lipemia. Because NF-κB activation is involved in the pathogenesis of atherosclerotic lesions, the inhibitory effect of red wine on NF-κB activation provides a further explanation of the beneficial effects of red wine intake in cardiovascular disease [49].

MAPKS FAMILY

MAPKs are a prominent group of serine/threonine protein Kinases that in mammalian cells consist of three families: p38 MAPK, ERK, and JNK. Mammalian ERK1 and ERK2 (ERK1/2) MAPKs predominantly mediate mitogenic and cellular differentiation signals: p38 and JNK MAPKs are mainly activated by exposure of cells to stress signal [160-162].

Numerous reports indicate that MAPK signaling pathways are affected by ethanol in a manner that depends on the organ or cell type, the duration of ethanol administration (acute vs chronic), and the type of stimulatory agents [163]. A study on the effect of ethanol exposure on MAPK activity monocytes/macrophages showed that LPS-induced p38 MAPK activation was inhibited in human blood cells cultured in the presence of ethanol [164].

Finally, results of MAPK-mediate processes depend on a length and a degree of activation of the MAPKs. Because MAPKs are activated by a double phosphorylation of a relevant threonine and tyrosine residues, a removal of a single phosphate from phosphothreonine or phosphotyrosine affects the activity of the enzymes.

MAPKs were studied as potential therapeutic targets in inflammatory and proliferative disorders [165]. Activation of MAPK pathways by LPS and cytokines represents a potential signaling mechanism for NO production during the inflammatory response.

Furthermore, the extracellular signal-regulated kinases (ERKs), c-jun N-terminal kinase/stress-activated protein kinases (JNK/SAPKs), and p38 MAPK pathways have been implicated in the activation of AP-1, which is involved in iNOS promoter activation by cytokines. The ERK and p38 MAPK pathways played a crucial role in human NOS transcriptional activation *via* modulation of AP-1 binding to specific promoter sequences.

In our *in vitro* experiments we have evaluated the molecular mechanisms elicited by polyphenols from red wine from healthy human PBMC [84]. In particular, our interest has been to investigate the putative activation of p38 and

ERK1/2 and expression of IκBα as an inhibitory of NF-κB. Also in this case we have observed a stronger activation of P-p38 and of PERK1/2 when mononuclear cells were treated in presence of polyphenols and alcohol and polyphenols alone but not with alcohol alone. Furthermore, stimulation of mononuclear cells in presence of a double stimulus (polyphenols plus LPS) gave rise to a lesser expression of these activated molecules in comparison with cells treated in the absence of LPS, thus confirming that a reduced expression of P-p38 and PERK1/2 should be beneficial for the host since it avoids an exaggerated release of proinflammatory cytokines that could be detrimental to the organism.

Regarding phosphorylated form of IκBα we have observed its decreased expression when cells were stimulated in presence of whole wine and polyphenols alone in comparison with LPS treated cells. These data are in accordance with the lower expression of p65/NF-κB than that of LPS treated cells.

As far as p38 activation is concerned, it has been observed that polyphenols and alcohol are responsible for p38 activation that acts on NF-κB which, in turn, stimulates release of t-PA, thus reducing the risk for acute coronary heart disease (CAD) by promoting fibrinolysis [166].

CYTOKINE RELEASE

Results on cytokine production obtained following stimulation of PBMC with red wine polyphenols seem to support the involvement of p38.

In previous experiments we found that Negroamaro polyphenols induced IFN-γ release from PBMC. It is known that p38 may act stimulating IFN-γ which, bound to its specific membrane receptor, activates the molecular nuclear factor STAT1, that, in turn, by binding to motifs at -5.2 and -5.8 kb in the iNOS promoter leads to NO production.

On the other hand, we have also observed [167] an increase in IL-12 which is known that acts as a key regulator of cell-mediated immune responses through the induction of T helper (h)1 type differentiation, and induces cellular immunity by promoting IFN-γ release. Furthermore, release of IL-1β, IL-6, and IL-10 (even if to a lesser extent) after stimulation of PBMC with red wine polyphenols was observed. On the other hand, IL-6 itself stimulates the release of IL-10, thus restoring the "equilibrium" between cytokines of Th1 and Th2 origin (Fig. **5**).

Briefly, these results, demonstrate that moderate intake of red wine polyphenols could be beneficial in terms of host protection. IL-10 seems to control the proinflammatory pathway even if limited biochemical and clinical evidence suggests a link between IL-10 and CHD [168]. In fact, in a randomized clinical trial, that examined the effects of hormone replacement therapy on post-menopausal women with known coronary atherosclerosis, elevated IL-10 concentration was associated with an increased risk for future cardiovascular events. However, our results did not provide evidence for an elevated production of IL-10.

On the other hand, IFN-γ and IL-12 can promote macrophage, natural killer and cytotoxic cells responses toward various pathogens such as bacteria, virus and parasites involved in atherosclerotic plaque formation.
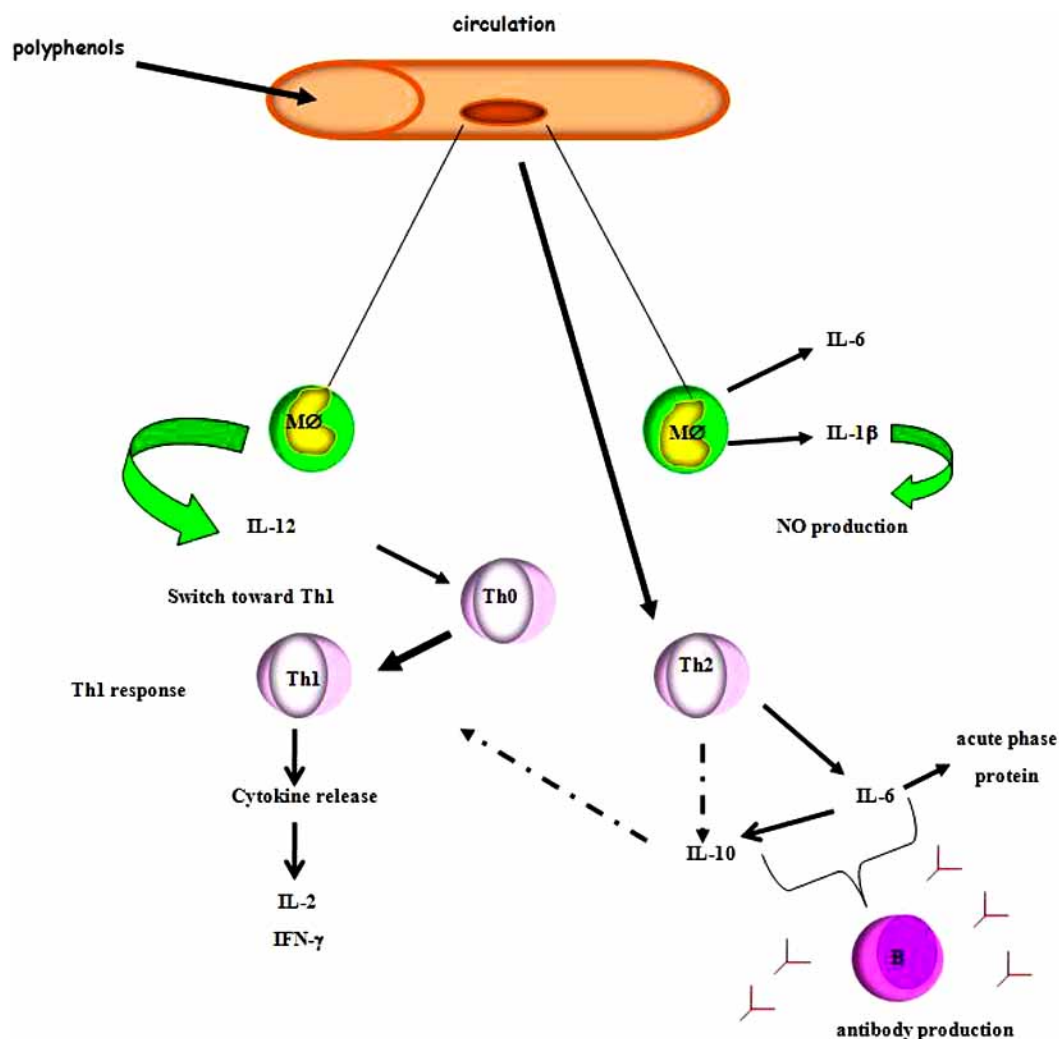
Fig. (5). Red wine polyphenols (Negroamaro) polarize the immune response toward the Th1 type *via* release of IL-12 and subsequent production of IFN-γ. IL-6 and IL-1β production is enhanced and counterbalanced by IL-10. Thus, moderate red wine assumption is able to promote Th1 cytokine release and the proinflammatory pathway with subsequent protection of the host against pathogens.

