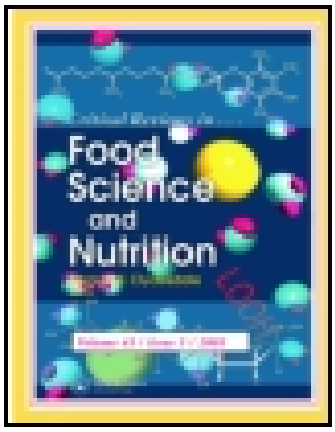

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Regulation of dendritic cell function by dietary polyphenols

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Abstract

Marked changes in socio-economic status, cultural traditions, population growth and agriculture have been affecting diets worldwide. Nutrition is known to play a pivotal role in the pathogenesis of several chronic diseases, and the use of bioactive food compounds at pharmacological doses is emerging as a preventive and/or therapeutic approach to target metabolic dysregulations occurring in ageing, obesity-related chronic diseases, and cancer. Only recently, data on the effects of specific nutrients or food on the immune system have become available, and studies regarding the human immune system are still at their infancy. Beyond providing essential nutrients, diet can actively influence the immune system. Understanding how diet and nutritional status influence the innate and adaptive arms of our immune system, represents an area of scientific need, opportunity and challenge. The insights gleaned should help to address several pressing global health problems. Recently, biologically active polyphenols, widespread constituents of fruit and vegetables, have gained importance as complex regulators of various cellular processes, critically involved in the maintenance of body homeostasis. This review outlines the potential effects of polyphenols on the function of dendritic cells (DCs), key players in the orchestration of the immune response. Their effects on different aspects of DC biology including differentiation, maturation and DC capacity to shift immune response toward tolerance or immune activation will be outlined.

Keywords: cell activation, dendritic cell, flavonoids, immune regulation, polyphenols

Abbreviations: dendritic cell (DC); antigen presenting cell (APC); major histocompatibility complex (MHC); T helper (Th); T regulatory (Treg); retinoic acid (RA); indoleamine 2,3-dioxygenase (IDO); aryl hydrocarbon receptor (AHR); FLICE inhibitory protein (c-FLIP); epigallocatechin gallate (EGCG); monocyte-derived DC (MD-DC); immunoglobulin-like transcript (ILT); bone marrow (BM) derived DC (BM-DC); polyinosinic:polycytidylic acid (poly I:C); Toll-like receptor (TLR); c-Jun N-terminal kinase (JNK); extracellular signal regulated kinase (ERK); prostaglandin (PG), mitogen-activated protein kinase (MAPK); nuclear factor κB (NF-κB); signal transducer and activator of transcription (STAT); interferon regulatory factor (IRF); cycloxygenase (COX); inhibitor κB (IκB); IκB kinase (IKK); protein kinase C (PKC); Janus kinase (JAK); aldehyde dehydrogenase (ALDH).

Introduction

The use of functional food, specific nutrient and/or food components able to beneficially affect one or more target functions in the body, as a form of preventive medicine, has been the subject of intense research over the last two decades. The evidence for therapeutic and preventive potential of food components comes from epidemiological studies demonstrating that the consumption of a diet rich in vegetables and fruit exerts beneficial healthy effects likely because of the high content in fibers, mineral salts, vitamins, and a large number of biologically active substances such as polyphenols (Diaz-Rubio et al. 2009; Saura-Calixto et al. 2009). Furthermore, basic science reports have shown that these compounds can efficiently modulate the oxidative, inflammatory and apoptotic imbalances in chronic disease metabolic pathways (Ramos 2008; Aggarwal 2010; Prasad et al. 2010).

Polyphenols are the most abundant antioxidants in our diet, widespread constituents of fruit, vegetables, cereals, olives, dry legumes, cocoa and beverages, such as tea, coffee and wine (D'Archivio et al. 2007). Current evidence strongly supports a contribution of polyphenols to the prevention of several oxidative stress-associated chronic-degenerative processes, diseases, and syndromes especially because of their strong antioxidant power able to protect cell constituents against oxidative damage (Scalbert et al. 2005; Covas 2007). It is worth of note that polyphenols can have opposite effects mainly due to the control of cell redox state. By acting as antioxidants these compounds can counteract cytotoxicity and apoptosis, conversely, by acting as pro-oxidants they become pro-apoptotic agents. However, emerging findings suggest that polyphenols, independently of their conventional antioxidant activities, can exert a variety of

potential mechanisms of action such as direct interactions with receptors or enzymes involved in signal transduction (Masella et al. 2005). Finally, a close relationship between nutrition and immune system does exist as well as a link between nutrition and chronic diseases (Wolowczuk et al. 2008). Consequently, it is not surprising that huge efforts and resources have been focused on the understanding of how dietary nutrients might impact specific targets of inflammation. In addition, since several synthetic drugs provided unknown side effects, there has been a need for new and safe anti-inflammatory agents. Targeting these inflammatory regulators by using food components may be thus a useful strategy to prevent, or to ameliorate, the development of chronic inflammation-related diseases. In this regard, polyphenols appear to be good candidates as they have been reported to exert anti-inflammatory activity both *in vitro* and *in vivo* (Santangelo et al. 2007).

Here, we discuss the current knowledge on the effects of polyphenols on dendritic cells (DCs), key players in the orchestration of the immune response.

