CHAPTER

22

Polyphenols and Immune System

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Abbreviations

AhR	aryl hydrocarbon receptor
AID	activation-induced cytosine deaminase
AIM2	absent in melanoma 2
AP-1	activator protein-1
APC	antigen presenting cell
Arg-1	arginase-1
BLYS	B lymphocyte stimulator
CAPS	cryopyrin-associated periodic syndrome
CARD	caspase activation and recruitment domain
COX-2	cyclooxygenase-2
CRP	C-reactive protein
CTLA-4	cytotoxic T-lymphocyte-associated antigen 4
DAMP	danger associated molecular pattern
DC	dendritic cell
DNMT1	DNA methyltranferase-1
EGCG	epigallocatechin-3-gallate
Elf-1	ETS like transcription factor
ESR	erythrocyte sedimentation rate
FMF	familial mediterranean fever
fMLP	formyl methionyl-leucyl-phenylalanine
FoxP3	forkhead winged helix protein-3; transcription factor for Tregs
GALT	gut-associated lymphoid tissue
GATA-3	transcription factor for Th2
GB	glabridin
GM-CSF	granulocyte macrophage colony-stimulating factor
GPx	glutathione peroxidase
GSH	glutation
HAT	histone acetyltranferase
HDAC	histone deacetylase
IBD	inflammatory bowel disease
ICAM-1	intercellular adhesion molecule-1
IDO	indoleamine 2,3-dioxygenase
IFN-γ	interferon-γ
Ig	immunoglobulin
IKK-a	I kappa B kinase a
IL	interleukin
ILC	innate lymphoid cell
iNOS	inducible nitric oxide synthase
IRAK	IL-1 receptor-associated kinase
iTreg	inducible Treg
KC	keratinocyte chemoattractant
LN	lymph node
LPS	lipopolysaccharide
LTB4	leukotriene B4
MAPK	major mitogen-activated protein kinase
MCP-1	monocyte chemoattractant protein-1
MHC class I	major histocompatibility complex class I
MHC class II	major histocompatibility complex class II
MIP-2	macrophage inflammatory protein-2
mTOR	mammalian target of rapamycin
MyD88	myeloid differentiation primary response 88
NF-IL-6	nuclear factor IL-6
NF-κB	nuclear factor κB
NK	natural killer
NLR	nucleotide-binding domain-like receptor
NO	nitric oxide
NOD-like	nucleotide oligomerization domain-like
nTreg	natural Treg
Ox-LDL	oxidatively modified LDL
PAAND	pyrin-associated autoinflammation with neutrophilic dermatosis
PAF	platelet activating factor
PAMP	pathogen-associated molecular pattern
PGE2	prostaglandin E2
PMN	polymorphonuclear leukocyte
PPARγ	peroxisome proliferator-activated receptor gamma
PRR	pattern recognition receptor
PYD	pyrin domain
RA	rheumatoid arthritis
RIG-I	retinoic acid-inducible gene I
RORγt	orphan retinoic acid receptor γt; transcription factor for Th17
ROS	reactive oxygen species
SAH	S-adenosylhomocysteine
SAM	S-adenosylmethionine
SIRT	silent information regulator
SLE	systemic lupus erythematosus
SOD	superoxide dismutase
STAT	factors signal transducer and activator of transcription
sVCAM-1	soluble vascular cell adhesion molecule-1
T-bet	transcription factor for Th1
TCR	T-cell receptor
TGF-β	transforming growth factor beta

Th	T helper cell
TIR	Toll/interleukin-receptor
TLR	Toll-like receptors
TNF-α	tumor necrosis factor-α
Tollip	Toll interacting protein
Treg	T regulatory cell

1 INTRODUCTION

Bioactive plants or phytochemicals have been used since ancient times as both primary and supplemental treatment for various illnesses as well as to support normal physiological functions in the healthy people. Isolated plant compounds remain one of the most significant sources for drug synthesis in the pharmaceutical industry. In the last decade, the demand for natural products has driven an increased interest in using natural phytochemicals for the prevention and treatment of different chronic human diseases. Phytochemicals can be broadly classified into three major groups: terpenoids, polyphenols, and alkaloids [1]. Thousands of compounds have been discovered, and it is expected that scientists will discover many more. This chapter will focus on main polyphenols and their effects on the immune system.

1.1 Polyphenols

Polyphenol is not a strict chemical term. It is used to refer to flavonoids, tannins, phenolic acids, lignans, stilbenes, and their various chemically modified or polymerized derivatives. Polyphenols are produced in plant cells through secondary metabolism and function in both plant reproduction and growth [2]. Secondary metabolites apparently act as defense (against herbivores, microbes, or competing plants) and signal compounds (to attract pollinating or seed-dispersing animals), as well as protecting the plant from ultraviolet radiation, oxidants, and various environmental hazardous. Therefore, they represent adaptive characters that have been subjected to natural selection during evolution [3].

The structure of polyphenols varies from simple molecules, such as phenolic acids, to highly polymerized compounds, such as proanthocyanidins, and several thousand different compounds have been identified with a large range of structures [4]. Polyphenols are found in a wide variety of fruits and vegetables. They are also found in tea and chocolate as catechins and flavanols [5]. It is estimated that flavonoids account for approximately two-thirds of the polyphenols in the diet. We can categorize the flavonoids as flavonols, isoflavones, flavanones, anthocyanins, flavan-3-ols, proanthocyanidins, and flavones. Phenolic acids, tannins, and stilbenes are called nonflavonoid phenolic compounds.

1.2 Innate Immune System

The human immune system can be divided into two parts as a didactic approach: the innate and the adaptive immune systems. The innate immune system is conserved throughout the evolution of plants and animals, including humans. Innate immunity is the first line of defense against infectious microorganisms in humans and relies on germ line-encoded pattern recognition receptors (PRRs) to recognize pathogen-derived substances [6]. The cells of the innate immune system do not use specific receptors to recognize individual foreign antigens; they respond quickly to pathogen invasion by recognizing the characteristic molecular patterns of microbes via PRRs, including Toll-like receptors (TLRs). TLRs were the first PRRs identified in mammals. These molecules have been evolutionarily conserved from the worms to mammals, and play an essential role in the early innate immune response. Upon recognizing a pathogen-associated molecular pattern (PAMP), TLRs mediate downstream signals through several adaptor proteins, including myeloid differentiation primary response 88 (MyD88) and the Toll/Interleukin-Receptor (TIR) domain-containing adapter-inducing IFN-β-dependent signaling pathway [7].