INTAKE OF RED WINE AND INTESTINAL ABSORPTION

The majority of polyphenols have a sugar residue linked to the carbon skeleton. Glucose is a common sugar residue; however, residues can include different monosaccharides, disaccharides, or oligosaccharides. Other compounds including amines, organic acids, carboxylic acids, lipids, and other polyphenols may also be linked to the basic polyphenolic structure [169].

Because of the abundance of polyphenols in nature, it is not surprising that they can be found in fruits, vegetables, coffee, tea, chocolate, and soy [4]. Once ingested, polyphenols have several possible fates, including absorption in the small intestine or colon, and/or excretion in the feces or urine. The site and rate of absorption depend on the chemical structure, degree of glycosylation/acylation, conjugation of other phenolics, molecular size, degree of polymerization, and solubility [169,170]. In the small intestine, polyphenols can enter the mucosa through passive diffusion. In some instances, hydrophobic moieties must be cleaved for absorption to take place. In the colon, polyphenols are initially digested into smaller phenolic structures by gut microflora. After this initial digestion is complete, the polyphenols and their metabolites may be absorbed [169].

Once absorption has taken place, polyphenols and their metabolites are transported to the liver. Further digestion may occur there, and then polyphenol metabolites may be transported to extrahepatic tissues or to the kidneys where they are excreted in the urine. For the majority of polyphenols, the maximum concentration in the plasma is apparent 1–2 h after ingestion. Polyphenols may also be incorporated into bile, return to the small intestine, and eventually be excreted in the feces [169].

In general terms, peak plasma concentrations of polyphenols were higher after aglycone ingestion than after glucoside ingestion, thus indicating a preferential absorption of polyphenols *via* the small intestine [171,172].

Intake of polyphenols can stimulate Gut Associated Lymphoreticular Tissue (GALT) that is rich in macrophages which generating NO can enter into circulation *via* lymphatic vessels and may contribute to the increase in plasma levels of NO along with the aliquot generated by endothelial cells.

Finally, we have also observed *in vitro* antibody production (IgA and IgG) from human healthy PBMC in presence of whole wine and polyphenols alone. This fact plays an important role in those pathological conditions where humoral and cellular immune responses are compromised. After intake of red wine polyphenols, GALT B cells are activated and their products can arrive to distant sites (spleen, glands and peripheral blood), thus providing specific immune protection (Fig. **6**) In particular, cytokine and immunoglobulin production could be very important in the case of geriatrics individuals, generally characterized by a series of immune dysfunctions, due to the involution of thymus (T cells deficiency) and altered antigen presentation with a defective phagocytosis and killing of microorganisms also due to a reduced NO [173].

Reequilibrium of immune balance in the elderly after moderate intake of red wine should be taken into serious consideration.

FUTURE TRENDS

As we have discussed in this review, a large body of evidence suggests that red wine polyphenols exert beneficial effects to human health when assumed at a moderate dosage. In particular, Negroamaro wine is a powerful inducer of NO and of proinflammatory cytokines, and its potential atherogenic property has been described. However, this finding is not supported by other reports dealing with single polyphenols and not with a mixture of them as in the case of whole wine. For instance, just recently evidence has been provided that (+)- catechin hampers tumor angiogenesis *via* inhibition of proinflammatory cytokines, NO, Vascular Endothelial Growth Factor, IL-2, and Tissue Inhibitor of Metalloproteinase-1 [174].

This implies that according to their polyphenol content different types of wine may exhibit a variety of biological effects into the host, and, therefore, investigations in this direction should be pursued. In fact, isolation and characterization of polyphenols from red grapes endowed with the strongest antiinflammatory and antineoplastic activities should represent the next target in this type of studies.

