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Meir Djaldetti*

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Review article

Sulforaphane – a Potent Immunomodulator, Widely Available, Yet Underutilized

Meir Djaldetti*

Laboratory for Immunology and Hematology Research, Rabin Medical Center, Hasharon Hospital, Petah-Tiqva, School of Medicine, Tel-Aviv University, Ramat Aviv, Israel.

***Corresponding Author:** Meir Djaldetti. Laboratory for Immunology and Hematology Research, Rabin Medical Center, Hasharon Hospital, Petah-Tiqva, School of Medicine, Tel-Aviv University, Ramat Aviv, Israel.

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Abstract

Vegetables play a crucial role as an important source of nutrients in global culinary traditions. Their rich content of vitamins, minerals and polyphenols makes them valuable contributors to overall human health. Broccoli, a member of the Brassica oleracea family is widely recognized for its diverse culinary uses and its beneficial impact on various health conditions, such as acute and chronic inflammation, cardiovascular diseases, diabetes and cancer. Broccoli's health-promoting qualities stem from its high polyphenol content, of which sulforaphane (SFN) is the most notable. Due to its strong antioxidant properties and ability to increase the activity of peripheral blood mononuclear cells, SFN plays a significant role in immune system modulation. It plays a catalytic role in the production of inflammatory cytokines by monocytes and polarization from M1 to M2 macrophages. Acting on lymphocytes, which are integral part of the adaptive immune system, SFN triggers the production of antibodies and cytokines, while enhancing the generation of natural killer cells. Based on in vitro and animal research it appears that there is substantial number of studies to explain the ways SFN modulates the function of the immune cells. However, although the results indicate that we have an easily available therapeutic tool for a number of serious diseases, more research is needed to elucidate the effect of SFN in humans. It is the aim of the present review to summarize the knowledge about the immune properties of SFN with expectation that the content will serve as a challenge for further research

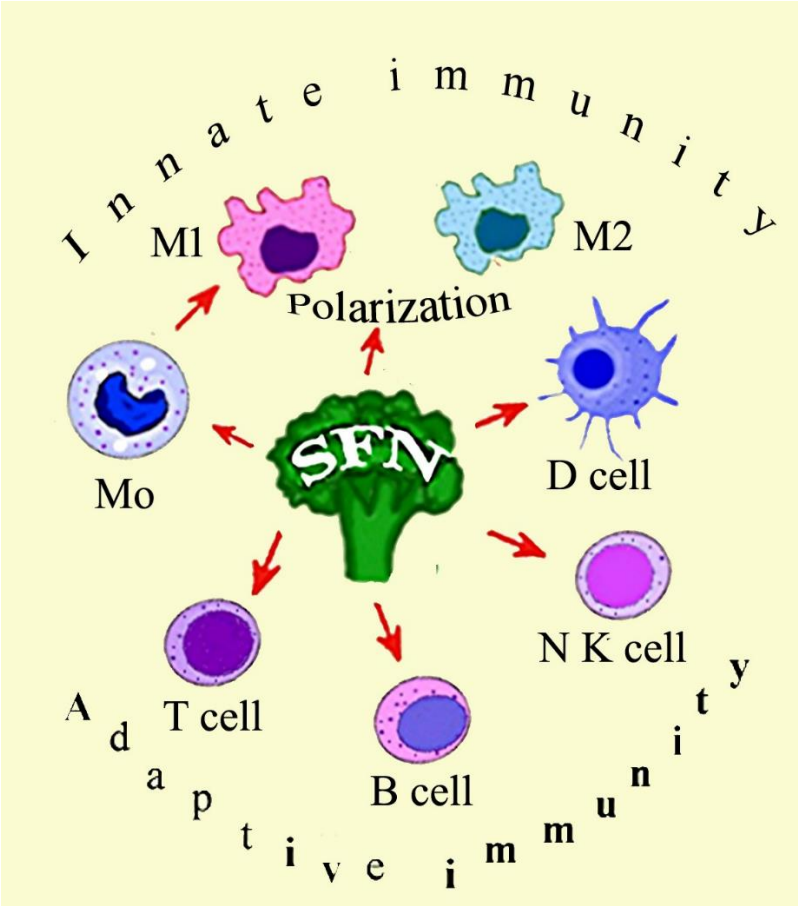
Key words: sulforaphane; mononuclear cells; cytokines; immunomodulation; immune response