The innate immune system consists of many cell types, such as polymorphonuclear leukocytes (PMNs), macrophages, Natural Killer (NK) cells, and soluble factors such as complement system, proinflammatory cytokines, and vasoactive mediators, which induce inflammatory reactions. Neutrophils are phagocytic cells that are the fastest responders to pathogen invasion. Neutrophils, together with eosinophils and basophils, are called granulocytes, because they contain large numbers of granules. At the earliest stage of immune response, active recruitment of neutrophils to sites of infection involves mobilization of PMNs from circulation in response to host- and pathogen-derived chemotactic factors. Phagocytes pass through the capillary wall, surveying tissue and lymphatic organs for signs of tissue distress. Endogenous substances such as interleukin 8 (IL-8 or CXCL8), leukotriene B4 (LTB4), platelet activating factor (PAF), and exogenous substances such as formyl methionyl-leucyl-phenylalanine (fMLP) derived from bacterial cell products are important neutrophil chemoattractants [8]. After adherence of the pathogen to the surface of phagocytes through a recognition receptor, the engulfment phase is initiated. The pathogens are then destroyed by the microbicidal mechanism. Elastase and other enzymes stored in cytoplasmic granules are released into the phagosome and contribute to the intracellular killing of ingested microorganisms [8]. Paradoxically, when released in a dysregulated manner into the extracellular compartments, these enzymes may damage host tissues and contribute to several inflammatory disorders. Thus, while well-controlled activation of neutrophils is necessary to

combat infections in healthy people, antiinflammatory strategies for decreasing neutrophil activation are necessary for preventing and treating inflammatory diseases in which neutrophils are involved. Eosinophils and basophils are involved in some inflammatory responses and in allergic reactions. Eosinophils are also important to defense against parasites. Mast cells, which also have granules, reside in the tissues. They play a central role in type I hypersensitivity [9].

Macrophages are phagocytic cells that ingest pathogens and dead cells. Circulating monocytes in the blood differentiate into macrophages after migrating into the tissues. Macrophages are highly plastic and versatile cells. They can differentiate into two subsets in response to stimuli from the microenvironment, the classical subtype of activated macrophage, M1, and the alternative subtype, M2. The M1 macrophages are typically induced by bacterial lipopolysaccharide (LPS) and/or interferon-γ (IFN-γ) and produce various proinflammatory cytokines, such as interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and monocyte chemoattractant protein-1 (MCP-1 or CCL2) [10]. The TNF family has a broad range of biological effects via two distinct TNF receptors (TNFR1 and TNFR2). TNF-α has a crucial role in initiating and regulating the cytokine cascade in the innate and adaptive immunity and play many roles in acute or chronic inflammatory conditions, cell proliferation, and apoptotic processes. TNFα is synthesized primarily by immune cells, such as macrophages, dendritic cells, and T lymphocytes [11]. IFN-γ is the main prototype of the Th1 cytokine, which induces cellular immunity by activation of macrophages, NK cells, and cytotoxic T-lymphocytes (CTLs) to act against cancers and intracellular microbes. Conversely, M2 macrophages are often polarized by IL-4 and/or IL-13 and secrete high levels of antiinflammatory cytokines, such as IL-10 [10]. M2 macrophages have arginase-1 (Arg-1) activity for their antiinflammatory and remodeling properties. Recent evidence suggests that macrophages can reversibly and progressively shift their functional phenotype in response to changes of microenvironment.

Macrophages and dendritic cells (DCs) are professional antigen presenting cells (APCs) that activate naive CD4+ T cells to become effector T cells. APCs are activated upon recognition of pathogens and process antigenic proteins into peptides, which are then presented to CD4+ T cells. DCs are the most potent APCs that play a pivotal role in switching the adaptive immune response toward inflammation or tolerance [12]. We can say that they are the center of the immune universe. DCs express a range of TLRs, which, when activated, initiate the DCs maturational program via Nuclear factor κB (NF-κB). TLR4 activation by LPS is among the best-known DC maturational stimuli. CD4+ T cells response is dependent on antigen presentation by major histocompatibility complex class II (MHC class II) and the up-regulation of costimulatory molecules (CD40, CD80, CD86) that stabilize the DCs T-cell contact in the immunological synapse [13]. Once inside the lymph nodes (LNs), mature DCs fulfill their function by efficiently priming naïve T cells toward effector T cells. The cytokine cocktail in the LNs microenvironment during DC-T cell interaction is the result of a stochastic production of various cytokines driving specific T helper cell lineage development. Not every antigen presented by the DCs is exogenous and indeed DCs commonly sample and present self-antigens in the LNs. This process is physiologically crucial in noninflammatory conditions as DCs promote tolerance toward self-antigens that are captured in the periphery [14]. However, dysregulated DC maturation and/or antigen presentation can lead to a number of pathological consequences, including those seen in chronic inflammatory responses. Traumatic events may alter tissue integrity with a resultant increase in the number of DCs bearing self and nonself antigens. Antigen availability is necessary but not sufficient to direct the adaptive immune response; antigen presentation capacity is also related to DCs maturation [14].

NK cells and some subpopulation of lymphocytes are innate immune cells that eliminate virus-infected cells and mutated cells. The lymphocyte subgroup called innate lymphoid cells (ILCs), although they are of lymphoid lineage, have no antigen-specific receptors. ILCs play important roles in the inflammatory response and in maintaining immune homeostasis. ILCs are classified into three subsets, ILC1, ILC2, and ILC3, which have different functions and cytokine-expression patterns [9]. NK cells are a component of the innate immune system, which does not directly attack invading microbes [15]. Rather, NK cells destroy compromised host cells, such as tumor cells or virus-infected cells, recognizing such cells by a condition known as *missing self*. This term describes cells with low levels of a cell-surface marker called MHC class I—a situation that can arise in viral infections of host cells. They were named *natural killer* because of the initial notion that they do not require activation in order to kill cells that are *missing self*.

The major way by which the innate immune system defends against infections and tissue injury is to stimulate acute inflammation. However, chronic inflammation is a sophisticated process that takes over from acute inflammation if the tissue injury is not repaired or prolonged [16]. Inflammation is a normal protective response of the innate immune system to tissue injury or detrimental external stimuli such as pathogens, allergens, and other irritants. Efficient control of inflammation is vital to protect the host from external stimuli. Defective resolution of inflammation and misreading of inflammatory signals increase the risk of developing autoimmune and chronic inflammatory diseases [17]. During the inflammatory reaction, immune system cells can recognize foreign

pathogenic molecules, such as LPS, which is recognized by TLRs or nucleotide oligomerization domain-like (NOD-like) receptors [18]. This process can lead to the activation of major mitogen-activated protein kinase (MAPK) cascades and translocation of the regulator NF-κB, resulting in the increased expression of proinflammatory cytokines such as interleukin-1β (IL-1β), interleukin 6 (IL-6), TNF-α, and MCP-1 (CCL-2) [19]. In addition, it will lead to increased expression of NO synthase and thus excess NO accumulation. The transcription factor NF-κB is crucial in a series of cellular processes including inflammation, immunity, cell proliferation, and apoptosis. In the resting state, NF-κB is sequestered in the cytoplasm of the cell through its tight association with inhibitory proteins called IκBs, comprising IκBa, IκBb, IκBg, IκBe, Bcl-3, p100, and p105 [19]. Upon cell stimulation, cytokine activation occurs when the IκB proteins are rapidly phosphorylated and degraded by the proteasome, and the freed NF-κB translocates into the nucleus to regulate the expression of multiple target genes. The activation of NF-κB in various immune cells, including T cells, B cells, macrophages, DCs, and neutrophils, leads to expression of proinflammatory cytokines. Proinflammatory cytokines play a key role in some cardiovascular disease and chronic inflammatory diseases, such as insulin resistance, diabetes, atherosclerosis, even depression [17]. Although M1-type macrophages, activated by IFN-γ, promote the adaptive immune response through the secretion of proinflammatory cytokines, M2-type macrophages activated by IL-4 and IL-13 have been linked to antiinflammatory signaling and wound healing [10]. NF-κB thus plays an important role in the immune response regardless of the specific macrophage type. NF-κB controls the expression of more than 500 different gene products that have been closely linked to inflammation, cellular transformation, tumor cell survival, proliferation, invasion, angiogenesis, and metastasis. In addition, this transcription factor is activated in response to a wide variety of stimuli that are shown to be lifestyle risk factors, such as stress, tobacco, radiation, asbestos, dietary agents, environmental pollutants, obesity, and various infectious agents closely linked to cancer [20].