ACKNOWLEDGEMENTS
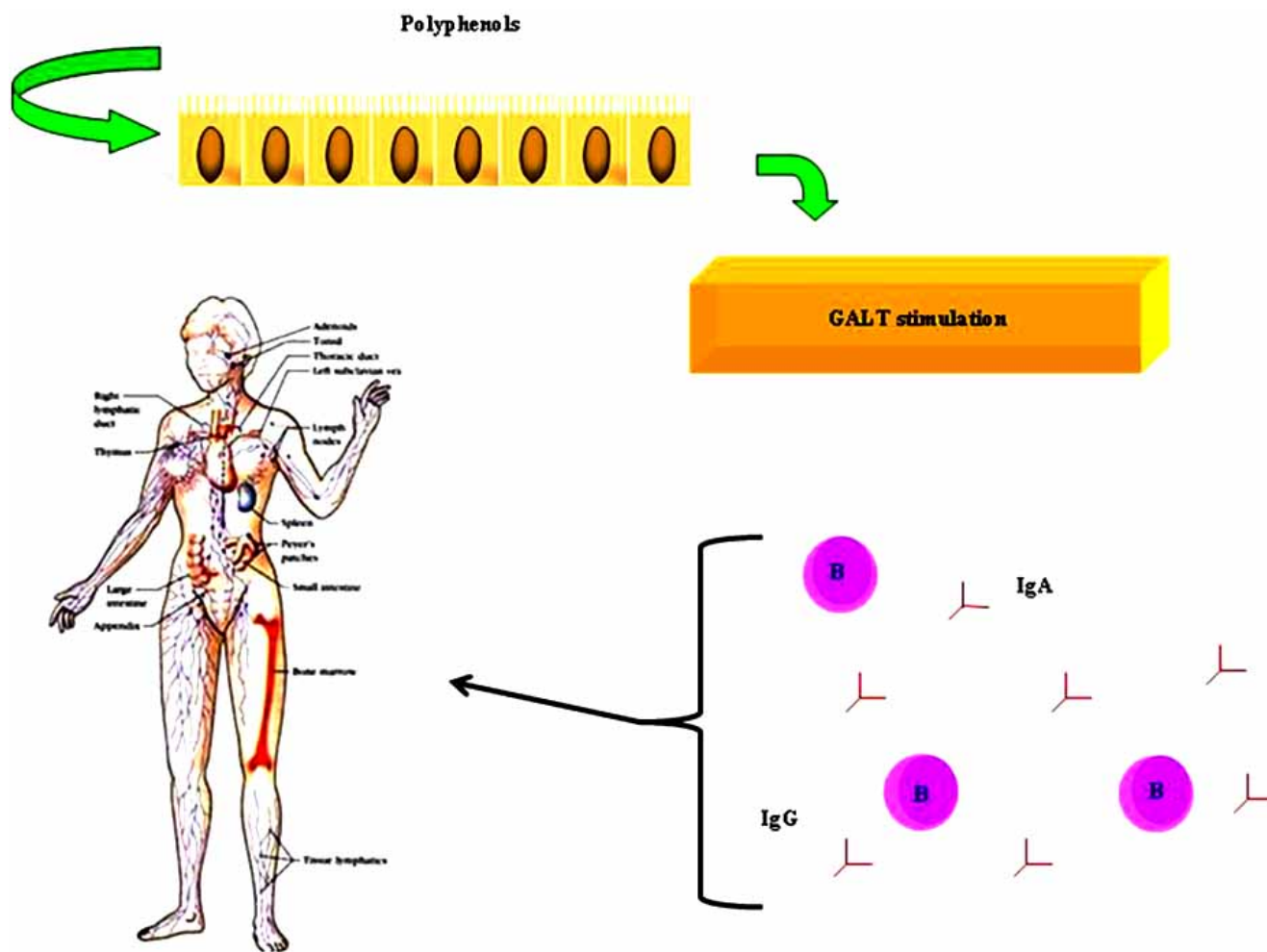
Fig. (6). Intake of red wine polyphenols stimulates release of immunoglobulins from B cells within GALT. Immunoglobulin reaches lymphoid organs and protects the host aganist various pathogens.

ABBREVIATIONS

BCRs	=	B cell receptors
CHD	=	Coronary Heart Disease
GALT	=	Gut Associated Lymphoreticular Tissue
HDL	=	High Density Lipoprotein
IL	=	Interleukin
IκBα	=	Inhibitor of NF-κB
iNOS	=	inducible Nitric Oxide Synthase
LDL	=	Low Density Lipoprotein
LPS	=	Lypopolysaccharide
MAPK	=	Mitogen Activated Protein Kinase
MCP-1	=	Monocyte Chemoattractant Protein-1
NO	=	Nitric Oxide
NF-κB	=	Nuclear Factor -κB
PBMC	=	Peripheral Blood Mononuclear Cell
RWPCs	=	Red Wine Polyphenol Compounds
SIRT	=	Sirtuitin
SRA	=	Scavenger Receptor A
TIMP-1	=	Tissue Inhibitor of Metalloproteinase-1
TLR	=	Toll like Receptor
TNF-α	=	Tumor Necrosis Factor-alpha
VEGF	=	Vascular Endothelial Growth Factor
VLDL	=	Very Low Density Lipoprotein

REFERENCES

References 175-177 are related articles recently published.

[1]   Manach C, Mazur A, Scalbert A. Polyphenols and prevention of cardiovascular diseases. Curr Opin Lipid 2005; 16: 77-84.

[2]   Scalbert A, Johnson IT, Saltmarsh M. Polyphenols: antioxidants and beyond. Am J Clin Nutr 2005; 81: S215-S217.

[3]   Cordova AC, Jackson LS, Berke-Schlessel DW, Sumpio BE. The cardiovascular protective effect of red wine. J Am Coll Surg 2005; 200: 428-39.

[4]   Bravo L. Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. Nutr Rev 1998; 56: 317-33.

[5]   Xiuzhen H, Tao S, Hongxiang L. Dietary polyphenols and their biological significance. Int J Mol Sci 2007; 8: 950-88.

[6]   Amorati R, Lucarini M, Mugnaini V, Pedulli GF, Roberti M, Pizzirani D. Antioxidant activity of hydroxystilbene derivatives in homogeneous solution. J Org Chem 2004; 69: 7101-7.

[7]   Baolin L, Inami Y, Tanaka H, Inagaki H, Iinuma M, Nagai H. Resveratrol inhibits the release of mediators from bone marrow-derived mouse mast cells *in vitro*. Planta Med 2004;70: 305-9.

[8]   Bode AM, Dong Z. Targeting signal transduction pathways by chemopreventive agents. Mutat Res 2004; 555: 33-51.

[9]   Alarcon de la Lastra C, Villegas I. Resveratrol as an anti-inflammatory and anti-aging agent: mechanisms and clinical implications. Mol Nutr Food Res 2005; 9: 405-30.

[10]   Han Y, Zheng WH, Bastianetto S, Chabot JG, Quirion R. Neuro-protective effects of resveratrol against beta-amyloid-induced neurotoxicity in rat hippocampal neurons: involvement of protein kinase C. Br J Pharmacol 2004; 141: 997-1005.

[11]   Hung LM, Chen JK, Huang SS, Lee RS, Su MJ. Cardioprotective effect of resveratrol, a natural antioxidant derived from grapes. Cardiovasc Res 2000; 47: 549-55.

[12]   Guarente L. Sirtuins as potential targets for metabolic syndrome. Nature 2006; 444: 868-74.

[13]   Zhu H, Guo Q, Mattson MP. Dietary restriction protects hippocampal neurons against the death-promoting action of a presenilin-1 mutation. Brain Res 1999; 842: 224-9.

[14]   Ingram DK, Weindruch R, Spangler EL, Freeman JR, Walford RL. Dietary restriction benefits learning and motor performance of aged mice. J Gerontol 1987; 42: 78-81.

[15]   Civitarese AE, Carling S, Heilbronn LK, Hulver MH, Ukropcova B, Deutsch WA, *et al.* Calorie restriction increases muscle mitochondrial biogenesis in healthy humans. PLoS Med 2007; 4: e 76.

[16]   Baur JA, Pearson KJ, Price NL, Jamieson HA, Lerin C, Kalra A, *et al.* Resveratrol improves health and survival of mice on a high-calorie diet. Nature 2006; 444: 337-42.

[17]   Lagouge M, Argmann C, Gerhart-Hines Z, Meziane H, Lerin C, Daussin F, *et al.* Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1alpha. Cell 2006; 127: 1109-22.