Introduction

Broccoli is a member of the Brassica oleracea family and has a lengthy history. Archeological evidence and seeds' examinations imply that the countries in the Mediterranean region were the first to cultivate broccoli [1]. Its widespread in agriculture is due both to its nutritional value being a rich source of vitamins C and K, as well as most of the B group and folate [2]. Broccoli exerts beneficial activities against a wide range of illnesses such as cardiovascular and gastrointestinal diseases, diabetes, autism, rheumatoid arthritis, inflammatory and neurodegenerative conditions and a many types of cancers [3- 6]. In actuality, broccoli's therapeutic properties stem from its thiocyanates, the most potent being sulforaphane (SFN) converted from glucoraphanin by myrosinase. It is ample documentation of SFN cytoprotective, antioxidant, anti-inflammatory and carcinopreventive effects [7]. Although SFN is highly concentrated in broccoli and its sprouts, it is also found in other cruciferous vegetables such as cauliflower and cabbage. In consumers of cruciferous vegetables SFN was able to suppress the production of pro-inflammatory cytokines by modulation of the toll-like receptor 4 (TLR4) [8]. The bioavailability, pharmacokinetics, dosage and toxicity of SFN have been extensively reviewed by Yagishita et al. [9]. The authors concluded that SFN is quickly absorbed with approximately 70% to 90% being eliminated through urine. However, SFN is not stable and its degradation is influenced by gastric acidity, temperature and enzyme activities [10-11].The pharmacological assets of sulforaphane have been recently evaluated by Baralik et al. [12]. Research has conclusively established the connection between chronic inflammation and the immunomodulatory potential of SFN, as well as other dietary phytochemicals to control the function of the antigen-presenting cells (APC) that include monocytes/macrophages, dendritic cells and lymphocytes [13], a process proceeding through several signaling pathways. In this sense the capacity of SFN to restrain the activity of the toll-like receptors (TLRs) located on the peripheral blood mononuclear cells (PBMC) and to inhibit the release of inflammatory mediators like TNFα and IL-1β plays an important role in preventing inflammation [14-15]. SFN is a potent activator of the of the nuclear erythrocyte relation factor (Nrf2) pathway which inhibits the upregulated overexpression of inflammatory process [16-18]. In a model of autoimmune encephalomyelitis in mice, animals treated with sulforaphane exhibited reduced demyelinating damage and improved clinical ratings in comparison to the control group [19]. In a mouse model of lipopolysaccharide (LPS) induced mastitis, SFN suppressed the

inflammatory response by downregulating the production of pro-inflammatory factors such as TNF-α, IL-1β, IL-6, COX2 and nuclear factor kappa-B (NF-kB) [20]. Researchers have long been intrigued by the ways in which SFN modulates the immune system. Immune cells, including monocytes, macrophages, dendritic cells, and those of the lymphocyte family, are the primary targets of its immuno-promoting activity [13]. Regrettably, the in vitro research on this topic is much more advanced than the clinical studies. This review aims to evaluate the current data supporting the immunomodulatory effects of SFN on the PBMC by its ability to control their cytokine and antibody production and to activate pathways involved in the course of the inflammatory process.

The impact of sulforaphane on the mononuclear family members (Figure1)



Legend to Figure1: Effect of sulforaphane on cells of innate and adaptive immunity. M1-classicaly activated macrophages producing pro-inflammatory cytokines. M2- alternative activated macrophages producing anti-inflammatory cytokines. Mo-monocytes. D cell-dendritic cell. N K cell-natural killer cell.

Monocytes

Monocytes play a crucial role in the immune system by producing cytokines, antibodies, and carrying out phagocytosis [21]. They are key players of the immune system and are actively involved in both acute and chronic inflammation. In cases of inflammation, researchers have focused on the ability of monocytes to differentiate into macrophages and their transition from classically activated M1 macrophages to alternatively activated M2, related to their ability to produce pro- and anti-inflammatory cytokines respectively [22]. At non-toxic concentrations of 10 μM SFN impeded the transition of M2 to M1 macrophages, thus inhibiting inflammatory markers' expression such as COX2, the inducible nitric oxide synthase (iNOS), CD14, CD197 and the regulator of innate immune response IL-12p70. Furthermore, mitogen-activated protein kinases (MAPKs), which regulate various cell processes, including cell differentiation, were found to be implicated in the process of macrophage polarization. [23]. When human monocytes were treated with non-toxic concentrations of SFN it inhibited the release of pro-inflammatory cytokines IL-1β, IL-6, TNFα, and NF-kB as well as the signaling pathways (MAPK) [24]. It also inhibited the heme oxygenase inhibitor (HO), and the macrophage-activating lipopeptide 2 (MALP-2) which promote pro-inflammatory cytokine production and NF-kB activation, while increasing Nrf2 expression [25]. In the presence of SFN at concentrations of 10 and 50 μM human PBMC showed a reduced production of the pro-inflammatory cytokines IL-6 and IL-1β and an increased differentiation towards dendritic cells [26].