1.3 Adaptive Immune System

The adaptive immune system evolved much later in higher species. In contrast to innate immunity, the adaptive immune system generates antigen specific receptors such as antibodies and T-cell receptors (TCRs) by somatic cell DNA rearrangement. These receptors recognize specific pathogen encoded proteins (antigens). In an adaptive immune system, lymphocyte subpopulations have pivotal roles for inducing appropriate immune responses. The majority of T cells express TCRs consisting of α- and β-chains (the others as a minor group of T cells express γδ-type chains) on their surfaces. These cells are divided into two subpopulations, depending on their surface expression of CD4 or CD8 molecules (TCR co-receptors). CD4+ T cells are mainly helper T cells helping and controlling the activity of other immune cells. CD8+ T cells are cytotoxic; they kill cells infected by viruses or mutated cells with the potential to cause cancer.

The T helper (Th) cells have the central role in all aspects of immune responses. They are important in B cell biology including B cell activation, proliferation, antibody class switching, macrophage activation, and activation of cytotoxic T cells. Cytokines are the main network for Th communications. Thus inappropriate activities play an important role in distinct inflammatory processes, particularly in asthma, allergy, atopic, dermatitis, and many forms of autoimmune diseases. Cytokines that mediate chronic inflammatory manners can be divided into those participating in cellular and humoral inflammation. Some of them, such as IL-1, IL-6, IL-11, IL-17, IL-18, TNF-a, and granulocyte-macrophage colony-stimulating factor (GM-CSF), significantly play roles in acute and chronic inflammation. Finally, in the immune system many biological events are induced by cytokines such as regulating hematopoiesis, inflammatory reactions, and T-cell differentiation from undifferentiated (Th0) cells to Th1, Th2, T regulatory (Treg), Th9, Th17, and Th22 cells [21]. The Th1 subtype, distinguished by nuclear factors signal transducer and activator of transcription 1 (STAT1), STAT4, and T-bet, produces IFN-γ, TNF-α, IL-2, and IL-12, resulting in the activation of effector cytotoxic CD8+ T-cells and innate immune cells such as macrophages and NK cells. Th2 cells, in which the nuclear factors STAT6 and GATA3 are present, secrete IL-4, IL-5, IL-10, and IL-13, which further activate B-cell transformation to plasma cells to provide humoral immunity through the production of antibodies [21]. In contrast to the aforementioned systemic helper T cells, IL-17-producing Th17 cells have been identified in local inflammation-prone sites, including the digestive tract. It has been reported that the orphan retinoic acid receptor (ROR) family transcription factor RORγt is essential for Th17 development and function, indicating that vitamin A, a precursor of retinoic acid, is a potential dietary inhibitor of Th17 cells and consequent inflammatory responses [21]. Indeed, abundant data have indicated that Th17 cells are highly relevant to the onset and propagation of local chronic inflammation, in part by producing inflammatory cytokines, including IL-17 and IL-21. Th17 cells appear to play a critical role in acute and chronic parasite, bacteria, and fungi infections, in addition to autoimmune and chronic inflammatory diseases, such as rheumatoid arthritis (RA), psoriasis, and psoriatic arthritis. IL-22-producing Th22 cells have been demonstrated to be central to host protection against bacterial infections at barrier sites.

The development of Th22 cells is dependent on the expression of the transcription factors T-bet and aryl hydrocarbon receptor (AhR). Th9 cells were identified by the production of IL-9, the in vivo significance of which has not been clearly identified, although Th9 cells are thought to be involved in allergic inflammation, antitumor immunity, and autoimmune inflammation [21].

Treg cells modulate innate and adaptive immune responses. Two distinctive regulatory mechanisms of Tregs have been discovered: direct cell-cell contact with inflammatory effector T-cells and secretion of antiinflammatory cytokines, such as transforming growth factor beta (TGF-β) and IL-10, which play a critical role in the maintenance of tolerance in the immune system. These cells comprise a predominate population of the CD4+ CD25+ T cells [12]. Additionally, the transcription factor forkhead winged helix protein-3 (FoxP3) is widely accepted as the most specific marker for Treg cells [21]. As for the development of the cell lineage, Tregs develop in either the thymus (natural Tregs, nTregs) or secondary lymphoid tissues (inducible Tregs, iTregs) such as spleen, lymph nodes, and intestinal mucosa. nTregs require CD28 costimulation during positive selection in the thymus in the presence of TGF-β, IL-2, and IL-15 [21]. iTregs, however, develop under subinflammatory conditions, such as in inflamed tissues or normal intestinal mucosa with costimulation of cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) in the presence of TGF-β and IL-2. iTregs exert antiinflammatory effects on activated effector T cells through the secretion of IL-10 and TGF-β1 [21]. Because iTregs develop from naïve CD4+ T-cells in the peripheral lymphoid tissues, the repertoire of iTregs is specific to allergens, commensal microbiota, neoantigens, alloantigens, and self-antigens. iTregs are specifically abundant in the digestive tract, respiratory tract, and other inflammatory sites, where the influx of exogenous materials is a common occurrence.

B cells produce antibodies, also known as immunoglobulins, against foreign antigens. During primary activation after encountering its specific antigen, a B cell undergoes differentiation into a cell that produces different classes of immunoglobulin, including immunoglobulin A (IgA), IgG, and IgE, through the mechanism of isotype switching. IgA plays a critical role in protection from infection and control commensal bacteria at mucosal sites and is secreted in dimeric form in mucosal secretions, as well as in tears, saliva, sweat, and breast milk. IgG is the most abundant class of immunoglobulin in serum and reaches the fetus via the placenta, thus protecting the fetus from infection. IgE is responsible for the onset of type I hypersensitivity [9].

Mammals have a complex immune response, which relies on communication between the innate and adaptive immune system. DCs are key players in immune surveillance and homeostasis in various organs, particularly those with large mucosal surfaces such as the gastrointestinal tract [14]. A number of specialized populations of DCs reside in the lamina propria and the gut-associated lymphoid tissue (GALT) such as the Peyer's patches [21]. Human intestinal DCs are not well characterized, but in mice different subsets are distinguished by their expression of CD11b, CD103, some chemokine receptors (CX3CR1 and CCR7), and they play an important role through antigen sampling from the intestinal lumen and subsequent presentation of pathogen antigens to T cells in the GALT [22]. Hence, DCs are exposed to both harmless gut flora and pathogenic intestinal microorganisms such as viruses, bacteria, and parasites, as well as dietary components [22]. They therefore play a key role in maintaining effective immune homeostasis; overt inflammatory responses by DCs such as excessive secretion of proinflammatory cytokines may lead to the development of chronic inflammation, while appropriate cytokine secretion and T-cell activation are also important for effective clearance of potentially harmful pathogens. Therefore, modulation of DC activity may be an effective strategy for ameliorating autoimmune diseases, as well as invoking a desirable immune response for protection against intestinal pathogens.