[18]   Milne JC, Lambert PD, Schenk S, Carney DP, Smith JJ, Gagne DJ, *et al.* Small molecule activators of SIRT1 as therapeutics for the treatment of type 2 diabetes. Nature 2007; 450: 712-6.

[19]   Liu L, Wang Y, Lam KSL, Xu A. Moderate wine consumption in the prevention of metabolic syndrome and its related medical complications. Endocr Metab Immune Disord Drug Targets 2008; 8: 89-98.

[20]   Oberdoerffer P, Sinclair DA. The role of nuclear architecture in genomic instability and ageing. Nat Rev Mol Cell Biol 2007; 8: 692-702.

[21]   Westphal CH, Dipp MA, Guarente L. A therapeutic role for sirtuins in diseases of aging? Trends Biochem Sci 2007; 32: 555-60.

[22]   Yamamoto H, Schoonjans K, Auwerx J. Sirtuin functions in health and disease. Mol Endocrinol 2007; 21: 1745-55.

[23]   Nisoli E, Clementi E, Carruba MO, Moncada S. Defective mitochondrial biogenesis: a hallmark of the high cardiovascular risk in the metabolic syndrome? Circ Res 2007; 100: 795-806.

[24]   Pecháňová O, Rezzani R, Babál P, Bernátová I, Andriantsitohaina R. Beneficial effects of Provinols™: cardiovascular system and kidney. Physiol Res 2006; 55: S17-S30.

[25]   Rimm EB, Williams P, Fosher K, Criqui M, Stampfer MJ. Moderate alcohol intake and lower risk of coronary heart disease: meta-analysis of effects on lipids and haemostatic factors. BMJ 1999; 319: 1523-8.

[26]   Renaud S, Guéguen R, Schenker J, d'Houtaud A. Alcohol and mortality in middle-aged men from eastern France. Epidemiology 1998; 9: 184-8.

[27]   Middleton E, Kandaswami C, Theoharides TC. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacol Rev 2000; 52: 673-751.

[28]   Zenebe W, Pecháňová O. Effects of red wine polyphenolic compounds on the cardiovascular system. Bratisl Lek Listy 2002; 103: 159-65.

[29]   Curin Y, Andriantsitohaina R. Polyphenols as potential therapeutical agents against cardiovascular diseases. Pharmacol Rep 2005; 57: 97-107.

[30]   Jendeková L, Kojšová S, Andriantsitohaina R, Pecháňová O. The time-dependent effect of Provinols™ on brain NO synthase activity in L-NAME-induced hypertension. Physiol Res 2006; 55: S31-S37.

[31]   Iijima K, Yoshizumi M, Hashimoto M, Akishita M, Kozaki K, Ako J, *et al.* Red wine polyphenols inhibit vascular smooth muscle cell migration through two distinct signaling pathways. Circulation 2002; 105: 2404-10.

[32]   Di Carlo G, Mascolo N, Izzo AA, Capasso F. Flavonoids: old and new aspects of a class of natural therapeutic drugs. Life Sci 1999; 65: 337-53.

[33]   Ross R. Atherosclerosis. An inflammatory disease. N Engl J Med 1999; 340: 115-26.

[34]   Yamashita S, Hirano K, Kuwasako T, Janabi M, Toyama Y, Ishigami M, *et al.* Physiological and pathological roles of a multi-ligand receptor CD36 in atherogenesis; insights from CD36-deficient patients. Mol Cell Biochem 2007; 299: 19-22.

[35]   Kuliczkowska-Płaksej J, Bednarek-Tupikowska G, Płaksej R, Filus A. Scavenger receptor CD36: its expression, regulation, and role in

the pathogenesis of atherosclerosis. Part I. Postepy Hig Med Dosw 2006; 60: 142-51.

[36]   Tabas I. Free cholesterol-induced cytotoxicity. A possible contributing factor to macrophage foam cell necrosis in advanced atherosclerotic lesions. Trends Cardiovasc Med 1997; 7: 256-63.

[37]   Sawayama Y, Tatsukawa M, Maeda S, Ohnishi H, Furusyo N, Hayashi J. Association of hyperhomocysteinemia and Chlamydia pneumoniae infection with carotid atherosclerosis and coronary artery disease in Japanese patients. J Infect Chemother 2008; 14: 232-7.

[38]   Watson C, Alp NJ. Role of Chlamydia pneumoniae in atherosclerosis. Clin Sci (Lond) 2008; 114: 509-31.

[39]   de Lorgeril M, Salen P. Wine ethanol, platelets, and Mediterranean diet. Lancet 1999; 353:1067.

[40]   Criqui MH, Ringel BL. Does diet or alcohol explain the French Paradox? Lancet 1994; 344: 1719-23.

[41]   Hertog MGL, Kromhout D, Aravanis C, Blackburn H, Buzina R, Fidanza F, *et al.* Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. Arch Intern Med 1995; 155: 381-6.

[42]   Brouillard R, George F, Fougerousse A. Polyphenols produced during red wine ageing. Biofactors 1997; 6: 403-10.

[43]   Soulat T, Philippe C, Bal dit Sollier C, Brèzillon C, Berge N, Teissedre PL, *et al.* Wine constituents inhibit thrombosis but not atherogenesis in C57BL/6 apolipoprotein E-deficient mice. Br J Nutr 2006; 96: 290-8.

[44]   Demrow HS, Slane PR, Folts JD. Administration of wine and grape juice inhibits *in vivo* platelet activity and thrombosis in stenosed canine coronary arteries. Circulation 1995; 91: 1182-8.

[45]   De Curtis A, Murzilli S, Di Castelnuovo A, Rotilio D, Donati MB, De Gaetano G, *et al.* Alcohol-free red wine prevents arterial thrombosis in dietary-induced hypercholesterolemic rats: experimental support for the 'French paradox'. J Thromb Haemost 2005; 3: 346-50.

[46]   Bell RA, Mayer-Davis EJ, Martin MA, D'Agostino RB Jr, Haffner SM. Associations between alcohol consumption and insulin sensitivity and cardiovascular disease risk factors: the insulin resistance and atherosclerosis study. Diabetes Care 2000; 23: 1630-6.

[47]   Ashen MD, Blumenthal RS. Clinical practice. Low HDL cholesterol levels. N Engl J Med 2005; 353: 1252-60.

[48]   Stocker R, O'Halloran RA. Dealcoholized red wine decreases atherosclerosis in apolipoprotein E gene-deficient mice independently of inhibition of lipid peroxidation in the artery wall. Am J Clin Nutr 2004; 79: 123-30.

[49]   Blanco-Colio LM, Valderrama M, Alvarez-Sala LA, Bustos C, Ortego M, Hernandez-Presa MA, *et al.* Red wine intake prevents nuclear factor-kappaB activation in peripheral blood mononuclear cells of healthy volunteers during postprandial lipemia. Circulation 2000; 102: 1020-6.

