Redox Biology 70 (2024) 103077

Contents lists available at ScienceDirect

Redox Biology

journal homepage: www.elsevier.com/locate/redox

Regulation of immunomodulatory networks by Nrf2-activation in immune cells: Redox control and therapeutic potential in inflammatory diseases

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ARTICLE INFO

Keywords:
Nuclear factor erythroid 2-related factor 2
Metabolic reprogramming
Oxidative stress
Immune modulation

ABSTRACT

Inflammatory diseases present a serious health challenge due to their widespread prevalence and the severe impact on patients' lives. In the quest to alleviate the burden of these diseases, nuclear factor erythroid 2-related factor 2 (Nrf2) has emerged as a pivotal player. As a transcription factor intimately involved in cellular defense against metabolic and oxidative stress, Nrf2's role in modulating the inflammatory responses of immune cells has garnered significant attention. Recent findings suggest that Nrf2's ability to alter the redox status of cells underlies its regulatory effects on immune responses. Our review delves into preclinical and clinical evidence that underscores the complex influence of Nrf2 activators on immune cell phenotypes, particularly in the inflammatory milieu. By offering a detailed analysis of Nrf2's role in different immune cell populations, we cast light on the potential of Nrf2 activators in shaping the immune response towards a more regulated state, mitigating the adverse effects of inflammation through modeling redox status of immune cells. Furthermore, we explore the innovative use of nanoencapsulation techniques that enhance the delivery and efficacy of Nrf2 activators, potentially advancing the treatment strategies for inflammatory ailments. We hope this review will stimulate the development and expansion of Nrf2-targeted treatments that could substantially improve outcomes for patients suffering from a broad range of inflammatory diseases.

1. Introduction

Immune-mediated inflammatory diseases (IMIDs) encompass a broad spectrum of disorders characterized by inflammation, which is an immune system response to infection, injury, or disease [1]. These conditions can range from chronic inflammatory diseases including autoimmune disorders such as rheumatoid arthritis [2,3], type 1 diabetes [4,5], gastrointestinal disorders like Crohn's disease and ulcerative colitis [6,7], skin conditions exemplified by psoriasis [8], and neurotoxicity triggered by certain pharmaceuticals (e.g., anesthetics) and environmental factors (such as alcohol and pesticides) [9–13]. Additionally, non-resolving immune response has been observed in exacerbating the pathogenesis of many chronic diseases including progressive neurodegenerative disorders like Alzheimer's Disease (AD) [14], as well as cardiovascular complications like atherosclerosis, and diabetic cardiomyopathy [15,16]. Unregulated inflammation has profound implications, exacerbating the pathology of numerous conditions and frequently resulting in diminished quality of life, persistent pain, organ dysfunction, heightened mortality rates, and a considerable burden on healthcare systems [17,18]. The frequency of these conditions varies; for instance, sepsis affects millions worldwide each year with a high fatality rate, while chronic diseases like AD are prevalent, particularly among the aging population, affecting approximately 1 in 9 people aged 65 and older. This underscores the growing public health concern associated with such inflammatory conditions [18]. Therefore, understanding the mechanisms of regulation of immunomodulatory networks offers a promising avenue for inflammation control and therapeutic intervention.

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https://doi.org/10.1016/j.redox.2024.103077
Received 30 December 2023; Received in revised form 26 January 2024; Accepted 5 February 2024
Available online 10 February 2024

The immune system is a complex network of different types of cells, tissues, and organs. The intricate interplay of the immune system characterized by the changes in the abundance and activity of distinct immune cells including macrophages, neutrophils, dendritic cells (DCs), natural killers (NKs), lymphocytes (T and B cells), and microglia in the proinflammatory and late reparative phases is paramount for the genesis, progression, and prediction of distinct IMIDs [1,19]. Intriguingly, with the rapid deployment of single-cell RNA sequencing, researchers have identified phenotypic and functional heterogeneity among infiltrating immune cell subsets in numerous IMIDs (Fig. 1) implicating immune activation and proinflammatory response [20–22]. Additionally, emerging evidence indicates that distinct immunological subsets commit to specific metabolic fates to suffice their bioenergetic demand via regulating the pathways of substrate utilization, redox homeostasis, and epigenetic landscape impacting their development, proliferation, differentiation, and function [22–25]. An effective therapeutic strategy for the modulation of signaling pathways of metabolic reprogramming and redox homeostasis that influence the phenotype and functional properties of immune cell subsets has not been established.