Polyphenol classification, content in human diet and bioavailability

Polyphenols comprise a wide variety of molecules that have a polyphenol structure (i.e. several hydroxyl groups on aromatic rings), but also molecules with one phenol ring, such as phenolic acids and phenolic alcohols (Perez-Jimenez et al.). Polyphenols are divided into several classes according to the number of phenol rings that they contain and to the structural elements that bind these rings each other. The main groups of polyphenols are flavonoids, phenolic acids, phenolic alcohols, stilbenes and lignans (**Figure 1**). Polyphenols are widely distributed in the plant kingdom and represent common constituents of vegetal food, such as fruit and vegetables, and beverages (fruit juice, red wine, tea, coffee, and chocolate). Some of them are specific to

particular food (flavanones in citrus fruit, isoflavones in soya, phlorizin in apples), whereas others, such as quercetin, are found in all plant products (D'Archivio et al. 2007). Generally, food contains complex mixtures of polyphenols, most of them in form of esters, glycosides, or polymers that cannot be absorbed in the native form.

Although the measurement of habitual dietary intake of nutrients is an essential component of research related to the healthy effect of diet, all the conventional tools for measuring dietary exposure, such as food frequency questionnaires (FFQ), 24-hour recalls, or food diaries, have well known limitations. In particular, as far as fruit and vegetables are concerned, estimating intakes as well as metabolism and physiological concentration of polyphenols are particularly challenging. This is mainly due to the great diversity of polyphenols present in different foods, the limited data on polyphenol content of specific foods, and the limited understanding of the extent of absorption, metabolic fate, and bioavailability of individual polyphenols from particular foods. Furthermore, the amount of polyphenols ingested can differ greatly among different populations, and even between male and female, depending on their dietary habits (Perez-Jimenez et al. 2011).

The absorption of some but not all dietary polyphenols occurs in the small intestine. Before the absorption, these compounds must be hydrolyzed by intestinal enzymes. The released aglycones may then enter the epithelial cell by passive diffusion as a result of their increased lipophilicity, or by active transport (Day et al. 2000; Gee et al. 2000). The polyphenols that are not absorbed in the small intestine reach the colon where they undergo substantial structural modifications by colonic microflora that hydrolyzes glycosides into aglycones and degrades them to simple phenolic acids (Kay 2006). Once absorbed, and prior to the passage into the blood stream, the

polyphenol-derived aglycones, undergo to other structural modifications due to the conjugation process. Therefore, any single polyphenol generates several metabolites, and the compounds that reach cells and tissues are chemically, biologically and, in many instances, functionally different from the original dietary form, which makes the identification of the active metabolites extremely difficult (Del Rio et al. 2010). These modifications affect also the polyphenol bioavailability. The term 'bioavailability' means the fraction of an ingested nutrient or compound that reaches the systemic circulation and the specific sites where it can exert its biological action. In this regard, it should be pointed out that most of the data published on the human bioavailability of polyphenols only refer to the release of these compounds from the food matrix with their subsequent absorption measured as concentration in blood or in urine. However, the plasma concentration profile of a phenolic compound should be more appropriately considered as a marker of release from the food matrix and absorption, than a parameter of bioavailability and biological activity. In fact, the most common approach used to carry out human studies is the "single-dose" design, which involves the intake of one portion of food containing the tested polyphenol. This means that the increase in the blood concentration is therefore transient and mainly reflects the ability of the organism to uptake the polyphenol from the food matrix, but cannot represent an index of physiological concentration. Consequently, it is not only important to know how much of a nutrient is present in specific food or dietary supplement, but it is even more important to know how much of it is bioavailable.

It must be underlined that the physiological *in vivo* context in which dietary polyphenols exert their influence is undoubtedly much more complex than that available from an *in vitro* system. The same compound might show strong activity *in vitro*, but could have little biological activity

in vivo if little or none of the compound gets to the target tissues (Porrini et al. 2008; D'Archivio et al. 2010). The biological activity indeed depends especially on the amount of polyphenols accumulated in target tissues. Unfortunately, human studies are still very scarce even if rapidly accumulating (Maubach et al. 2003; Henning et al. 2006; Veeriah et al. 2008).

Dendritic cells: biology and functions

An extraordinary feature of the mammalian immune system is its capacity to generate a repertoire of T and B cell receptors that can recognize virtually any antigen. This delicate balance of responding to foreign antigens while remaining tolerant to self-antigens is critical, because its breakdown can lead to autoimmunity which results from a failure of tolerogenic mechanisms and excessive inflammatory responses, or to chronic infections and tumors, caused by insufficient immunity and excessive tolerance. Research over the last decade has revealed a fundamental role for innate immune system in initiating protective responses against different pathogens as well as in tuning the quality of such responses. Emerging studies are now beginning to reveal a key role for the innate immune system in regulating immunity against self components such as tissue antigens, food antigens, and commensals (Banchereau et al. 2003; Buckwalter et al. 2009). The innate immune system consists of a diverse array of cells, including DCs, macrophages, natural killer cells, mast cells, basophils, neutrophils, eosinophils, and γδ T cells (Medzhitov 2007). DCs, which are the most potent antigen presenting cells (APCs), are critical sentinels of the immune system and have the unique ability to integrate a wide array of incoming signals and convey them to lymphocytes, thus initiating and regulating adaptive