Macrophages

Circulating monocytes that enter the tissues differentiate into macrophages and play a dominant role in innate immune responses by functioning as phagocytes and producing cytokines. When LPS stimulated Raw 264.7 macrophages were incubated with SFN at concentrations ranging between 0-50 μM, three was a significant inhibition of pro-inflammatory cytokines and nitric oxide (NO) expression though modulation of the inflammatory enzyme iNOS and activation of Nrf2/HO-1 transduction pathway [27]. In a mouse model and in vitro hyperoxia induced macrophage dysfunction SFN was able inhibit the oxidative stress by inducing Nrf2 generation and reducing the high mobility group box 1 protein (HNGB1) [28]. Furthermore, exposure of human macrophages to 25μM of SFN for 24 hours downregulated the production of inflammatory cytokines [29]. Qin et al. [30] discovered that administering of 50 mg/kg SFN to mice after hemorrhagic shock resulted in reduced inflammatory cytokine secretion by their splenic macrophages, along with increased Nrf2 activation, indicating a shift from M1 to M2 macrophage polarization. Treatment with 5 or 10 μM of SFN preserved the

mitochondrial activity and maintained a normal tricarboxylic acid (TCA) cycle in bone marrow-derived M1 macrophages stimulated with LPS, 25 ng/ml [31]. Macrophages play a pivotal role in chronic inflammation linked to obesity. Administering 0.5 mg/kg of SFN to obese mice for six weeks, and treating RAW 264.7 LPS-stimulated macrophages with 2.5-5 μM of the polyphenol, resulted in the inhibition of iNOS, COX-2 expression, NO, TNFα, IL-1β, and IL-6 production [32]. Mouse bone marrow-derived M1 and M2 macrophages stimulated with LPS and IL-4, and IL-12 respectively treated with 10 μM of SFN, showed a shift towards M2 macrophages with a decrease of IL-12, IL-6 and TNFα secretion, as well as a simultaneous increase of low ability IgFc receptors (CS16/32) percentage in M-1 cells. The levels of heme oxidase (HO-10) and Nrf2 were also elevated. Conversely, SFN raised the levels of a few cytolytic enzymes such as YM1, Fizz1 and Arg1 in M2 macrophages [33]. On mouse LPS-stimulated peritoneal macrophages, it has been demonstrated that the SFN enantiomer (R)-S induces an immunomodulatory impact manifested by suppression of pro-inflammatory cytokines and enzymes, along with ROS species production. On the other hand Nrf-2/HO axis was activated whereas the inflammasome signaling pathways were inhibited [34]. It's interesting to note that inflammasome gene expression was inhibited in adipose tissue macrophages (ATP) exclusively, but it was reduced when 40 μM of SFN, TNFα and IL-1β were incubated with macrophages derived from healthy individuals and palmitic acid-stimulated ATP [35]. Notably, several members of the Brassica oleracea family are SFN and polysaccharides producers that have the potential to immunomodulate macrophage function. Thus, Sim et al. [36] have found that sprout extract from purple head broccoli (Brassica oleracea L., var. italica) contains more SFN compared to other species of the same family and when applied to LPS-stimulated RAW 264.7 macrophages, it significantly decreased the production of the pro-inflammatory cytokines IL-1β, IL-6, IL-1, TNFα and INOS.

Dendritic cells

Dendritic cells, together with macrophages, are an essential part of the innate immune system [37]. Furthermore, as antigen-presenting cells being specialized in activating the elements of the adaptive system they are actively involved in inflammatory responses and cancer cell development [38]. Their origin, development and relation to T cell immune responses have been reviewed by Puhr et al. [39]. Similar to macrophages and monocytes, dendritic cells react quickly to SFN. By reducing the expression of the activating markers CD80, CD83 and CD86, that are potent regulators of the T cells immune function, SFN restrains Th2 proliferation and decreases the secretion of the pro-inflammatory cytokines IL-9 and IL-12 [40].