Nuclear factor erythroid 2-related factor 2 (Nrf2) is a ubiquitously expressed transcription factor implicated in modulating multiple metabolic pathways in distinct immune cell subsets specifically those at the interface of proliferation and redox homeostasis [26–28]. Cellular metabolism and redox signaling pathways within immune cells are intimately linked via complex mechanisms [29]. Considering the critical role of Nrf2 in modulating the cellular redox status it is possible that Nrf2 expression and signaling might regulate activity of redox sensitive immunoregulatory factors influencing the functional state of immune cell subsets. Intriguingly, multitude of studies suggest that augmented Nrf2 expression within immune cells plays an important role in normalization of oxidative stress and mitigate the proinflammatory activity of the immune cells infiltrating into various tissues during infections [30–32]. For instance, studies using experimental preclinical models have demonstrated favorable effects of T-cell-specific activation of Nrf2 that promotes antioxidative response, further inhibiting inflammation by modulating Treg-mediated immune suppression [33]. Additionally, knocking out Kelch-like ECH-associated protein 1 (Keap1) within $CD4^{+}$ T cells resulting in Nrf2 activation reduced cytokine production with no signs of T cell exhaustion [34]. While most of the published studies have demonstrated an inverse relationship between immune cell-specific Nrf2 activation and their contribution to promote an elevated inflammatory state, it was recently reported that Keap1 deletion specifically within regulatory T cells (Tregs) promotes spontaneous accumulation of interferon-gamma (IFN-γ), producing effector T cells and inflammation, which was found to break immune tolerance [35]. While this apparent discrepancy of Nrf2 mediated immune responses in distinct immune cell subsets challenges our current understanding of the immunoresponsive mechanisms, development of pharmacologically potent compounds that can efficiently induce Nrf2 activation and it's downstream signaling pathways continues to elevate.

Considering the emerging role of Nrf2 in immune response, specifically its influence on the suppressive and inflammatory states of distinct immune cells via regulation of immunometabolism and redox homeostasis, in this review we summarize the pharmacologically relevant Nrf2