immune responses (Banchereau et al. 2003; Buckwalter et al. 2009). These cells are endowed with a remarkably high functional plasticity allowing the critical decision between immune activation or tolerance (**Figure 2**). DCs are comprised of a heterogeneous population of cells with DCs in various organs possessing unique sets of cell surface markers. It has become evident that many distinct DC subtypes exist, each with a particular location and a specialized function in the immune system (Kushwah et al. 2011). Under homeostatic conditions, peripheral DCs typically display an immature phenotype characterized by expression of low surface levels of major histocompatibility complex (MHC) class II and co-stimulatory molecules. These cells induce suboptimal T cell priming, often leading to T cell anergy or tolerance. Upon receipt of a variety of activation signals, DCs are induced to mature and migrate to secondary lymphoid organs. This process is accompanied by a marked upmodulation of co-stimulatory and MHC molecule expression, secretion of effector cytokines, as well as by a concomitant decrease in their capacity for antigen uptake and a marked increase in their ability to present antigens and activate naive T cells (Banchereau et al. 2000). Emerging evidence suggests that DCs are also critical in suppressing immune responses and maintaining peripheral tolerance, through the generation of anergic and/or T regulatory (T_{reg}) cells and fine-tuning the response by altering the T helper 1 (Th1)/ Th2/Th17 balance (Steinman et al. 2003; Pulendran et al. 2010; Manicassamy et al. 2011). It has been postulated that immature DCs promote tolerogenic responses, whereas mature DCs promote immunogenic responses (Steinman et al. 2003; Manicassamy et al. 2011). However, certain stimuli can promote DC activation and maturation and yet induce tolerogenic T cells (Jiang et al. 2007). Likewise, a broad range of microbial stimuli can program DCs to acquire tolerogenic properties (Pulendran et al. 2010).

Although there has been much progress in understanding the role of innate immunity in inducing protective responses against pathogens, very little is known about its role in promoting tolerogenic responses and suppressing autoimmune responses. The tolerogenic properties of DCs can depend on their maturation state, the exposure to anti-inflammatory and immunosuppressive agents, the nature of the microbial stimuli, and environmental cues from the tissue microenvironment (Steinman et al. 2007; Pulendran et al. 2008; Pulendran et al. 2010). It is now clear that tolerogenic responses are controlled by multiple parameters of the innate immune system: (i) the innate receptors on DCs that sense microbial and non-microbial stimuli, (ii) the intracellular signaling pathways that program DCs to a tolerogenic state, (iii) the intercellular interactions between DCs and multiple cell types, and (iv) the inductive signals from the local microenvironment (Manicassamy et al. 2011).

The observation that DC subsets in specific microenvironments, such as those in the gut, lung, eye, and placenta, display tolerogenic propensities suggests that the local environment imparts inductive signals to program tolerogenic DCs *in situ*. At mucosal surfaces, the immune system has a particularly challenging task of maintaining tolerance to self-antigens and commensals, while launching robust immunity to pathogens. Tolerogenic APCs in the mucosal compartment prevent excessive inflammation and immunity against commensals and food or environmental antigens. For example, in the intestine, $CD11c^{high}$ DCs that express CD103 have been shown to efficiently induce $Foxp3^{+}$ T_{reg} cells *in vitro, via* a mechanism dependent on retinoic acid (RA) (Coombes et al. 2007; Scott et al. 2011). Mucosal DCs are continuously exposed to dietary constituents and some specific nutrients have striking effects on the regulation of mucosal immune responses. The most extensively studied of these factors is vitamin A (whose only

source in mammals is the diet) which is responsible for several functions of $CD103^+$ DCs. Tryptophan is also only derived from the diet in mammals and is needed for the indoleamine 2,3-dioxygenase (IDO)-dependent tolerogenic effects of mucosal DCs (Fallarino et al. 2006). Other dietary metabolites might have immunomodulatory effects on DCs, including lipid mediators, that activate anti-inflammatory peroxisome proliferator-activated receptor γ (Varga et al. 2008), and ligands of the aryl hydrocarbon receptor (AHR), that regulate the balance between Th17 and T_{reg} cell differentiation (Elgueta et al. 2008; Chmill et al. 2010; Takamura et al. 2011).

Effects of polyphenols on DC biology

Discoveries in recent years have provided evidence that, beyond nutrition, diet may also modulate various bodily functions, including immunity, that are relevant to the host's health (Hoyles et al. 2008). It is now well established that nutrition plays a vital role in chronic diseases, but it is only recently that data relating to the effects on specific nutrients or food on the immune system have become available. It should be noted, however, that studies into the role of functional food with regard to the human immune system are still at their beginning, and very few studies, mostly carried out with murine cell models, are currently available on the effects of specific polyphenols on DCs. In particular, among the polyphenols were tested curcumin, a curcuminoid contained in turmeric; epigallocatechin gallate (EGCG), the green tea catechin with the strongest biological activity; flavones (apigenin, luteolin); flavonols (fisetin, quercetin); resveratrol, a stilbene contained especially in red wine and grapes; hydroxycinnamic-derivative phenolic acids (*p*-coumaric acid, rosmarinic acid); and silibinin, the major active component of silymarin, a mixture of flavolignans extracted from milk thistle. A summary of the main *in vitro*

studies demonstrating functional effects of selected polyphenols on DC functional activities is shown in **Table 1**.