[50]   Shoji T, Masumoto S, Moriichi N, Akiyama H, Kanda T, Ohtake Y, *et al.* Apple procyanidin oligomers absorption in rats after oral administration: analysis of procyanidins in plasma using the porter method and high performance liquid chromatography/tandem mass spectrometry. J Agric Food Chem 2006; 54: 884-92.

[51]   Hayek T, Fuhrman B, Vaya J, Rosenblat M, Belinky P, Coleman R, *et al.* Reduced progression of atherosclerosis in apolipoprotein E-deficient mice following consumption of red wine, or its polyphenols quercetin or catechin, is associated with reduced susceptibility of LDL to oxidation and aggregation. Arterioscler Thromb Vasc Biol 1997; 17: 2744-52.

[52]   Hertog MG, Feskens EJ, Hollman PC, Katan MB, Kromhout D. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen elderly study. Lancet 1993; 342: 1007-11.

[53]   Auger C, Gerain P, Laurent-Bichon F, Portet K, Bornet A, Caporiccio B, *et al.* Phenolics from commercialized grape extract prevent early atherosclerotic lesions in hamsters by mechanisms other than antioxidant effect. J Agric Food Chem 2004; 52: 5297-302.

[54]   Nardini M, Natella F, Scaccini C. Role of dietary polyphenols in platelet aggregation. A review of the supplementation studies. Platelets 2007; 18: 224-43.

[55]   Nam NH. Naturally occurring NF-kappaB inhibitors. Mini Rev Med Chem 2006; 6: 945-51.

[56]   de Lange DW, van de Wiel A. Drink to prevent: review on the cardioprotective mechanisms of alcohol and red wine polyphenols. Semin Vasc Med 2004; 4: 173-86.

[57]   Andriambeloson E, Magnier C, Haan-Archipoff G, Lobstein A, Anton R, Beretz A *et al.* Natural dietary polyphenolic compounds cause endothelial-dependent vasorelaxation in rat thoracic aorta. J Nutr 1998; 128: 2324-33.

[58]   Zenebe W, Pecháňová O, Andriantsitohaina R. Red wine polyphenols induce vasorelaxation by increased nitric oxide bioactivity. Physiol Res 2003; 52: 425-32.

[59]   Duarte J, Andriambeloson E, Diebolt M, Andriantsitohaina R. Wine polyphenols stimulate superoxide anion production to promote calcium signaling and endothelial-dependent vasodilatation. Physiol Res 2004; 53: 595-602.

[60]   Wallerath T, Poleo D, Li H, Forstermann U. Red wine increases the expression of human endothelial nitric oxide synthase: a mechanism that may contribute to its beneficial cardiovascular effects. J Am Coll Cardiol 2003; 41: 471-8.

[61]   Wollny T, Aiello L, Di Tommaso D, Bellavia V, Rotilio D, Donati MB, *et al.* Modulation of haemostatic function and prevention of experimental thrombosis by red wine in rats: a role for increased nitric oxide production. Br J Pharmacol 1999; 127: 747-55.

[62]   Pecháňová O, Bernátová I, Babál P, Martinez MC, Kyselá S, Štvrtina S, *et al.* Red wine polyphenols prevent cardiovascular alterations in L-NAME-induced hypertension. J Hypertens 2004; 22: 1551-9.

[63]   Bernátová I, Pecháňová O, Babál P, Kyselá S, Štvrtina S, Andriantsitohaina R. Wine polyphenols improve cardiovascular remodeling and vascular function in NO-deficient hypertension. Am J Physiol Heart Circ Physiol 2002; 282: H942-8.

[64]   Cai H, Harrison DG. Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. Circ Res 2000; 87: 840-4.

[65]   Lusis AJ. Atherosclerosis. Nature 2000; 407: 233-41.

[66]   Luscher TF. Endothelium-derived nitric oxide: the endogenous nitrosovasodilator in the human cardiovascular system. Eur Hearth J 1991; 12 Suppl E: 2-11.

[67]   Abbey M, Kerry NL. Flavonoids and their effects on LDL oxidation. Proc Nutr Soc Austr 1997; 21: 91-7.

[68]   da Luz PL, Serrano Junior CV, Chacra AP, Monteiro HP, Yoshida VM, Furtado M, *et al.* The effect of red wine on experimental atherosclerosis: lipid-independent protection. Exp Mol Pathol 1999; 65: 150-9.

[69]   Coleman JW. Nitric oxide in immunity and inflammation. Int Immunopharm 2001; 1: 1397-406.

[70]   Bogdan C. Nitric oxide and the regulation of gene expression. Trends in Cell Biol 2001; 11: 66-75.

[71]   Nappi AJ, Vass E, Frey F, Carton Y. Nitric oxide involvement in Drosophila immunity. Nitric oxide 2000: 4: 423-30.

[72]   Klessig DF, Durner J, Noad R, Navarre DA, Wendehenne D, Kumar D, *et al.* Nitric oxide and salicylic acid signaling in plant defense. Proc Natl Acad Sci USA 2000; 97: 8849-55.

[73]   van der Veen RC. Nitric oxide and T helper cell immunity. Int Immunopharmacol 2001; 1: 1491-500.

[74]   Kronke KD, Fehsel K, Suschek C, Kolb-Bachofen V. Inducible nitric oxide synthase-derived nitric oxide in gene regulation, cell death and cell survival. Int Immunopharmacol 2001: 1: 1407-20.

[75]   Bogdan C, Rollinghoff M, Diefenbach A. Reactive oxygen and reactive nitrogen intermediates in innate and specific immunity. Curr Opin Immunol 2000; 12: 64-76.

[76]   Thomassen MJ, Kavuru MS. Human alveolar macrophages and monocytes as a source and target for nitric oxide. Int Immunopharmacol 2001: 1: 1479-90.

[77]   Cifone MG, Ulisse S, Santoni A. Natural killer cells and nitric oxide. Int Immunopharmacol 2001; 1: 153-24.

[78]   Magrone T, Tafaro A, Jirillo F, Panaro MA, Cuzzuol P, Cuzzuol AC, *et al.* Red wine consumption and prevention of atherosclerosis: An *in vitro* model using human peripheral blood mononuclear cells. Curr Pharm Des 2007; 13: 3718-25.

[79]   Vogel RA. Vintners and vasodilators: are French red wines more cardioprotective? J Am Coll Cardiol 2003; 41: 479-81.

[80]   Räthel TR, Samtleben R, Vollmar AM, Dirsch VM. Activation of endothelial nitric oxide synthase by red wine polyphenols: impact

of grape cultivars, growing area and the vinification process. J Hypertens 2007; 25: 541-9.

[81] Wallerath T, Li H, Godtel-Ambrust U, Schwarz PM, Forstermann U. A blend of polyphenolic compounds explains the stimulatory effect of red wine on human endothelial NO synthase. Nitric Oxide 2005; 12: 97-104.

[82] Caimi G, Carollo C, Lo Presti R. Wine and endothelial function. Drugs Exp Clin Res 2003; 29: 235-42.