1.4 Oxidative Stress

Oxidative stress, induced by an imbalance in the body's natural antioxidant defenses and increased free radical production, leads to the damage of key cellular molecules including lipids, DNA, and proteins [23]. Oxidative stress is directly linked with inflammation due to the fact that the previously mentioned oxidants are activators of NF-κB, the key regulator of inflammation [24].

Oxidant molecules, produced during the inflammatory response, up-regulate cytokine production through the activation of nuclear transcription factors such as NFκB, nuclear factor IL-6 (NF-IL-6), and activator protein-1 (AP-1). The transcription factor NFκB preexists within the cell cytoplasm in an inactive form, by virtue of its binding to an inhibitory subunit, called IkB. Cellular signals induce dissociation of the IkB, to reveal a nuclear recognition site, which, after a series of phosphorylation steps, causes the NFκB subunit to move into the cell nucleus and turns on gene transcription [14]. There are large ranges of genes, which have been shown to be regulated through NFκB. Their products include cytokines, adhesion molecules, enzymes, and other inflammatory mediators. The process of dissociation and phosphorylation has a redox sensitive step, which means that oxidant molecules promote NFκB activation and antioxidants inhibit it. Up-regulation of NFκB controls many of the cytokines implicated in the inflammatory responses that are seen during infection and injury. Nutrients with antioxidant properties contribute to the body's antioxidant

defenses and thereby limit the ability of oxidants, released during inflammation, to activate NFκB directly or damage host tissue [25].

A typical feature of atherosclerosis is the accumulation of oxidatively modified LDLs (Ox-LDLs) within plaques. Also, these lipoproteins are considered to contribute to the inflammatory state of atherosclerosis and to play a key role in its pathogenesis. The cellular uptake of ox-LDL leads to the generation of reactive oxygen species (ROS) [26]. ROS are potentially very harmful substances, because they can react with proteins, DNA, or lipids. In other words, the accumulation of ox-LDL can be a starter of oxidative stress. Ox-LDL is an important marker of oxidative stress and the lipid peroxidation process. And, also, ox-LDL, by itself, may induce inflammation. The accumulation of ox-LDL in the tissues may directly affect inflammation in some inflammatory diseases, such as atherosclerosis, fatty liver, and psoriasis [27].

1.5 Inflammation

Inflammation is the normal, protective, and usually temporary response of the innate immune system to pathogens or injury. It has five cardinal signs: redness, swelling, heat, pain, and loss of function. These responses occur as a result of increased blood flow and increased permeability across blood capillaries, which increases the movement of leukocytes and large molecules (e.g., antibodies, cytokines) from the blood into the surrounding tissue. The aim of inflammation is to induce immunological processes to eliminate invading pathogens and toxins and to repair damaged tissue [11]. Inflammation is triggered by the production of a broad spectrum of cytokines, chemokines, adhesion molecules, eicosanoids, and complement protein. These molecules form complex regulatory networks to promote increased blood flow to the infected tissue, immune cell infiltration and activation, and systemic responses, including increased body temperature, increased heart rate, and decreased appetite. At the molecular level, the inflammatory process is mainly regulated by NF-κB, the previously mentioned key regulator of inflammation. NF-κB is activated by ROS, which explains the mechanistic link between oxidative stress and inflammation, but also by viruses, bacterial toxins, proinflammatory cytokines, and many other stressors. Upon activation, NF-κB target genes, such as proinflammatory cytokines, chemokines, inflammatory enzymes, adhesion molecules and various receptors, are induced [25].

1.6 Aging and Inflammaging

Aging is associated with complex changes and dysregulation of the immune system, including its inflammatory component. The aging of the immune system, termed immunosenescence, has been suggested to be a consequence of continuous attrition caused by chronic antigenic overload and an inability of immune cell output, for example from the thymus, to keep up with the demand for naïve cells. Another phenomenon that accompanies the aging process is a low grade, chronic inflammatory state. The lifelong exposure to antigens and inflammatory stimuli accumulate and affect this phenomenon [28]. The elevated inflammatory state that occurs with aging can potentially trigger or facilitate the onset of the most important age-related diseases [29]. Many possible triggers of low-grade inflammation have been proposed, ranging from dysfunctional mitochondria to an imbalance in gut microbiota (termed dysbiosis). Another important contributor to the onset and maintenance of low-grade inflammation is cellular senescence, defined as an irreversible block of the cell cycle. Actually, aging is accompanied by the accumulation of senescent cells in many organs and tissues. It is also hypothesized that failure of antiinflammatory and inflammation-resolving mechanisms to neutralize inflammatory processes plays a role in the development of chronic low-grade inflammation in the elderly. The terminal activators of the inflammatory response are the NF-κB pathway and the inflammasome platform [19]. Briefly, NF-κB is a multimeric transcription factor that modulates gene expression by binding to specific DNA sequences, known as κB response elements, in gene promoters and enhancers. NF-κB can be activated by over 150 different stimuli, including cytokines, ultraviolet irradiation, and bacterial or viral antigens. Moreover, it has a unique sensitivity to oxidative stress, as many of the agents activating NF-κB are either modulated by oxidative stress or are prooxidants themselves or are oxidized molecules, such as ox-LDL.

Inflammasomes are cytoplasmatic platforms that trigger the maturation and release of proinflammatory cytokines such as IL-1β. Inflammasome assembly mostly results from the oligomerization of a nucleotide-binding domain-like receptor (NLR) upon the recognition of different types of PAMPs from bacteria, viruses, or fungi, or danger associated molecular patterns (DAMPs), including ATP, nucleotides, cholesterol crystals, beta-amyloid, and hyaluronan. Other proteins such as absent in melanoma 2 (AIM2), retinoic acid-inducible gene I (RIG-I), and pyrin may be able to form inflammasome platforms. However, the NLR proteins are considered the main inflammasome [30,31]. They contain either a pyrin domain (PYD) or a caspase activation and recruitment domain (CARD). Inflammasomes activate procaspase-1 to caspase, which in turn leads to the maturation of pro-IL-1β and pro-IL-18 to the respective mature forms [31]. Dysregulation of inflammasomes leads to well-recognized autoinflammatory diseases such as the cryopyrin-associated periodic syndrome (CAPS) for the NLRP3 inflammasome and the familial Mediterranean fever (FMF) and pyrin-

associated autoinflammation with neutrophilic dermatosis (PAAND) for the pyrin inflammasome [32]. However, inflammasomes are involved in the pathophysiology of many other illnesses, including chronic inflammatory diseases, degenerative processes, fibrosis, or metabolic diseases. Age-related increase in low-grade inflammation is termed inflammaging and this is seen to contribute to many of the common declines in function, health, and well-being that accompany aging.