Polyphenol effects on DC differentiation

The capacity of polyphenols to influence DC differentiation is illustrated by studies carried out with resveratrol. In particular, Svajger and colleagues demonstrated that resveratrol treatment during the differentiation stage is of crucial importance in obtaining human monocyte-derived DCs (MD-DCs) with strong regulatory potential. Resveratrol does not merely block DC maturation but redirect human DC differentiation from monocytes, which, in turn, leads to alternative activation by maturation signals (Svajger et al. 2009). Indeed, DCs generated in the presence of resveratrol were resistant to the LPS-induced activation towards Th1 polarizing DC type but acquired the potential to induce allogeneic IL-10-secreting $CD4^+$ T cells. Interestingly, resveratrol-treated DCs upmodulated the expression of surface tolerogenic markers such as immunoglobulin-like transcript (ILT)3 and ILT4. Similar results have also been achieved with EGCG. In the presence of EGCG, the conversion of adherent monocytes to cells with the typical morphology of DCs was altered. Specifically, the typical cluster formation by DCs disappeared, cells remained adherent and developed elongated pseudopods and a fusiform body (Yoneyama et al. 2008). In addition, exposure of monocytes to EGCG, together with the DC differentiation promoting cytokines GM-CSF and IL-4, inhibited their differentiation to DCs and induced apoptosis of DC-precursors and immature DCs. Polyphenol-induced apoptosis was also reported in splenic DCs from SNF1 mice with established lupus-like disease, exposed *in vitro* to apigenin (Kang et al. 2009).

Polyphenol effects on DC maturation

Most of the studies aimed at evaluating the effects of selected polyphenols on DC functional activities indicate that a number of polyphenols, such as EGCG (Ahn et al. 2004; Yoneyama et al. 2008), curcumin (Kim et al. 2005a; Shirley et al. 2008), quercetin (Huang et al. 2010), apigenin (Yoon et al. 2006), fisetin (Liu et al. 2010), silibinin (Lee et al. 2007b), and blackberry polyphenols (Dai et al. 2007), strongly inhibit murine bone marrow (BM) derived DC (BM-DC) maturation induced by the Toll-like receptor (TLR)4 agonist lipopolysaccharide (LPS). These compounds were reported to profoundly inhibit co-stimulatory and MHC molecule expression, restore the decreased antigen uptake activity, and inhibit enhanced IL-12 and pro-inflammatory cytokine production typically observed in LPS stimulated BM-DCs (see also Figure 3, pathway 1). Likewise, murine splenic DCs stimulated with TLR7 or TLR9 agonists failed to produce IFN-α and IL-6 in the presence of apigenin (Kang et al. 2009). Polyphenol exposed murine BM-DCs were poor stimulators of naïve allogenic T cell proliferation and reduced levels of IL-2 were found in the responding T cells. Similar immunosuppressive effects were observed in human MD-DCs exposed to EGCG, resveratrol or curcumin and stimulated to mature with LPS or polyinosinic:polycytidylic acid (poly I:C) (Shirley et al. 2008; Yoneyama et al. 2008; Svajger et al. 2009). The mechanisms underlying polyphenol capacity to affect the phenotypic and functional maturation of DCs have been partially characterized. TLR ligation induces the activation of mitogen-activated protein kinase (MAPK), Akt, and nuclear factor (NF)-κB pathways, resulting in DC activation (Arrighi et al. 2001; An et al. 2002). Activation of both NF-κB- and MAPK-signaling pathways are important events in DC maturation (Rescigno et al.

1998). Interestingly, different polyphenols (i.e. curcumin, quercetin, fisetin, silibinin, apigenin, luteolin and resveratrol) were reported to target the NF-κB pathway, strongly impairing IκB kinase (IKK) activation (Kim et al. 2005b), inhibitor κB (IκB) degradation (Kim et al. 2005b; Huang et al. 2010), and NF-κB p65 nuclear translocation (Ahn et al. 2004; Kim et al. 2005a; Yoon et al. 2006; Lee et al. 2007b; Svajger et al. 2009). The activation of certain members of the MAPK family, including p38, c-Jun N-terminal kinase (JNK), and extracellular signal regulated kinase (ERK1/2), was also reported to be targeted by several polyphenols, thus representing an additional mechanism by which these compounds affects DC functional maturation. In particular, EGCG (Ahn et al. 2004), curcumin (Kim et al. 2005a), silibinin (Lee et al. 2007b), and apigenin (Yoon et al. 2006) were reported to impair the LPS-induced activation of all members of the MAPK family whereas other polyphenols, such as quercetin (Huang et al. 2010), only affected JNK and ERK1/2 but not MAPK p38. Lastly, quercetin was also reported to block the LPS-induced Akt activation in murine BM-DCs (Huang et al. 2010).