[83] Wallerath T, Deckert G, Ternes T, Anderson H, Li H, Witte K, *et al.* Resveratrol, a polyphenolic phytoalexin present in red wine, enhances expression and activity of endothelial nitric oxide synthase. Circulation 2002; 106: 1652-8.

[84] Magrone T, Panaro MA, Jirillo E, Covelli V. Molecular effects elicited *in vitro* by red wine on human healthy peripheral blood mononuclear cells: potential therapeutical application of polyphenols to diet-related chronic disease. Curr Pharm Des 2008; 14(26): 2758-66.

[85] Andriambeloson E, Kleschyov AL, Muller B, Beretz A, Stoclet JC, Andriantsitohaina R. Nitric oxide production and endothelium-dependent vasorelaxation induced by wine polyphenols in rat aorta. Br J Pharmacol 1997; 120: 1053-8.

[86] Andriambeloson E, Stoclet JC, Andriantsitohaina R. Mechanism of endothelial nitric oxide-dependent vasorelaxation induced by wine polyphenols in rat thoracic aorta. J Cardiovasc Pharmacol 1999; 33: 248-54.

[87] Ralay Ranaivo H, Dielbolt M, Andriantsitohaina R. Wine polyphenols induce hypotension, and decrease cardiac reactivity and infarct size in rats: involvement of nitric oxide. Br J Pharmacol 2004; 142: 671-8.

[88] Kwon KH, Murakami A, Tanaka T, Ohigashi H. Dietary rutin, but not its aglycone quercetin, ameliorates dextran sulphate sodium-induced experimental colitis in mice: attenuation of pro-inflammatory gene expression. Biochem Pharmacol 2005; 69: 395-406.

[89] Graefe EU, Wittig J, Mueller S, Riethling AK, Uehleke B, Drewelow B, *et al.* Pharmacokinetics and bioavailability of quercetin glycosides in humans. J Clin Pharmacol 2001; 41: 492-9.

[90] Erlund I, Kosonen T, Alfthan G, Maenpaa J, Perttunen K, Kenraali J, *et al.* Pharmacokinetics of quercetin from quercetin aglycone and rut in healthy volunteers. Eur J Clin Parmacol 2000; 56: 545-53.

[91] Hollman PC, Bijsman MN, van Gameren Y, Cnossen EP, de Vries JH, Katan MB. The sugar moiety is a major determinant of the absorption of dietary flavonoids glycosides in man. Free Radic Res 1999; 31: 569-73.

[92] Akira S, Takeda K, Kaisho T. Toll-like receptors: critical proteins linking innate and acquired immunity. Nat Immunol 2001; 2: 675-80.

[93] Medzhitov R, Preston-Hurlburt P, Janeway CA. A human homologue of the Drosophila Toll protein signals activation of adaptive immunity. Nature 1997; 388: 394-7.

[94] O'Neill LA. TLRs: Professor Mechnikov, sit on your hat. Trends Immunol 2004; 25: 687-93.

[95] Janeway CA, Medzhitov R. Innate immune recognition. Annu Rev Immunol 2002; 20: 197-216.

[96] Kopp E, Medzhitov R. Recognition of microbial infection by Toll-like receptors. Curr Opin Immunol 2003; 15: 396-401.

[97] Takeda K, Kaisho T, Akira S. Toll-like receptors. Annu Rev Immunol 2003; 21: 335-76.

[98] Dunne A, O'Neill LA. The interleukin-1 receptor/Toll-like receptor superfamily: signal transduction during inflammation and host defense. Sci STKE 2003: 25 re3.

[99] Zhang D, Zhang G, Hayden MS, Greenblatt MB, Bussey C, Flavell RA, *et al.* Toll-like receptor that prevents infection by uropathogenic bacteria. Science 2004; 303: 1522-6.

[100] Hayashi F, Smith KD, Ozinsky A, Hawn TR, Yi EC, Goodlett DR, *et al.* The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. Nature 2001; 410: 1099-103.

[101] Hemmi H, Takeuchi O, Kawai T, Kaisho T, Sato S, Sanjo H, *et al.* A Toll-like receptor recognizes bacterial DNA. Nature 2000; 408: 740-5.

[102] Rhee SH, Hwang D. Murine Toll-like receptor 4 confers lipopolysaccharide responsiveness as determined by activation of NF-κB

and expression of the inducible cyclooxygenase. J Biol Chem 2000; 275: 34035-40.

[103] Poltorak A, He X, Smirnova I, Liu MY, Van Huffel C, Du X, *et al.* Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. Science 1998; 282: 2085-8.

[104] Heil F, Hemmi H, Hochrein H, Ampenberger F, Kirschning C, Akira S, *et al.* Species-specific recognition of single-stranded RNA *via* toll-like receptor 7 and 8. Science 2004: 303: 1526-9.

[105] Diebold SS, Kaisho T, Hemmi H, Akira S, Reis e Sousa C. Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA. Science 2004: 303: 1529-31.

[106] Alexopoulou L, Holt AC, Medzhitov R, Flavell RA. Recognition of double-stranded RNA and activation of NF-κB by Toll-like receptor 3. Nature 2001; 413: 732-8.

[107] Lien E, Sellati TJ, Yoshimura A, Flo TH, Rawadi G, Finberg RW, *et al.* Toll-like receptor 2 functions as a pattern recognition receptor for diverse bacterial products. J Biol Chem 1999; 274: 33419-25.

[108] Ohashi K, Burkart V, Flohe S, Kolb H. Cutting edge: heat shock protein 60 is a putative endogenous ligand of the Toll-like receptor-4 complex. J Immunol 2000; 164: 558-61.

[109] Okamura Y, Watari M, Jerud ES, Young DW, Ishizaka ST, Rose J, *et al.* The extra domain A of fibronectin activates Toll-like receptor 4. J Biol Chem 2001; 276: 10229-33.

[110] Kurt-Jones EA, Popova L, Kwinn L, Haynes LM, Jones LP, Tripp RA, *et al.* Pattern recognition receptors TLR4 and CD14 mediate response to respiratory syncytial virus. Nat Immunol 2000; 1: 398-401.

[111] Lee JY, Sohn KH, Rhee SH, Hwang D. Saturated fatty acids, but not unsaturated fatty acids, induce the expression of cyclooxygenase-2 mediated through Toll-like receptor 4. J Biol Chem 2001; 276: 16683-9.

[112] Byrd-Leifer CA, Block EF, Takeda K, Akira S, Ding A. The role of MyD88 and TLR4 in the LPS-mimetic activity of Taxol. Eur J Immunol 2001; 31: 2448-57.

[113] Lee JY, Ye J, Gao Z, Youn HS, Lee WH, Zhao L, *et al.* Reciprocal modulation of Toll-like receptor-4 signaling pathways involving MyD88 and phosphatidylinositol 3-kinase/AKT by saturated and polyunsaturated fatty acids. J Biol Chem 2003; 278: 37041-51.