Mouse bone marrow derived dendritic cells exposed to 0.3 μM of SFN showed inhibited TLR4-induced IL-12 and IL-23 cytokine generation by regulating the activity of TLR4-induced NF-kB factor. In addition, there was a significant hindrance to the development of Th1 and Th17 cells [41]. Similarly, SFN-treated porcine monocyte-derived dendritic cells demonstrated a controlled activity of TLR4-induced NF-kB transcription factor and suppressed histone deacetylases (HDACs) 6 and 10 [42,43]. Additional mechanisms by which SFN stimulates dendritic cells to increase T-cell activation include modulation of the regulatory proteins Janus kinase/transcription activator 3 (JAK/STAT3) leading to downregulation of the anti-apoptotic receptor B1-H1 and microRNA-signaling. Furthermore, SFN improved the activity of the dendritic cells by affecting their co-stimulatory molecules CD80 and CD83 [37]. Notably, as demonstrated in mice with induced colitis, broccoli-derived nanoparticles may also have an anti-inflammatory impact by activating dendritic cell adenosine monophosphate-activated protein kinase (AMPK) [44]. Results on the function of SFN on the immune activity of dendritic cells indicate that there is a challenge to mobilize them in the prevention of inflammatory processes and the immune defense.

The effect of SFN on lymphocytes

Being members of the antigen-presenting cells (APC), lymphocytes play a crucial role in the adaptive immune response. According to Checker et al. [45] treating mitogen stimulated T and B cells with SFN reduced their proliferation and upregulation as well as their ability to produce IL-2, IL-4, IL-6 and IFNγ cytokines. Furthermore, SFN stimulated the production of phosphoinositide kinase/protein kinase/glycogen synthase kinase 3β (PI3K/Akt/GSK-3β) expression leading to Nrf-2 activation. Human T cells are readily affected by SFN expressed by an increase in ROS species, suppression of the transcription factor RORyt and the T helper 17 (Th17) cells that are specialized in IL-17 and IL-22 secretion [46]. Mice with induced airway inflammation showed down-regulation of Th17 capacity to secrete pro-inflammatory cytokines overturned by Nrf2 activation after treatment with SFN. In addition, the corticosteroid resistance was inverted [47]. Activation of Nrf2 was the main cause for the beneficial effect of SFN when it was used in vitro and when given intragastric to mice with trichloroethylene-mediated necrotizing enterocolitis (NEC). At a dose of 20 mg/kg., it improved the colitis and the survival rate of the animals [48]. In another study with NEC mice the reduced the proportion of the helper Th17 to regulatory T cells (Th17/Treg cells' ratio) and the levels of TLR4 and NF-kB, TNFα and IL-6 were decreased after treatment with SFN. Additionally, by controlling the PI3K/Akt/GSK-3β signaling pathway, both in vivo and in vitro, SFN reduced apoptosis [49].

The Th17/Treg cells' ratio was observed to be downregulated in mice with B hepatitis following administration of 100 μM of SFN for 4 weeks [50] and in BTBR mice with idiopathic autism-like symptoms [51]. Notably it was found that SFN lessens the toxicity caused by cadmium. Compared to 69% cell death induced by cadmium alone, treating lymphocytes and monocytes damaged by IC35 of cadmium with 100 μM of SFN restored 17–20% of cell viability [52].

B cells upon specific activation are able to produce antibodies and to secrete cytokines, though the cells themselves are impacted by a number of interleukins such as IL-7, IL-4, IL-6, IL-10 and the interferons IFN-α, IFN-β, and IFN-γ [53]. SFN, being a potent Nrf2 activator and an inhibitor of NF-kB activity may suppress B cells from producing pro-inflammatory cytokines [54-56].

The designation of the natural killer cells (NKC), active auxiliary members of the innate immune defense, suggests that they possess the capacity to kill other cells, particularly those related with cancer. Their role in biology and therapy has been thoroughly reviewed by Wu et al. [57]. When activated they secret both pro- and anti-inflammatory cytokines, such as IFN-γ, TNF-α, IL-10, IL-5, and IL-13, which act in the proceeding of inflammation and its control [58]. L-SFN incubated with PBMC reduced the secretion of pro-inflammatory cytokines and the proportion of NKC with a shift in the number of T, B, and dendritic cells [26]. In both control and Ehrlich ascites tumor-bearing mice, NKC cells responded with increased activity when treated with SFN. Furthermore, the number of spleen, thymus, and bone marrow cells rose along with the generation of IL-2 and IFN-γ [59]. Notably, most of the studies are focused on the effect of SFN in the role of NKC in chemoprevention, although they are an important factor in the process of inflammation.