Polyphenol effects on tolerogenic marker expression

IDO, the enzyme catalyzing the initial and rate-limiting step in the catabolism of tryptophan along the kynurenine pathway, is now well established as a key enzyme in T cell suppression and induction of immune tolerance to tumor. DCs expressing significant IDO activity can mediate inhibition of T cell proliferation through tryptophan depletion in microenvironment (Mellor et al. 2004). Studies carried out in murine BM-DCs clearly indicate that a number of polyphenols can influence the expression of IFN-γ- or LPS-induced IDO by different mechanisms. EGCG (Jeong et al. 2007), curcumin (Jeong et al. 2009), rosmarinic (Lee et al. 2007a) and *p*-coumaric (Kim et

al. 2007) acids inhibit the induction of IDO expression and activity by IFN-γ (see also Figure 3, pathway 3). In keeping with this inhibitory effect on IFN-γ-induced IDO expression, these polyphenols reversed IDO-mediated suppression of T cell responses (Jeong et al. 2007; Kim et al. 2007; Lee et al. 2007a; Jeong et al. 2009). The mechanisms underlying the polyphenol-mediated IDO inhibition rely on the capacity of these compounds to transcriptionally impair IDO expression by suppressing signal transducer and activator of transcription (STAT)1 activation and its subsequent binding to the interferon regulatory factor (IRF)-1 promoter (Jeong et al. 2007; Kim et al. 2007; Lee et al. 2007a; Jeong et al. 2009). In curcumin-exposed BM-DC, suppression of STAT1 activation was directly linked to the curcumin-mediated inhibition of Janus kinase (JAK)1/2 and protein kinase (PK)Cδ phosphorylation (Jeong et al. 2009). Lastly, an additional mechanism leading to the suppression of IDO expression was described in EGCG treated murine BM-DCs. Specifically, it was reported that EGCG inhibits the constitutive expression of cycloxygenase (COX)-2 mRNA and protein, and attenuates the downstream synthesis of prostaglandin (PG)E_2. Since PGE_2 was described to up-regulate functional IDO during DC maturation (Braun et al. 2005), polyphenol-mediated reduction of IDO expression in DCs may derive from the suppressive activity of EGCG on COX-2/PGE_2 pathway.

In sharp contrast to the effects of polyphenols on IFN-γ-activated IDO expression DCs, resulting in a dominant mechanism by which T cell proliferation is restored, the inhibition of IDO expression induced by LPS leads to the suppression of LPS-induced DC maturation (see also Figure 3, pathway 2). In particular, it was found that curcumin suppresses LPS-induced IDO expression in a COX-2/PGE_2-dependent manner, thus inhibiting murine BM-DC maturation (Jung et al. 2010). In addition, curcumin was found to enhance LPS-induced expression of COX-

2 and PGE_2 production. This latter interferes with the LPS-induced IDO expression in DCs, thereby contributing to the inhibition of expression of the surface molecules (CD80, CD86 and MHC class I) and the production of the proinflammatory cytokines (IL-12p70 and TNF-α) induced by LPS stimulation. LPS-induced IDO expression is not regulated by curcumin in BMDCs-derived from COX-$2^{-/-}$ mice adding further evidence to the role of COX-2/PGE_2 pathway as a critical intrinsic modulator of DC maturation through the regulation of IDO expression (Jung et al. 2010).

Polyphenol effects on DC-mediated induction of T_{reg} cells

Several recent reports have demonstrated that the ability of mucosal DC to induce T_{reg} development relies on RA production (Scott et al. 2011). However, the factors that induce the tolerogenic mucosal DC phenotype in the intestine remain unclear. In this regard, it has been reported that immature DCs exposed to curcumin can generate T_{reg} (Cong et al. 2009). In particular, murine BM-DCs exposed to curcumin upmodulate the expression of aldehyde dehydrogenase (ALDH)1a and IL-10, and induce differentiation of naive $CD4^+$ $CD25^-$ T cells into $CD25^+$ T_{reg} resembling T_{reg} in the intestine. Such T_{reg} induction requires IL-10, TGF-β and RA produced by curcumin-modulated DCs. Cell contact as well as IL-10 and TGF-β production were involved in the function of such induced T_{reg}. More importantly, these T_{reg} inhibited antigen-specific T cell activation *in vitro* and inhibited colitis due to antigen-specific pathogenic T cells *in vivo* (Cong et al. 2009). In keeping with these results, it has been reported that human MD-DCs exposed to resveratrol are able to generate allogeneic IL-10-secreting T cells. However, differently from the effects observed for curcumin treated murine BM-DCs,

resveratrol treated human MD-DCs were not competent to induce T_{reg} cells expressing the transcription factor Foxp3 (Svajger et al. 2009).

Immunomodulatory effects of polyphenols: *in vivo* studies

Evidence for the capacity of polyphenols to modulate DC functional activities has also been provided in a number of interventional studies performed in animal models. A summary of the main studies is showed in **Table 2**. Similarly to the results achieved *in vitro*, rosmarinic and *p*-coumaric acids markedly suppressed IDO expression in splenic DCs of tumor-draining lymph node of tumor-bearing mice (Kim et al. 2007; Lee et al. 2007a). Likewise, curcumin was reported to down-modulate IDO and co-stimulatory molecule expression in splenic DCs from mice injected with this compound prior to LPS challenge (Jung et al. 2010). Interestingly, T_{reg} cells generated *in vitro* upon co-culture of curcumin-treated murine BM-DCs were functional *in vivo* and able to regulate colitis development induced by antigen-specific Th1 cells (Cong et al. 2009). In keeping with these results, it has been demonstrated that apigenin inhibits autoantigen-presenting and stimulatory functions of the APCs necessary for activation and expansion of autoreactive Th1 and Th17 cells and B cells in a murine model of lupus (Kang et al. 2009). Apigenin also causes apoptosis of the hyperactive lupus APCs, T cells, and B cells, likely by inhibiting expression of NF-κB-regulated anti-apoptotic molecules, especially COX-2 and FLICE-inhibitory protein (c-FLIP), which are persistently hyper-expressed by the lupus immune cells (Kang et al. 2009). Interestingly, parallel *in vivo* data showed that intraperitoneal administration of apigenin inhibits LPS-induced DC maturation and impairs IFN-γ production in splenic $CD4^+$ T cells (Yoon et al. 2006). Although apigenin-treated DCs have been shown to