1.7 Polyphenols, Inflammation, and Immunity

Polyphenols are able to scavenge free radicals and inactivate other prooxidants but they also have antiinflammatory actions by inhibiting the activation of NF-κB and related cell signaling pathways that trigger systemic inflammation. They have important antiinflammatory effects by regulating innate and adaptive immunity through the modulation of different cytokines [33]. Polyphenols have been demonstrated to modulate the inflammatory process and stimulators via several individual and synergistic mechanisms: (a) by altering signaling and enzymatic processes involved in inflammation such as tyrosine and serine-threonine protein kinases, which have been known to be involved in B-lymphocyte activation and T-cell proliferation (they have also been known to inhibit the key inflammatory mediator, NF-κB, inducible nitric oxide synthase (iNOS), proinflammatory enzymes such as COX-2, MAPK and protein kinase-C); (b) by exhibiting a blunting effect on inflammatory cell secretions; (c) by protecting oxidative stress by scavenging free radicals and inflammatory prooxidants such as superoxide anions, hydrogen peroxide; and (d) by modulating inflammatory mediators such as cytokines, peptides, arachidonic acid [34].

1.8 Flavonoids on Immunity and Inflammation

The most important class of polyphenols is flavonoids, which are synthesized by plants from the aromatic amino acids phenylalanine and tyrosine, and from malonate as well. Flavonoids appear to show various antioxidant properties; they are able not only to scavenge ROS but also to quench their formation either by inhibiting a number of enzymes or chelating trace elements that participate in generation of free radicals. This metal chelating property is mostly propounded for quercetin, an abundant flavonoid in onion, broccoli, apples, grapes, and soybeans, which exerts both iron chelating and iron stabilizing abilities [35].

From a chemical viewpoint, flavonoids can be considered as fairly reactive compounds. They typically display π-electron-rich aromatic nuclei and labile phenolic —OH groups that confer on them a reducing (electron- and hydrogen-donating) character [35]. The inhibition of ROS production—a possible antioxidant mechanism for flavonoids—can proceed by direct binding to the enzyme and/or by ROS scavenging. Flavones and flavonols display a general affinity to ATP-binding proteins as a consequence of their formal structural analogy with ATP. Within this protein class, protein kinases are especially important targets [36]. Protein kinases catalyze the transfer of a phosphate group from the ATP cofactor to a protein or peptide substrate. Serine/threonine kinases and tyrosine kinases are distinguished according to the ligand residue accepting the phosphate group. Kinase-catalyzed phosphorylation is a very important cell-signaling mechanism, especially involved in cell growth, proliferation, survival, and apoptosis. Within inflammatory processes, flavonoids increase in the number, mobility, lifespan, tissue influx ability, and phagocytic activity of neutrophils.

1.8.1 Quercetin

The antiinflammatory action of quercetin is caused by the inhibition of enzymes such as lipoxygenase, and the inhibition of inflammatory mediators. Quercetin affects immunity and inflammation by acting mainly on leukocytes and targeting many intracellular signaling kinases and phosphatases, enzymes, and membrane proteins often crucial for a cellular specific function [37]. Quercetin inhibits the production and release of histamine and other allergic and inflammatory substances, possibly by stabilizing cell membranes of mast cells. In particular, quercetin is an inhibitor of allergic (IgE-mediated) mediator release from mast cells and basophils [38]. It is also an inhibitor of human mast cell activation through the inhibition of Ca^{2+} influx, histamine, leukotrienes, and prostaglandins release and protein kinase activation. Mast cells are influential immune cells important for the pathogenesis of allergic responses and autoimmune disorders. They also affect release of many cytokines involved in the inflammatory reactions such as IL-8 and TNF. This is a reason why quercetin is suitable for the treatment of mast cell-derived allergic inflammatory diseases such as asthma, sinusitis, and rheumatoid arthritis [38].

Antiinflammatory and antiallergic properties of quercetin have been proven in the treatment of respiratory and food allergies. Quercetin is a frequently studied phenolic compound due to its known great antioxidant properties. It seems to be one of the most powerful flavonoids for protecting the body against reactive oxygen species [37,38]. Quercetin inhibits the initiation step in chain oxidation and prevents chain propagation. This may also include the termination of a chain by the reaction of two radicals. Endogenous antioxidant ability of quercetin modifies the range of cellular injury during the allergic damage that is caused by free radicals. Enzymes, such as repair and de novo ones (lipases, DNA repair enzymes,

proteases and transferases), act as the third line of defense by repairing damage and reconstituting membranes [38].

Nowadays, attention is focused on immunomodulation and antiinflammatory properties of quercetin, such as stimulation of the immune system, antiviral activity (antiherpes virus type I), inhibition of histamine release, inhibition of nuclear factor activation (NF-κB), proinflammatory cytokines and leukotrienes [38]. Quercetin induces significant gene expression and production of Th-1-derived IFN-γ, as well as down-regulating Th-2-derived IL-4 production by normal peripheral blood mononuclear cells [39]. The antiinflammatory profile of quercetin is known to impact on the recruitment of immune cells to the skin and in preventing the development of secondary infections following disruption of the skin barrier [37].

Quercetin was reported as a long-lasting antiinflammatory substance that possesses strong antiinflammatory capacities. It possesses antiinflammatory potential that can be expressed on different cell types, both in animal and human models. It is known to possess both mast cell stabilizing and gastrointestinal cytoprotective activity. It can also play a modulating, biphasic and regulatory action on inflammation and immunity. Additionally, quercetin has an immunosuppressive effect on dendritic cells function [37].

1.8.2 Anthocyanins, Procyanidins, Epicatechin, Catechin

Anthocyanins, a class of flavonoids and a phenylalanine derivative, are distributed widely in brightly colored fruits including berry fruits, concord grapes, and grape seeds. Anthocyanins account for a variety of different colors in fruits and vegetables and are considered to have powerful antioxidant and antiinflammatory potency [40]. It seems that they inhibit lipid peroxidation and the inflammatory agents such as COX-1 and COX-2. It seems that anthocyanidins have higher ability to scavenge free radicals than anthocyanins, and this activity reduces with increase in the number of sugar molecules [41]. Epigallocatechin-3-gallate (EGCG), the main polyphenolic component in green tea, is capable of scavenging free radicals and protecting against DNA damage. It plays a central role in the antiinflammatory effects of green tea polyphenols [42]. EGCG inhibits the up-regulation of NO synthase by suppressing LPS-induced NFkB activation and inflammation in vitro and in vivo [43,44]. Furthermore, recent studies have shown that Toll interacting protein (Tollip) up-regulation plays an indispensable role in the antiinflammatory effect of EGCG. EGCG up-regulates Tollip via its receptor, 67-kDa laminin receptor (67LR) or by Elf-1 expression [20,45]. Oral EGCG administration significantly suppressed Elf-1 expression levels. EGCG down-regulates the TLR4- independent signal-elicited phosphorylation of MAPK and also down-regulates AP-1 [20]. Given the suppressing effect of EGCG on TLR4-independent inflammation stimuli, there may be several antiinflammation mechanisms. EGCG has been reported to inhibit STAT3, a transcription factor essential for the differentiation of Th17. Modulation of STAT3 suppresses Th17 differentiation and increases Tregs in rheumatoid synovial T cells. According to a study, EGCG suppressed p-STAT3 727 and p-STAT3 705. STAT3 and STAT5 compete for binding to the IL-17 loci, which may explain the reciprocal regulation of STAT3 and STAT5 on IL-17 activation. Because EGCG suppressed the activity of STAT3, the chance of STAT5 binding to the IL-17 gene locus increased, and IL-17 gene and protein expression was inhibited [42].