Based on the experiments by which SFN modulates the responses of the immune cells, it is evident that the polyphenol exerts its activity by several ways. In this regard Nrf2 plays a critical role. Previous research has demonstrated that SFN increases Th1 cell activity, which declines with age [60]. Due its Nrf2 anti-oxidant effect, mice with induced hyperoxia-induced pulmonary injury showed improvement after treatment with SFN [61], which in addition restored mitochondrial dysfunction allied with airway disorders [62]. In SH-SYSY neuroblastoma cells, SFN inhibited the inflammatory events caused by hydrogen peroxide (HO) as demonstrated by a decrease COX-2 and in pro-inflammatory cytokine production, which was mediated by the Nrf2/HO-1 pathway [63]. In mice with rheumatoid arthritis, activation of Nrf2 by SFN resulted in a considerable improvement in the inflammatory changes in the joints concomitantly with a reduced production of IL-6, IL-17, and TNFα, both in the joints' fluid and their secretion by PBMC [54]. The SFN's ability to activate Nrf2 has been beneficial in treating morbid conditions in addition to reducing inflammatory responses [12].

Dosage and side effects

Table 1 outlines the range of SFN doses applied to immune cells in vitro or in animal models. According to Yagishita et al. [9] the median effective dose of SFN given orally in animal models is 175 µMol/kg, and 113 µM/kg via intraperitoneal injection. Sestilli at al. [64] referenced earlier research that a 50 µmol oral dose of SFN corresponds to a peak plasma concentration of roughly 20 µM, while following dietary consumption in humans, SFN levels are approximately 3 µM.

Type of immune cells	SFN concentration	Effects	Ref.
Human monocytes	10 µM	Inhibits COX-2, iNOS, regulates MAPK, induces M1-M2 polarization	23
Human monocytes	Non-toxic	Suppresses IL-1β, IL-6, TNFα, NF-kB, MAPK	24
Monocytes and dendritic cells	10 and 50 µM	Suppress IL-6 and IL-1β production, promotes differentiation to dendritic cells	26
Human macrophages	25 µM for 24 hrs	Prevents inflammatory cytokine secretion	29
Murine splenic macrophages	50 mg/kg. intraperitoneal.	Decreases inflammatory cytokine secretion, activates Nrf2 and prompts the M1 to M2 polarization	30
BM-derived macrophages	5 and 10 µM	Preserves TCA cycle and mitochondrial activity	31
Obese mice and Raw 264.7 macrophages	0.5 mg/kg and 2.5-5 µM	Attenuates iNOS, COX-3,NO, TNFα, IL-1β, IL-6 production	32
Murine BM derived macrophages	10 µM	Inhibits inflammatory cytokines, Increases Nrf2, NQO1 and HO-1	33
Human dendritic cells	10-30 µM	Modulates JAK/STAT3 and microRNA	37
Mouse BM-derived dendritic cells	0.3 µM	Inhibits IL-12 and IL-23 production and Th cell development	41
Porcine dendritic cells	5-50 µM	Inhibits HDAC6, HDAC10, regulates TLR4 induced NF-kb activity	42
Th17 and Treg cells in NEC mice	20 mg/kg	Improves survival, reduces Th17/Treg cells' ratio, regulates PI3K/Akt/GSK-β	49
Th17/Treg cells' ratio in mice with hepatitis B	100 µM for 4 weeks	Increases the number of Th17, decreases Treg cells	50
Nrf2 deficient mice with HILI	9 µM for 1,3,5 days	Nrf2 activation, improveslung damage	62
SH-SYSY neuroblastoma cells	Pretreatment with 5 µM	Attenuates H_2O_2 induces damage through Nrf2/ HO-1 pathway	63

Table 1. Dosage and effect of sulf0raphane (SFN) on the immune cells.

HILI - Hyperoxia Induced Lung Injury. HO – heme oxidase.

In an attempt to establish a toxicity profile Socata et al. [65] documented that given to mice at doses ranging from 150-300 mg/kg SFN induced sedation and hypothermia, while 200-300 mg/kg resulted in fatality. Latte et al. [66] warn that broccoli and SFN may induce specific genotoxic changes identified in vitro and animal research, yet emphasize that their significance in humans remains uncertain. The overall impression is that the positive effects of SFN outweigh the undesirable effects [6].

In summary, broccoli and its thiocyanates-SFN in particular, seem to be a valuable potential tool for treating a wide range of diseases, such as autoimmune conditions, acute and chronic infections and cancer. Given its availability, minimal side effects, and encouraging results from both in vitro and animal studies, it is surprising that SFN did not receive a higher ranking place among the therapeutic agents in medicine. We agree with a number of researchers who have been listed in this study that additional animal and human studies are necessary to accomplish this goal, and we hope that the review will attract the attention of scientists working in this area.

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