[114] Adachi O, Kawai T, Takeda K, Matsumoto M, Tsutsui H, Sakagami M, *et al.* Targeted disruption of the MyD88 gene results in loss of IL-1- and IL-18-mediated function. Immunity 1998; 9: 143-50.

[115] Kawai T, Adachi O, Ogawa T, Takeda K, Akira S. Unresponsiveness of MyD88-deficient mice to endotoxin. Immunity 1999; 11: 115-22.

[116] Horng T, Barton GM, Flavell RA, Medzhitov R. The adaptor molecule TIRAP provides signalling specificity for Toll-like receptors. Nature 2002; 420: 329-33.

[117] Yamamoto M, Sato S, Hemmi H, Sanjo H, Uematsu S, Kaisho T, *et al.* Essential role for TIRAP in activation of the signalling cascade shared by TLR2 and TLR4. Nature 2002; 420: 324-9.

[118] McGettrick AF, O'Neill LA. The expanding family of MyD88-like adaptors in Toll-like receptor signal transduction. Mol Immunol 2004; 41: 577-82.

[119] Medzhitov R, Preston-Hurlburt P, Kopp E, Stadlen A, Chen C, Ghosh S, *et al.* A. MyD88 is an adaptor protein in the hToll/IL-1 receptor family signaling pathways. Mol Cell 1998; 2: 253-8.

[120] Muzio M, Natoli G, Saccani S, Levrero M, Mantovani A. The human Toll signaling pathway: divergence of nuclear factor kappa B and JNK/SAPK activation upstream of tumor necrosis factor receptor-associated factor 6 (TRAF6). J Exp Med 1998; 187: 2097-101.

[121] Barton GM, Medzhitov R. Toll-like receptor signaling pathways. Science 2003; 300: 1524-5.

[122] Karin M. The beginning of the end. IKB kinase (IKK) and NF-κB activation. J Biol Chem 1999; 274: 27339-42.

[123] Li Q, Verma IM. NF-κB regulation in the immune system. Nat Rev Immunol 2002; 2: 725-34.

[124] Cook DN, Pisetsky DS, Schwartz DA. Toll-like receptors in the pathogenesis of human disease. Nat Immunol 2004; 5: 975-9.

[125] Michelsen KS, Wong MH, Shah PK, Zhang W, Yano J, Doherty TM, *et al.* Lack of Toll-like receptor 4 or myeloid differentiation factor 88 reduces atherosclerosis and alters plaque phenotype in

mice deficient in apolipoprotein E. Proc Natl Acad Sci USA 2004; 101: 10679-84.

[126] Bjorkbacka H, Kunjathoor VV, Moore KJ, Koehn S, Ordija CM, Lee MA, *et al.* Reduced atherosclerosis in MyD88-null mice links elevated serum cholesterol levels to activation of innate immunity signaling pathways. Nat Med 2004; 10: 416-21.

[127] Jobin C, Sartor RB. The IκB/NF-κB system: a key determinant of mucosal inflammation and protection. Am J Physiol Cell Physiol 2000; 278: C451-62.

[128] Haller D, Jobin C. Interaction between resident luminal bacteria and the host: can a healthy relationship turn sour? J Pediatr Gastro-enterol Nutr 2004; 38: 123-36.

[129] Kraehenbuhl JP, Corbett M. Immunology. Keeping the gut micro-flora at bay. Science 2004; 303: 1624-5.

[130] Neish AS. The gut microflora and intestinal epithelial cells: a continuing dialogue. Microbes Infect 2002; 4: 309-17.

[131] Kotanidou A, Xagorari A, Bagli E, Kitsanta P, Fotsis T, Papapetro-poulos A, *et al.* Luteolin reduces lipopolysaccharide-induced lethal toxicity and expression of proinflammatory molecules in mice. Am J Resp Crit Care Med 2002; 165: 818-23.

[132] Kim JS, Jobin C. The flavonoid luteolin prevents lipopolysaccha-ride-induced NF-kappaB signalling and gene expression by block-ing IκB kinase activity in intestinal epithelial cells and bone-marrow derived dendritic cells. Immunology 2005; 115: 375-87.

[133] Bouma G, Strober W. The immunological and genetic basis of inflammatory bowel disease. Nat Rev Immunol 2003; 3: 521-33.

[134] Strober W, Fuss IJ, Blumberg RS. The immunology of mucosal models of inflammation. Annu Rev Immunol 2002; 20: 495-549.

[135] Hoffmann A, Natoli G, Ghosh G. Transcriptional regulation *via* the NF-κB signaling module. Oncogene 2006; 25: 6706-16.

[136] Bonizzi G, Karin M. The two NF-κB activation pathways and their role in innate and adaptive immunity. Trends Immunol 2004; 25: 280-8.

[137] Malek S, Chen Y, Huxford T, Ghosh G. I kappa B beta, but not IκBα, functions as a classical cytoplasmic inhibitor of NF-κB di-mers by masking both NF-kappaB nuclear localization sequences in resting cells. J Biol Chem 2001; 276: 45225-35.

[138] Malek S, Huang DB, Huxford T, Ghosh S, Ghosh G. X-ray crystal structure of an IκBβ x NF-κB p65 homodimer complex. J Biol Chem 2003; 278: 23094-100.

[139] Huang TT, Kudo N, Yoshida M, Miyamoto S. A nuclear export signal in the N-terminal regulatory domain of I kappa B alpha con-trols cytoplasmic localization of inactive NF-κB/IκBα complexes. Proc Natl Acad Sci USA 2000; 97: 1014-9.

[140] Renard P, Percherancier Y, Kroll M, Thomas D, Virelizier JL, Arenzana-Seisdedos F, *et al.* Inducible NF-κB activation is permit-ted by simultaneous degradation of nuclear IκBα. J Biol Chem 2000; 275: 15193-99.

[141] Ghosh S, May MJ, Kopp EB. NF-κB and Rel proteins: evolutionar-ily conserved mediators of immune responses. Annu Rev Immunol 1998; 16: 225-60.

[142] Turpin P, Hay RT, Dargemont C. Characterization of IκBα nuclear import pathway. J Biol Chem 1999; 274: 6804-12.

[143] Hayden MS, Ghosh S. Signaling to NF-κB. Genes & Dev 2004; 18: 2195-224.

[144] Silverman N, Maniatis T. NF-κB signaling pathways in mammal-ian and insect innate immunity. Genes & Dev 2001; 15: 2321-42.

[145] Grimm S, Baeuerle PA. The inducible transcription factor NF-κB: structure-function relationship of its protein subunits. Biochem J 1993; 290: 297-308.

[146] Henkel T, Machleidt T, Alkalay I, Krönke M, Ben-Neriah Y, Baeuerle PA. Rapid proteolysis of I kappa B-alpha is necessary for activation of transcription factor NF-κB. Nature 1993; 365: 182-5.

[147] Ritchie ME. Nuclear factor-κB is selectively and markedly acti-vated in humans with unstable angina pectoris. Circulation 1998; 98: 1707-13.