Cocoa extracts or single flavonoids, both as monomers (epicatechin, catechin) or polymers (procyanidins) have demonstrated in vitro their antiinflammatory potential, although there have been some controversial results [46]. A cocoa flavonoid-enriched extract and the monomers epicatechin and isoquercitrin were able to decrease the production of inflammatory molecules such as TNF-α and MCP-1 by macrophages under stimulation with LPS [47]. Similarly, epicatechinin stimulated whole blood cells culture suppressed the production of IL-6 and IL-8 [48]. Aside from cytokines, other inflammatory molecules can be influenced by cocoa. Epicatechin, procyanidin B1, procyanidin B2, and a cocoa extract reduced NO release by stimulating macrophages. Likewise, the in vitro treatment with cocoa fractions or flavonoids alone decreased the production of ROS from several kinds of cells [49].

Certain flavanols and procyanidins isolated from cocoa moderated some signaling pathways induced by LPS on neutrophils, particularly those of oxidative bursts and activation markers, and cocoa could influence selected apoptosis mechanisms. Regarding the mechanisms of action, it has been reported that hexameric cocoa procyanidins have the capacity to modulate TNF-α-induced NF-κB activation in intestinal epithelial cells [49]. NF-κB is a transcription factor involved in the regulation of genes encoding cytokines (IL-1, IL-2, IL-6, IL-8, TNF-α, among others), adhesion molecules (e.g., ICAM-1, VCAM-1), acute phase proteins, and inducible enzymes.

1.8.3 Chrysin, Apigenin, Luteolin

Oral chrysin significantly reduced myeloperoxidase (MPO) activity as an active biochemical marker of neutrophil infiltration in a murine inflammatory bowel disease (IBD) model [50]. Chrysin has antiinflammatory effects by inhibiting superoxide anion generation and elastase release for neutrophils in human. Chrysin treatment could increase the up-regulation of the M2 marker genes such as CD206, Arg1, and Ym1 and the reduction of the mRNA levels in M1 markers genes such as CCL3 and IL-12b, respectively. In addition, chrysin suppresses M1 phenotype and induces an antiinflammatory M2

phenotype, both in peritoneal macrophages of obese mice and cultured macrophages in vitro study [51]. These activities introduce the chrysin as an antiinflammatory factor by changing the phenotype of the responding macrophage toward M2 phenotype. The studies on the M2 model of macrophages indicated that chrysin increased the transcriptional activation of the mRNA expression of peroxisome proliferator-activated receptor gamma (PPARγ) in macrophages and could increase the expression of the PPARγ dependent genes CD36, Arg1 in primary macrophages. In another study, the effect of flavonoids on macrophage physiology revealed that most of the flavonoids reduced macrophage M-CSF-induced proliferation without affecting cellular viability [51].

Some flavones such as chrysin, luteolin, apigenin, and baicalein can inhibit eosinophilic inflammatory reactions in human asthma. Also, histological studies showed that crysin could decrease the infiltration of the inflammatory cells in the airway. Chrysin showed dose-dependent effects on eosinophils, neutrophils, macrophages, lymphocytes, and monocytes and also reduced the total number of inflammatory cells in the bronchoalveolar lavage fluid (BALF) [52]. The flavones, apigenin, luteolin, and chrysin, with a double bond at C2–3 with an H group at R3 are capable of inhibiting any of the proinflammatory cytokines. Whereas, naringenin, kaempferol, morin, and quercetin lacking those features and unable to inhibit proinflammatory cytokines [53].

Major antiinflammatory cytokines include IL-1Ra, IL-4, IL-10, IL-11, IL-13, and TGF-β. These cytokines can suppress the production of proinflammatory cytokines. Some specific cytokine receptors for IL-1, TNF-α, and IL-18 also function as proinflammatory cytokine inhibitors. Blocking IL-1 or TNF has been highly successful in patients with rheumatoid arthritis (RA), IBD, or graft versus host disease (GVHD). For antiinflammatory effects of flavonoids, apigenin and its structural analogues such as chrysin and luteolin evaluated their capacity to inhibit the production of proinflammatory cytokines by LPS-stimulated human peripheral blood mononuclear cells (PBMC) [53]. IL-4 and IL-10 have marked inhibitory effects on the expression and release of proinflammatory cytokines. IL-10 can inhibit I-kB kinase (IKK) activity and block DNA binding of NF-kB, thus blocking NF-kB nuclear translocation.

Alternatively, MAPK pathway is another signal transduction. Three main MAPK subfamily members are extracellular signal-regulated kinase (ERK), p38 MAPK, and JNK. ERK, p38 JNK as three MAPK activities, c-fos and c-jun mRNA expression and the AP-1 transcriptional activity were inhibited by apigenin and luteolin, but kaempferol and chrysin only inhibited JNK activity [54]. Some proinflammatory cytokines such as the IL-1, IL-18, and TNF family can activate NF-kB and MAPK signaling pathways. Antiinflammatory effects of several flavonoids have been related to the suppression of the NF-kB signal transduction pathway. It has been clearly demonstrated that chrysin exerted potent properties by acting as an antagonist of NF-kB, which down-regulated the production of iNOS and COX-2. Chrysin could decrease the NF-kB p65 unit and TNF-α level. Chrysin acted as an antagonist of NF-kB and down-regulated the production of iNOS and COX-2. The iNOS expression is responsible for the prolonged production of NO that mediates the tumoricidal and bactericidal functions of activated macrophages [54].

The antioxidant properties of flavonoids have been associated with their ability to scavenge free radicals and inhibit the production of protein oxidation and lipid peroxidation products. Many investigations have reported the antiinflammatory functions of different flavonoid molecules such as suppressing inflammatory cytokines, modulating transcription factors, and inflammation-related pathways, and reducing accumulation of NO or ROS [55]. Glabridin (GB) is a species-specific isoflavan from *Glycurrhiza glabra* L. roots. It is reported that its biological activities include antimicrobial, antiinflammatory, and cardiovascular protection. GB was found to attenuate LPS-induced production of inflammatory mediators, including NO, TNF-α, and IL-1β in THP-1 cells, RAW 264.7 cells, and J774a.1 cells. GB inhibited dendritic cell maturation by blocking NF-κB and MAPK signaling [56].

Flavonoids are known to inhibit histamine release from human basophils and murine mast cells. Flavonoids inhibit the release of chemical mediators, and further suppress interleukin (IL)-4 and IL-13 synthesis (Th2 type cytokines) by allergen or anti-IgE antibody-stimulated receptor-expressing cells (e.g., peripheral blood basophils or mast cells). They can also affect the differentiation of naïve glycoprotein CD4 positive T cells due to the inhibitory effect on the activation of the aryl hydrocarbon receptor. The inhibitory activity of flavonoids on IL-4 and CD40 ligand expression is probably related through their inhibitory action on activation of nuclear factors of activated T cells and AP-1 [57]. Flavonols extracted from plants inhibit histamine and some cytokines release from rodent basophils and mast cells. Basophils are more responsible for this balance than tissue mast cells, so they could be considered potent natural substances for allergy cure.