migrate to the T cell areas of secondary lymphoid tissues, they fail to induce a normal cell-mediated contact hypersensitivity reaction when injected in sensitized animals (Yoon et al. 2006). Migration of DCs into spleens, as well as DC-mediated T cell activation, were instead attenuated by *in vivo* administration of fisetin to LPS-treated mice (Liu et al. 2010). Polyphenols, in particular those contained in white and green tea, have received much interest because of their beneficial effect in skin cancer prevention. In a recently published article, it was reported that the photoprotective effects of polyphenols rely on their capacity to prevent the depletion of skin resident DCs, the Langerhans cells, which is induced upon *in vivo* ultraviolet ray irradiation of human skin, as well as by an *in vitro* skin explant model (Camouse et al. 2009).

Concluding remarks

The interaction between diet, environment, and genome ultimately defines health status and can be critical in influencing chronic diseases. It is now clear that a number of food and food components beneficially stimulate the immune system and confer health benefits upon the consumer. Although studies into functional food and its action on the immune system are still at their infancy, the potential biological activity of many dietary polyphenols on cells of critical importance in the orchestration of the immune response, such as the DCs, represents an exciting area of research. As schematically represented in **Figure 3**, the capacity of several polyphenols to affect DC maturation by decreasing T cell proliferative responses and favoring the expansion of T cell subsets endowed with regulatory activity argues for a major role of these compounds in the attenuation of inflammation. On the other side, these compounds are also capable to promote immunoactivation by rescuing immunosuppressive T lymphocytes. However, the extent and

precise nature of the role played by polyphenols in human health is yet to be elucidated. In this regard, although some polyphenols have previously been demonstrated to affect cytoskeleton organization in different cell systems (Azios et al. 2005; Kim et al. 2009; Lu et al. 2009), their capacity to affect cytoskeleton changes occurring during DC maturation has not yet been reported. Since cytoskeleton rearrangements play a pivotal role in DC biology by governing different aspects of their functions including migration and phagocytosis, future studies should be specifically addressed to unravel these critical aspects in the regulation of the immune response. The past five years witnessed a shift of basic research from *in vitro* analyses of antioxidant activity to more complex cellular investigations, notably as related to cell signaling. We are gaining a better understanding of the fate of polyphenols once ingested, both in terms of absorption, distribution, metabolism, excretion and in terms of target tissues and biological effects. Nonetheless, several areas require dedicated research. In particular, information on the qualitative/quantitative composition of food items is still scant and the methodology to assess it still far from being agreed upon worldwide. These are important steps for the development of a 'functional diet' that confers maximal health benefits and obviate the need for resorting to medicines for the treatment of certain pathological conditions.

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Table 1. *In vitro* effects of polyphenols on murine and human DCs

Compound	Concentration	Cell system	Effect	Molecular mechanism	References
EGCG	25-100 μM	Human MD-DCs	↓ differentiation and LPS-induced maturation ↑ apoptosis ↑ IL-10	Unknown	(Yoneyama et al. 2008)
	25-100 μM	Mouse BM-DCs	↓ LPS-induced maturation	Inhibition of MAPK p38, JNK, ERK1/2 and NF-κB activation	(Ahn et al. 2004)
	25-50 μM		↓ IFN-γ induced IDO expression	STAT1 and COX-2 inhibition ↓ PGE_2	(Jeong et al. 2007)
Curcumin	50 μM	Mouse BM-DCs	↑ tolerogenic DC ↑ regulatory T cells	ALDH1, IL-10	(Cong et al. 2009)
	1-25 μM		↓ IFN-γ induced IDO expression	STAT1 inhibition via JAK1/2 and PKCδ	(Jeong et al. 2009)
	25 μM		↓ LPS-induced maturation	Inhibition of MAPK p38, JNK, ERK1/2 and NF-κB activation	(Kim et al. 2005a)

	20-30 µM§	Human MD-DCs	↓ Poly I:C and LPS-induced maturation ↓ ICAM-1 and CD11c ↓ chemokine secretion ↓ migration	Unknown	(Shirley et al. 2008)
	1-25 µM	Mouse BM and splenic DCs	↓ LPS and IFN-γ induced IDO expression	↑ COX-2 and PGE_2	(Jung et al. 2010)
Apigenin	20 µM	Mouse BM-DCs	↓ LPS-induced maturation	Inhibition of MAPK p38, JNK, ERK1/2and NF-κB activation	(Yoon et al. 2006)
Apigenin	1-100 µM	Mouse spleen DCs	↑ DC apoptosis ↓ IFN-α and IL-6 production	Unknown	(Kang et al. 2009)
Quercetin	50 µM	Mouse BM-DCs	↓ LPS-induced maturation	↓ Akt, NF-κB, ERK1/2, JNK activation	(Huang et al. 2010)
Resveratrol	3-50 µM	Human MD-DC	↓ differentiation and LPS-induced maturation ↑ ILT3 and ILT4 ↑ IL-10-producing T cells	↓ NF-κB activation	(Svajger et al. 2009)
Blackberry polyphenols	12.8 – 37.3 µM*	Mouse BM-DC	↓ lipid A-induced IL-12	Unknown	(Dai et al. 2007)
p-coumaric		Mouse BM-	↓ IFN-γ	↓ STAT1	(Kim et al.