[148] Brand K, Page S, Rogler G, Bartsch A, Brandl R, Knuechel R, *et al.* Activated transcription factor nuclear factor-κB is present in the atherosclerotic lesion. J Clin Invest 1996; 97: 1715-22.

[149] Bourcier T, Sukhova G, Libby P. The nuclear factor-κB signaling pathway participates in dysregulation of vascular smooth muscle cells *in vitro* and in human atherosclerosis. J Biol Chem 1997; 272: 15817-24.

[150] Ebenbichler CF, Kirchmair R, Egger C, Patsch JR. Postprandial state and atherosclerosis. Curr Opin Lipidol 1995; 6: 286-90.

[151] Choi JS, Choi YJ, Park SH, Kang JS, Kang YH. Flavones mitigate tumor necrosis factor-alpha-induced adhesion molecule upregula-tion in cultured human endothelial cells: role of nuclear factor-κB. J Nutr 2004; 134: 1013-9.

[152] Hernandez-Presa MA, Bustos C, Ortego M, Tunon J, Renedo G, Ruiz-Ortega M, *et al.* Angiotensin-converting enzyme inhibition prevents arterial nuclear factor-κB activation, monocyte chemoat-tractant protein-1 expression, and macrophage infiltration in a rab-bit model of early accelerated atherosclerosis. Circulation 1997; 95: 1532-41.

[153] Schreck R, Meier B, Mannel DN, Droge W, Bauerle PA. Dithio-carbamates as potent inhibitors of nuclear factor-κB activation in intact cells. J Exp Med 1992; 175: 1181-94.

[154] Feng AN, Chen YL, Chen YT, Ding YZ, Lin SJ. Red wine inhibits monocyte chemotactic protein-1 expression and modestly reduces neointimal hyperplasia after balloon injury in cholesterol-Fed rab-bits. Circulation 1999; 100: 2254-9.

[155] Xu R, Yokohama WH, Irving D, Rein D, Walzem RL, German JB. Effect of dietary catechin and vitamin E on aortic fatty streak ac-cumulation in hypercholesterolemic hamsters. Atherosclerosis 1998; 137: 29-36.

[156] Williams RJ, Motteram JM, Sharp CH, Gallagher PJ. Dietary vita-min E and the attenuation of early lesion development in modified Watanabe rabbits. Atherosclerosis 1992; 94: 153-9.

[157] Iijima K, Yoshizumi M, Hashimoto M, Kim S, Eto M, Ako J, *et al.* Red wine polyphenols inhibit proliferation of vascular smooth muscle cells and downregulate expression of cyclin A gene. Circu-lation 2000; 101: 805-11.

[158] Kerry NL, Abbey M. Red wine and fractioned phenolic compounds prepared from red wine inhibit low density lipoprotein oxidation *in vitro*. Atherosclerosis 1997; 135: 93-102.

[159] Nigdikar SV, Williams NR, Griffin BA, Howard AN. Consumption of red wine polyphenols reduces the susceptibility of low-density lipoproteins to oxidation *in vivo*. Am J Clin Nutr 1998; 68: 258-65.

[160] Goral J, Kovacs EJ. *In vivo* ethanol exposure down-regulates TLR2-, TLR4-, and TLR9-mediated macrophage inflammatory re-sponse by limiting p38 and ERK1/2 activation. J Immunol 2005; 174: 456-63.

[161] Johnson GL, Lapadat R. Mitogen-activated protein kinase path-ways mediated by ERK, JNK, and p38 protein kinases. Science 2002; 298: 1911-2.

[162] Davies RJ. Signal transduction to the nucleus by MAP kinase. In: Gutkind JS, Finkel T Eds. Signaling networks and cell cycle con-trol: the molecular basis of cancer and other diseases. Humana Press Inc.: Totowa 2003: 153.

[163] Aroor AR, Shukla SD. MAP kinase signalling in diverse effects of ethanol. Life Sci 2004; 74: 2339-64.

[164] Arbabi S, Garcia I, Bauer GJ, Maier RV. Alcohol (ethanol) inhibits IL-8 and TNF: role of the p38 pathway. J Immunol 1999; 162: 7441-5.

[165] Kristof AS, Marks-Konczalik J, Moss J. Mitogen-activated protein kinases mediate activator Protein-1-dependent human inducible ni-tric-oxide synthase promoter activation. J Biol Chem 2001; 276: 8445-52.

[166] Booyse FM, Pan W, Grenett HE, Parks DA, Darley-Husmar VM, Bradley KM, *et al.* Mechanism by which alcohol and wine poly-phenols affect coronary heart disease risk. Ann Epidemiol 2007; 17: S24-31.

[167] Magrone T, Tafaro A, Jirillo F, Amati L, Jirillo E, Covelli V. Elici-tation of immune responsiveness against antigenic challenge in age-related disease: effects of red wine polyphenols. Curr Pharm Des 2008; 14(26): 2749-57.

[168] Lakoski SG, Liu Y, Brosnihan KB, Herrington DM. Interleukin-10 concentration and coronary heart disease (CHD) event risk in the estrogen replacement and atherosclerosis (ERA) study. Atheroscle-rosis 2008; 197: 443-7.

[169] Hollman PC, Bjsman MN, van Gameren Y, Cnossen EP, de Vries JH, Katan MB. The sugar moiety is a major determinant of the ab-sorption of dietary flavonoids glycosides in man. Free Radic Res 1999; 31: 569-73.

[170] Day AJ, DuPont MS, Ridley S, Rhodes M, Rhodes MJ, Morgan MR, *et al.* Deglycosylation of flavonoid and isoflavonoid glycosides by human small intestine and liver beta-glucosydase activity. FEBS Lett 1998; 436: 71-5.

[171] Scalbert A, Williamson G. Dietary intake and bioavailability of polyphenols. J Nutr 2000; 130: S2073-85.

[172] Hollman PC, de Vries JH, van Leeuwen SD, Mengelers MJ, Katan MB. Absorption of dietary quercetin glycosides and quercetin in healthy ileostomy volunteers. Am J Clin Nutr 1995; 62: 1276-82.

[173] Stout RD, Suttles J. Immunosenescence and macrophage functional plasticity: dysregulation of macrophage function by age-associated microenvironmental changes. Immunol Rev 2005; 205: 60-71.

[174] Guruvayoorappan C, Kuttan G. (+)-Catechin inhibits tumor angiogenesis and regulates the production of nitric oxide and TNF-{α} in LPS-stimulated macrophages. Innate Immun 2008; 14, 160-74.

[175] Cossarizza A. Apoptosis and HIV infection: about molecules and genes. Curr Pharm Des 2008; 14(3): 237-44.

[176] Motilva V, Talero E, Calvo JR, Villegas I, Alarcon-de-la-Lastra C, Sanchez-Fidalgo S. Intestinal immunomodulation. Role of regulative peptides and promising pharmacological activities. Curr Pharm Des 2008; 14(1): 71-95.

[177] O'Sullivan DJ. Genomics can advance the potential for probiotic cultures to improve liver and overall health. Curr Pharm Des 2008; 14(14): 1376-81.