Polyphenols are considered effective antiallergy agents capable of influencing multiple biological pathways and immune cell functions in the allergic immune response. Among the most investigated plant-derived polyphenolic compounds (flavonoids), quercetin, together with resveratrol, EGCG, and genistein, have exhibited potent effects on cellular and humoral immune functions in preclinical investigations. The interaction of polyphenols with proteins can modulate the process of allergic sensitization and their direct effect on allergic effector cells such as mast

cells inhibit mediator release, resulting in the alleviation of symptoms. Polyphenols inhibit histamine release from human basophils and murine mast cells. Intake of polyphenols such as flavones, flavone-3-ols, catechins, anthocyanidins, flavanones, procyanidins, and resveratrol can improve a skewed balance of T-helper (Th) type 1 and 2 cells (Th1/Th2) and suppress antigen-specific IgE (Immunoglobulin E) antibody formation [57].

Epidemiological studies suggest that a high intake of fruits and vegetables rich in polyphenols may be associated with a decreased risk of a range of human chronic disorders including cardiovascular disease, inflammatory and metabolic diseases, neurodegenerative diseases, and some cancers.

1.8.4 Curcumin

Curcumin is a polyphenolic dietary phytochemical, and the bioactive pigment present in the roots of *Curcuma longa* L. (turmeric). Turmeric and its chemical constituents have been used in traditional medicine and Asian cooking as a food color and food additive for thousands of years. Curcumin shows a wide range of pharmacological activities, including antioxidant, chemopreventive, proapoptotic, antiinflammatory, antiischemic, hepatoprotective, antimicrobial, and chemotherapeutic [12]. Furthermore, there is accumulating scientific evidence suggesting the immunomodulatory potential of curcumin. Curcumin has been shown to improve systemic markers of oxidative stress. There is evidence that it can increase serum activities of antioxidants, such as superoxide dismutase (SOD). The effect of curcumin on free radicals is carried out by several different mechanisms. It can scavenge different forms of free radicals, such as reactive oxygen and nitrogen species (ROS and RNS, respectively); it can modulate the activity of glutation (GSH), catalase, and SOD enzymes active in the neutralization of free radicals; also, it can inhibit ROS-generating enzymes such as lipoxygenase/cyclooxygenase and xanthine hydrogenase/oxidase. In addition, curcumin is a lipophilic compound, which makes it an efficient scavenger of peroxyl radicals; therefore, like vitamin E, curcumin is also considered a chain-breaking antioxidant [13].

Curcumin has been found to modulate the activation of T cells, B cells, macrophages, neutrophils, NK cells, and dendritic cells, as well as the secretion of immune cytokines in the normal body. A variety of pharmacological activities of curcumin stem from its ability to interact with different biological targets and signaling pathways. Some studies suggest that the immunomodulatory activity of curcumin may involve direct targeting (activation) of TLRs (such as TLR4: a receptor of LPS) by PAMPs [13]. Other mechanisms underlying curcumin modulation of immune responses are attributed to the regulation of various transcription factors such as NF-κB, AP-1, STAT, and also their downstream signaling pathways. NF-κB exerts a key role in producing proinflammatory cytokines such as IL-1, IL-2, and interferon-γ (IFNγ) in T-cells [13]. Pleiotropic effects of curcumin (such as inhibition of IL-1 production) are believed to stem from suppression of NF-κB activity via inhibition of I kappa B kinase a (IKK-a) phosphorylation and prevention of nuclear translocation of the NF-κB p65 subunit [13]. Curcumin induces the polarization of M2 macrophages through inducing secretion IL-4 and/or IL-13, and a STAT6- dependent pathway. Curcumin has emerged as a new antiinflammatory and immunomodulatory agent with clinical potential. Additional understanding of the molecular regulation by which curcumin exert its effects in several inflammatory diseases will be important for exploring potential new therapies to ameliorate inflammatory disorders [13].

B Lymphocyte Stimulator (BLYS) is an important cytokine for B cell proliferation and autoantibody secretion in autoimmune diseases. Curcumin has the potential to serve as a successful therapy for autoimmune disorders such as systemic lupus erythematosus (SLE) and RA by targeting BLYS. The inhibitory effect of curcumin on BLYS expression is due to reduction of NF-κB activity by inhibiting DNA binding of NF-κB and the nuclear translocation of p65 [58].

Curcumin has been found to inhibit the maturation of DCs and to reduce costimulatory molecules (CD80 and CD86), MHC class II, and CD40 expression on the surfaces of DCs in a dose-dependent manner [13]. The curcumin-treated DCs have a high capacity at antigen capture through mannose receptor mediated endocytosis. The maturation inhibition by curcumin is similar to the activity of corticosteroids, IL-10, TGF-β, cyclosporine, 1,25-dihydroxyvitamin D3, and aspirin. Human DCs expressing indoleamine 2,3-dioxygenase (IDO) are capable of maturation. Curcumin has been found to suppress maturation of DCs by inhibition of IDO expression through a COX-2/prostaglandin E2 (PGE2) dependent pathway [13]. Adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1; CD54) are pivotal in regulating cellular adhesion and T cell responses [13]. CD11c, a protein belonging to the integrin family, is also an important regulator of cell adhesion, and is highly expressed on DCs. Curcumin significantly reduces the expression of both markers mentioned previously on the DC surface. The reduced CD11c could be the result of curcumin-induced AP-1 inhibition [59]. Curcumin attenuates LPS-stimulated expression and secretion of macrophage inflammatory protein-2 (MIP-2), IL-1b, keratinocyte chemoattractant (KC), IL-8 (mediates chemotaxis of human neutrophils), and MIP-1a in neutrophils and macrophages. It also reduces the levels of iNOS, IFN-γ, and IL-12 through inhibition of the NF-κB signaling pathway in monocytes/macrophages. In several studies, a significant increase in macrophage phagocytic

activity in the presence of curcumin has also been observed [13]. These studies suggest the potential benefits of curcumin for reducing oxidative and lipid-mediated damages in macrophages. Furthermore, pretreatment with curcumin was reported to significantly inhibit IL-12 production by down-regulating NF-κB in LPS-stimulated macrophages, which resulted in inhibition of IFN-γ production and enhancement of IL-4 production by CD4+ T cells [13].

The effects of curcumin on B cell activity have been investigated in several studies. Curcumin can decrease antibody production (IgG2a, IgE, and IgG1, and particularly IgG1) in response to LPS by rat splenocytes [60]. The TLRs signaling leading to NF-κB activation can involve B cell activation. Curcumin can also inhibit NF-κB activation through inhibiting the TLR signaling pathway [13]. Curcumin has also been reported to enhance intestinal immune function in high-fat fed animals by increasing IgA production or suppression of IgA degradation [61]. These observations suggest the potential effects of curcumin in the treatment of B cell-mediated autoimmune diseases. The immunomodulatory effect of curcumin on CD8+ and CD4+ T cell subsets has been frequently found in previous studies. Curcumin can inhibit the production of the Th1 cytokine profile in CD4+ T cells by suppressing IL-12 production in macrophages; therefore curcumin may possess a possible therapeutic effect on Th1-mediated immune diseases [13]. Some evidence indicates that curcumin inhibits differentiation and development of Th17 cells by down-regulating the expression of IL-6, IL-21, IL-17, and RORγt signaling, as well as inhibiting STAT3 phosphorylation [62]. It was reported that treatment of DCs with curcumin may induce development of FoxP3+ Treg cells [63]. A key mechanism for the modulatory effects of curcumin on proinflammatory cytokines is the suppression of NF-κB. Upon binding of IL-1 to IL-1 Receptor-I (IL-1RI), NF-κB is activated via various signaling pathways, such as activation of several mediators, for example IRAK, MyD88, and Tollip [64]. Curcumin blocks IL-1-mediated recruitment of IL-1 receptor-associated kinase (IRAK) to the IL-1RI in murine Thymoma EL-4 cells and thereby inhibits NF-κB activation [65]. Curcumin has been found to reduce serum levels of various inflammatory mediators such as cytokines, chemokines, and surface receptors including IL-1β, IL-6, soluble CD40 ligand, IL-8, MIP-1, MCP-1, TNF-α, adhesion molecules, C-Reactive Protein (CRP), CXCR-4, PGE2, and soluble vascular cell adhesion molecule-1(sVCAM-1), as well as the erythrocyte sedimentation rate (ESR) [13].