acid		DC	induced IDO expression		2007)
Rosmarinic acid	100 μM	Mouse BM-DC	↓ IFN-γ induced IDO expression	↓ STAT1	(Lee et al. 2007a)
Fisetin	1-30 μM	Mouse BM-DC	↓ LPS-induced maturation	Unknown	(Liu et al. 2010)
Luteolin	10-50 μM	Mouse BM-DC	↓ LPS-induced IL-12p40	↓ LPS-induced NF-κB transcriptional activity	(Kim et al. 2005b)
Silibinin	50 μg/ml	Mouse BM-DC	↓ LPS-induced maturation	Inhibition of MAPK p38, JNK, ERK1/2 and NF-κB activation	(Lee et al. 2007b)

A summary of the main studies demonstrating *in vitro* effects of specific polyphenols and the biology of DCs is shown. In most studies, polyphenols have been added concomitantly or shortly before LPS (concentration range 100 ng/ml - 1μg/ml) or IFN-γ (100 units/ml) treatment, and analyzed 24 h later.

*Lipid A was used as activation stimulus (concentration range 0.1 – 10 μg/ml)

§ Poly I:C was also used as activation stimulus (concentration 25 μg/ml)

Table 2. *In vivo* immunomodulatory effects of polyphenols on DCs

Compound	Concentration	*In vivo* model	Effect	Mechanism	References
Apigenin	3-20 mg/kg	Lupus prone SFN1 mice	↓ Th1 and Th17 response to auto-antigens	↓ COX-2 ↓ auto-antigen presentation	(Kang et al. 2009)
Apigenin	5 mg/kg	C57BL/6, BALB/C	↓ LPS-induced maturation	Unknown	(Yoon et al. 2006)
Fisetin	50 mg/kg	C3H/HeN	↓ DC migration ↓ DC allostimulatory capacity	Unknown	(Liu et al. 2010)
Curcumin	50 mg/kg	C57BL/6 colitis model	↑ regulatory T cells	Colitis inhibition *via* T_{reg} cells	(Cong et al. 2009)
Curcumin	50 µM*	C57BL/6	↓ IDO and co-stimulatory molecule expression	Unknown	(Jung et al. 2010)
Rosmarinic acid	5 mg/kg	Tumor-bearing C57BL/6	↓ IDO expression	Unknown	(Lee et al. 2007)
p-coumaric acid	5 mg/kg	Tumor-bearing C57BL/6	↓ IDO expression	Unknown	(Kim et al. 2007)
Green and white tea polyphenols	2.5mg/cm^{2} #	Human volunteers	↓ skin DC depletion	Unknown	(Camouse et al. 2009)

A summary of the main studies demonstrating *in vivo* effects of specific polyphenols and the biology of DCs is shown.

*Curcumin was added to BMDC *in vitro*

This study was also carried out on a human skin explant model at the concentration of 2 mg/cm^{2}

Legends to the Figures

Figure 1. Main classes of polyphenols.

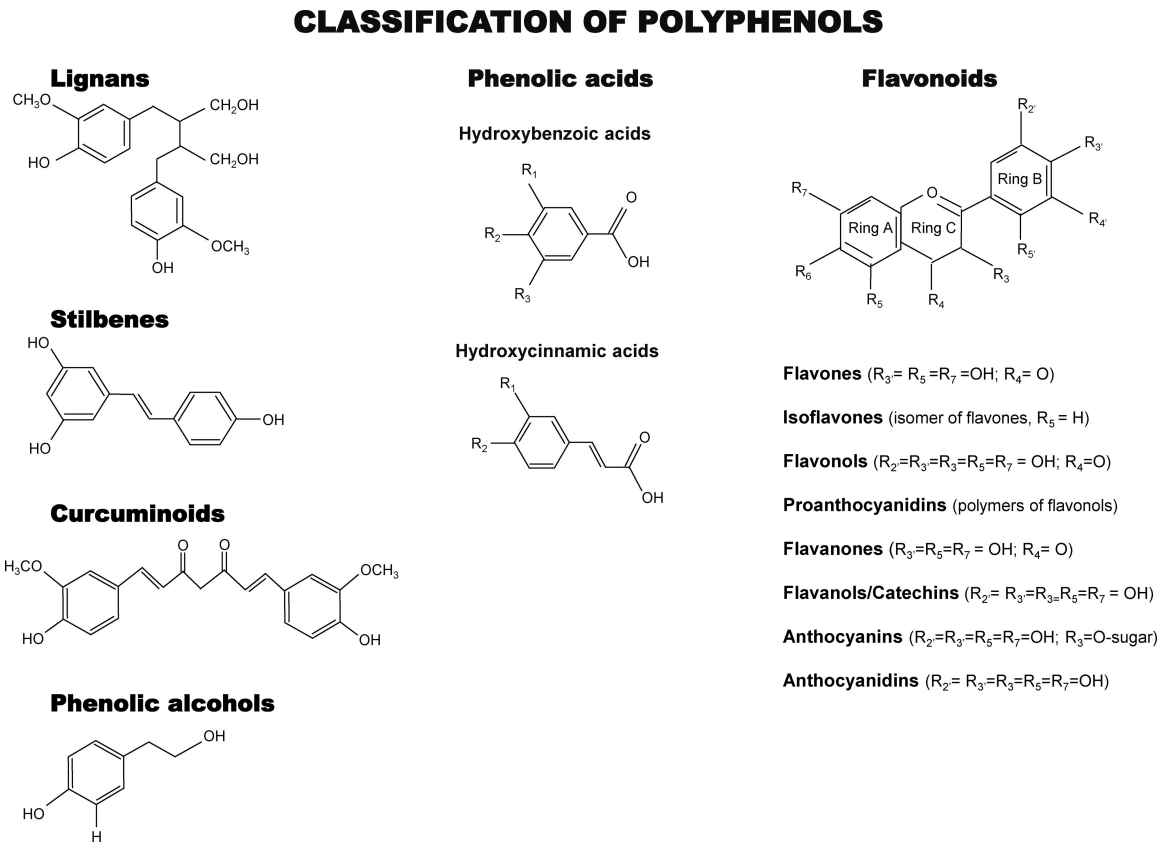

Figure 2. Functional plasticity of DCs: induction of tolerance *versus* immunity. DCs are accessible to activating and/or inhibitory factors present in the microenvironment that favor their immunogenic or tolerogenic function, respectively. In steady-state conditions, immature DCs, DCs exposed to anti-inflammatory and immunosuppressive agents or activated by T_{reg} cells, convert into tolerogenic DCs with immunosuppressive function. Conversely, proinflammatory cytokines, microbial products and DC cross-talk with activated T effector lymphocytes induce terminal maturation of immature DCs into immunogenic DCs, fully capable to activate and expand effector T lymphocytes.

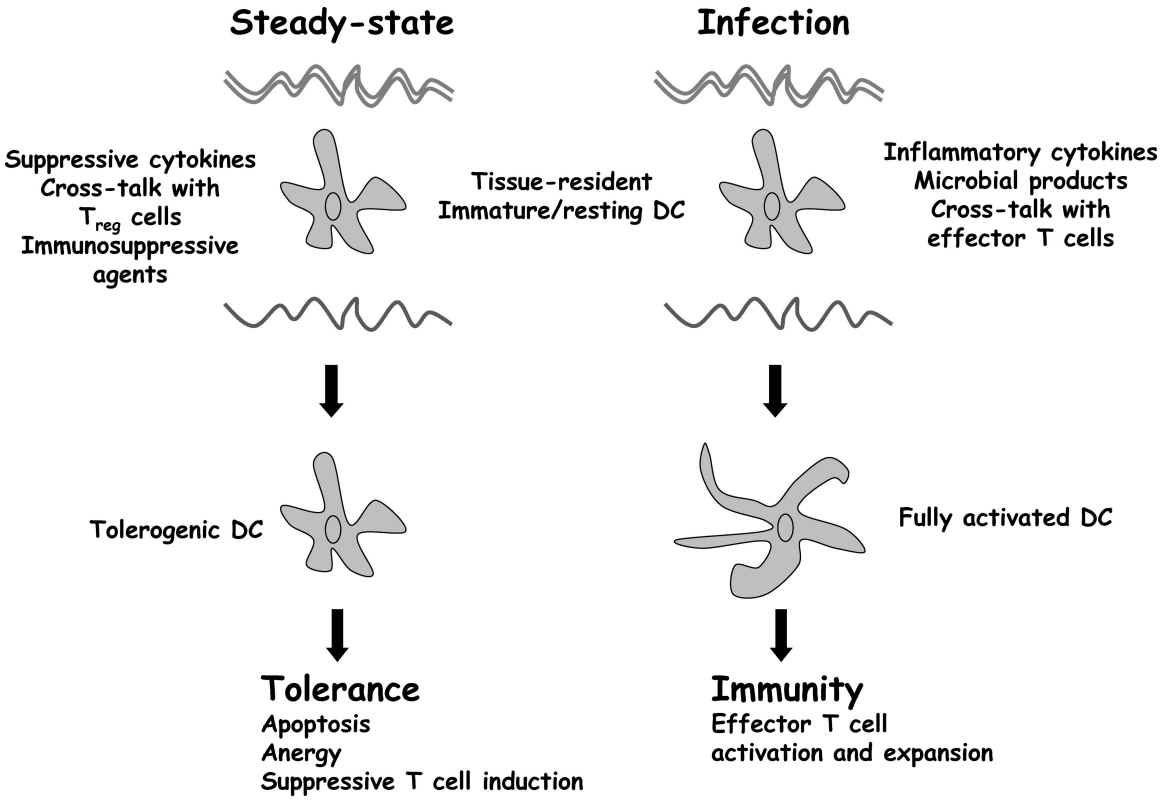

Figure 3. A schematic representation of the immunomodulatory effects of polyphenols on DCs.

Polyphenols can regulate DC functional activities in a negative or positive manner by attenuating inflammation or rescuing immunological anergy, respectively. Polyphenol abrogation of specific signaling pathways (i.e. MAPK, NF-κB) or *vice versa* synergy with other pathways ultimately leading to the expression of tolerance-associated molecules (i.e.IDO), impair DC maturation and, consequently, strongly reduce T cell proliferative responses. On the other side, the capacity of polyphenols to suppress the activity of some transcription factors (i.e. STAT1) may counteract the suppressive activity of T cell derived factors thus favoring immune activation.