1.8.5 Phenolic Acids

Phenolic acids possess antioxidant activity due to the hydroxylated aromatic rings, which are capable of donating hydrogen atoms and quenching singlet oxygen. Some phenolic acids are capable of inhibiting transcriptional activity of AP-1, which is an activator protein that has been linked to the control of inflammation, cell differentiation, and proliferation. Specifically, caffeic acid has been discovered to be a selective blocker of the biosynthesis of leukotrienes, components involved in immunoregulation of diseases, asthma, and allergic reactions.

Phenolic acids, in particular hydroxy-cinnamic acid derivatives, have attracted considerable attention because of their broad biological and pharmacological effects, at least in part, due to their antioxidant effects and a suggested efficacy in the treatment of pathologies such as cardiovascular diseases, cancer, and inflammatory disorders. Moreover, many phenolic compounds have been reported to modulate the immune system via suppression of mitogen-activated protein kinases and NF-kB signaling pathways. p-Coumaric acid blocks NF-kB and MAPKs signaling pathways by inhibiting LPS-induced inflammatory cytokines [66]. Hence, p-coumaric acid has the potential of being used as an immunosuppressive agent in treating autoimmune inflammatory diseases like RA. Other studies showed that caffeic acid decreased the inflammatory cytokines and reduced the induction of the inflammatory pathway, c-jun-N-terminal kinase, NFκB, and COX-2 expression. Thus, phenolic acids such as caffeic acid, gallic acid, p-coumaric acids, and ferulic acid showed a remarkable antioxidant effect, such as glutathione peroxidase (GPx), SOD, and catalase, as well as antimicrobial activity. In addition, chicoric acid, a derivative of both caffeic acid and tartaric acid, which is the main phenolic compound found in *Echinacea purpurea*, has been shown to have immunostimulatory properties, to promote phagocyte activity in vitro and in vivo, and to inhibit hyaluronidase, a key enzyme involved in bacterial infection [66].

1.8.6 Polyphenols Modulate Epigenetic Mechanism

Several studies have shown that polyphenols are able to modulate epigenetic mechanisms including either DNA methylation or histone modifications. In this regard, a number of natural compounds have been identified as histone deacetylase (HDAC) inhibitors (EGCG, curcumin, genistein, quercetin), histone acetyltranferase (HAT) activators (genistein), HAT inhibitors (EGCG, curcumin), silent information regulator (SIRT) activator (resveratrol), or SIRT inhibitor (genistein) [67].

The modulation of epigenetic mechanisms by polyphenols has been reported showing an inhibitory effect of EGCG on DNA methyltranferase-1 (DNMT1) together with transcriptional reactivation of suppressed genes. This inhibitory effect could be determined by a direct interaction between EGCG and DNMT1 in according to in silico molecular modeling studies [68]. Later studies described two mechanisms of DNMT1 regulation. Catechol-containing polyphenols showed an inhibitory

effect by S-adenosylhomocysteine (SAH) production derived from its own methylation process, using S-adenosylmethionine (SAM) as methyl donor [69,70]. This process promotes SAH accumulation, acting as a non-competitive inhibitor of DNMTs. Curcumin showed a similar inhibiting effect, probably by a covalent interaction. In relation to the histone modulation by polyphenols, it was shown that EGCG is able to induce reexpression of the silenced tumor suppressor genes, p16INK4a and Cip1/p21, by partial inhibition of HDAC activity and increased acetylation of lysines 9 and 14 on H3 histone (H3-K9 and 14) and acetylated lysine 5, 12 and 16 on H4 histone, and also to decrease the levels of methylated H3-Lys 9. Moreover, curcumin inhibited HAT activity by inducing proteasome-dependent degradation of p300 in cancer cells and also inhibiting the expression of p300, HDAC1, HDAC3, and HDAC8 proteins in Raji cells, modulating the NFκB signaling pathway. Furthermore, quercetin induced HAT activation and HDAC inhibition in HL60 leukemia cells promoting increased histone H3 acetylation and inducing FasL-related apoptosis [67].

1.8.7 Polyphenols and Immunonutrition

Immunonutrition can be defined as modulation of either the activity of the immune system, or modulation of the consequences of activation of the immune system by nutrients or specific food items fed in amounts above those normally encountered in the diet. Immunonutrients are nutrients that have an effect on the immune system. There are many nutrients covered in this definition, including various essential and nonessential amino acids as the macronutrients, some vitamins and trace elements as the micronutrients, and also prebiotics and probiotics as well as microbial nucleosides in medical practice.

Over two decades ago, a class of compounds (termed prebiotics) were recognized for their ability to manipulate host microbiota to the benefit of the host. At that time fructans (fructooligosaccharides (FOS) and inulin) and galactans (galactooligosaccharides or GOS) fit that category, with their effects acting through enrichment of *Lactobacillus* and/or *Bifidobacterium* spp. FOS and GOS currently dominate the prebiotic category as evidenced by numerous studies on their prebiotic effects. Today, the prebiotic concept has expanded, in part because of advances in tools for microbiome research (for example, high-throughput sequencing), which have improved our knowledge of the composition of the microbiota and enabled identification of additional substances influencing colonization. Currently established prebiotics are carbohydrate-based, but other substances such as polyphenols and polyunsaturated fatty acids converted to respective conjugated fatty acids might fit the updated definition, assuming convincing weight of evidence in the target host [71].

2 CONCLUSION

Plant polyphenols are a major group of phytochemicals and there are many studies on their effects on the immune system. Some of these are limited to animal experiments or cell culture studies. Some are clinical and even meta-analytic studies. Polyphenols have been demonstrated to modulate the inflammatory process and stimulators via several individual and synergistic mechanisms. They can alter signaling and enzymatic processes involved in inflammation such as tyrosine and serine-threonine protein kinases, which have been known to be involved in B-lymphocyte activation and T-cell proliferation. They have also been known to inhibit the key inflammatory mediator, NFκB, iNOS, proinflammatory enzymes such as COX-2, MAPK and protein kinase-C. They exhibit mostly a blunting effect on inflammatory cytokines and augment antiinflammatory cytokines. Polyphenols protect oxidative stress by scavenging free radicals and inflammatory prooxidants such as superoxide anions and hydrogen peroxide. The epigenetic importance of polyphenols has been extensively studied. Since 2017, the prebiotic properties of polyphenols have been officially accepted. The capacity exists for these "magic molecules" to be used therapeutically in the management of diseases, inflammatory in particular, and to promote health preventively.

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