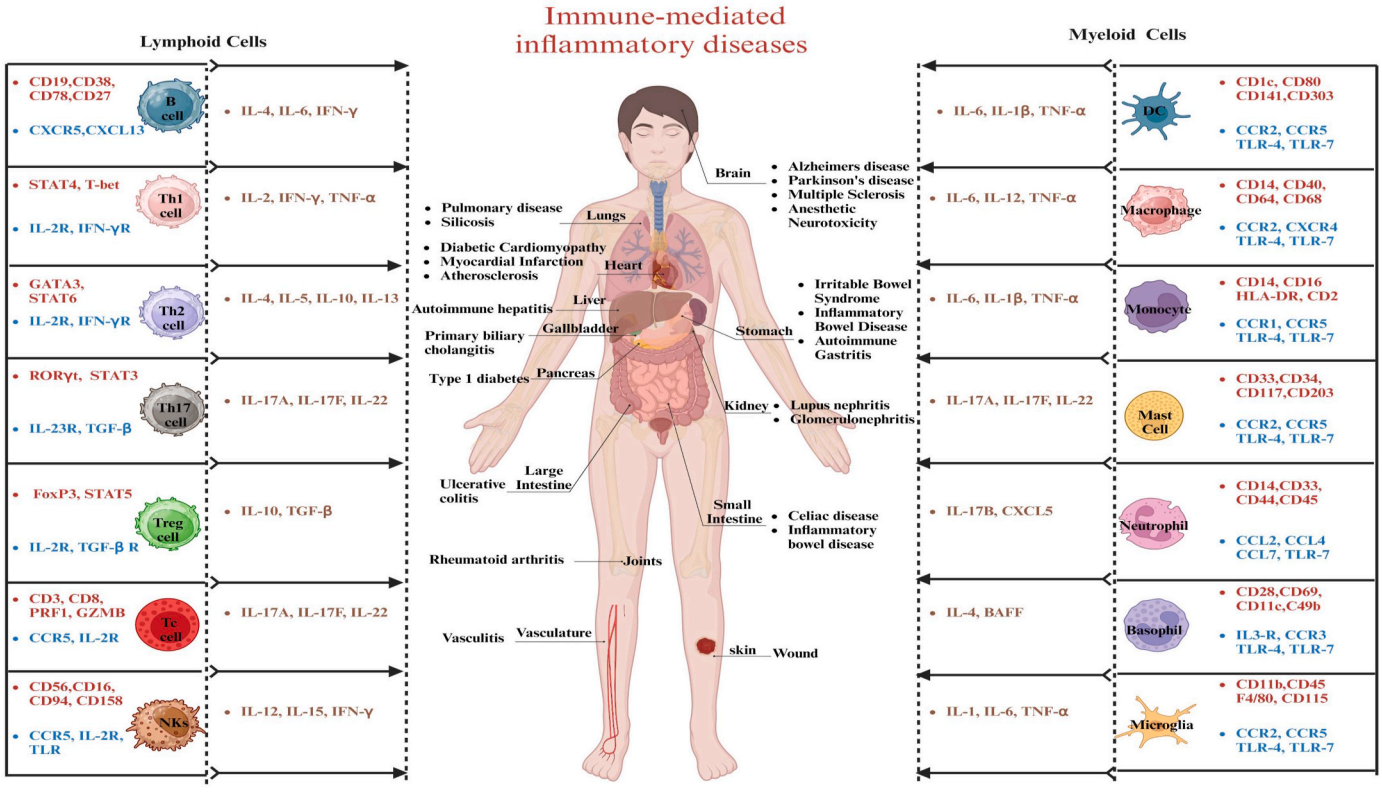


Fig. 1. Distinct Immune cell subsets and their secreted cytokines in immune mediated inflammatory diseases (IMIDs). CXCR4:C-X-C motif chemokine receptor 4; CXCR5:C-X-C motif chemokine receptor 5; CXCL13:C-X-C motif chemokine ligand 13; CCR1: C–C motif chemokine receptor 1; CCR2: C–C motif chemokine receptor 2; CCR3: C–C motif chemokine receptor 3; CCR5: C–C motif chemokine receptor 5; STAT3:Signal transducer and activator of transcription 3; STAT4:Signal transducer and activator of transcription 4; STAT5:Signal transducer and activator of transcription 5; STAT6:Signal transducer and activator of transcription 6; T-bet: T-box transcription factor TBX21; GATA-3:GATA binding protein 3; IL2-R: Interleukin-2 receptor; IL-23R: Interleukin-23 receptor; IL-1β: Interleukin (IL)-1β; IL-2: Interleukin (IL)-2; IL-4: Interleukin (IL)-4; IL-6: Interleukin (IL)-6; IL-10: Interleukin (IL)-10; IL-12: Interleukin (IL)-12; IL-17A: Interleukin (IL)-17A; IL-17F: Interleukin (IL)-17F; IL-22: Interleukin (IL)-22; Igγ: Ig gamma; (TNF)-α: tumor necrosis factor; IFNγ: Interferon γ; CXCL5:C-X-C motif chemokine ligand 5; BAFF:B-cell-activating factor; Th1: Type 1 T helper; Th2: Type 2 T helper; Th17: Type 17 T helper; Th22:Type 22 T helper; Tc: T cytotoxic cell; Tregs: Regulatory T cells; DCs: Dendritic cells; DC: dendritic cells.

modulators that could emerge as therapeutic interventions in the clinical setting for the treatment and prevention of distinct IMIDs. We also discuss emerging strategies of Nrf2 targeted drug delivery that could efficiently improve the therapeutic benefits of Nrf2 activators in inflammatory diseases.

2. Nrf2 signaling and its role in modulating immune responses through controlling cellular redox status and metabolic reprograming

Nrf2 is a member of the Cap 'n' Collar (CNC) family of basic leucine zipper transcription factors that modulate the temporal expression of many genes associated with cellular defense pathways under normal physiological conditions and chronic diseases [36,37]. It comprises approximately 605 amino acids and seven conserved domains in humans referred to as Nrf2-ECH (erythroid cell-derived protein with CNC homology) homologies (Neh 1–7) required for its regulatory functions [38]. Under normal physiological conditions, Keap1 tightly regulates the abundance of Nrf2 within the cell via anchoring to its DLG and ETGE motifs within the NEH-2 domain to attach Cullin-3, promoting its polyubiquitination and proteasomal degradation, thus, ensuring dynamic and precise control over its expression and activity [39–42]. Keap1 contains 27 redox-sensitive cysteine residues, which undergo chemical modification by either oxidative or electrophilic insults resulting in a reduced affinity of Keap1 for Nrf2 [43–45]. Under such conditions, Nrf2 escapes the regulatory arm of Keap1-mediated ubiquitination, resulting in its accumulation and translocation from the cytosol to the nucleus (Fig. 2), where it binds to the antioxidant response element (ARE) to regulate the expression of target genes [46,47]. These genes encode antioxidant and phase II detoxifying enzymes which collectively protect the cellular milieu from oxidative and electrophilic insults. The negative regulator Keap1, with its redox-sensitive residues, functions as an actual redox sensor that under more oxidizing conditions dictates the resultant localization of Nrf2, as opposed to signaling to or directly activating the transcription factor. When free from its negative regulator, Nrf2 is able to accumulate and then translocate to the nucleus to illicit its effect. Nrf2 is, therefore, an effector for redox regulation based on its subcellular distribution, rather than its induction, to regulate expression of its gene targets [48,49]. The activities/mechanisms of the Nrf2-regulated antioxidant defense systems include either catabolism or conversion of reactive oxygen species (ROS), reduction of oxidized cofactors and proteins back to their reduced states, increased production of reducing factors, the increase of redox transport, metal chelation and expression of multiple antioxidant enzymes [50]. Many of these proteins are localized in specific compartments within the cell to regulate redox signaling in the local environment [32].

2.1. Nrf2 signaling-mediated redox control in IMIDs

Under physiological conditions of redox homeostasis, distinct immune cell subsets display a controlled immune response [51]. However, alterations in the level of cellular oxidants has been implicated in inducing immune responses, subsequently contributing to increased systemic inflammation and impaired biological function of different organs [52]. While the molecular mechanisms of the interplay between the redox and inflammatory pathways remain to be deciphered, typically, the functional capacity of individual immune cells is highly sensitive to redox changes and is also orchestrated and regulated by the activity of Nrf2 and cellular antioxidant system [53].

Several studies thus far have substantiated the protective role of Nrf2 in attenuating inflammation-driven pathology via controlling the expression of antioxidant and cell protective enzymes. For instance,

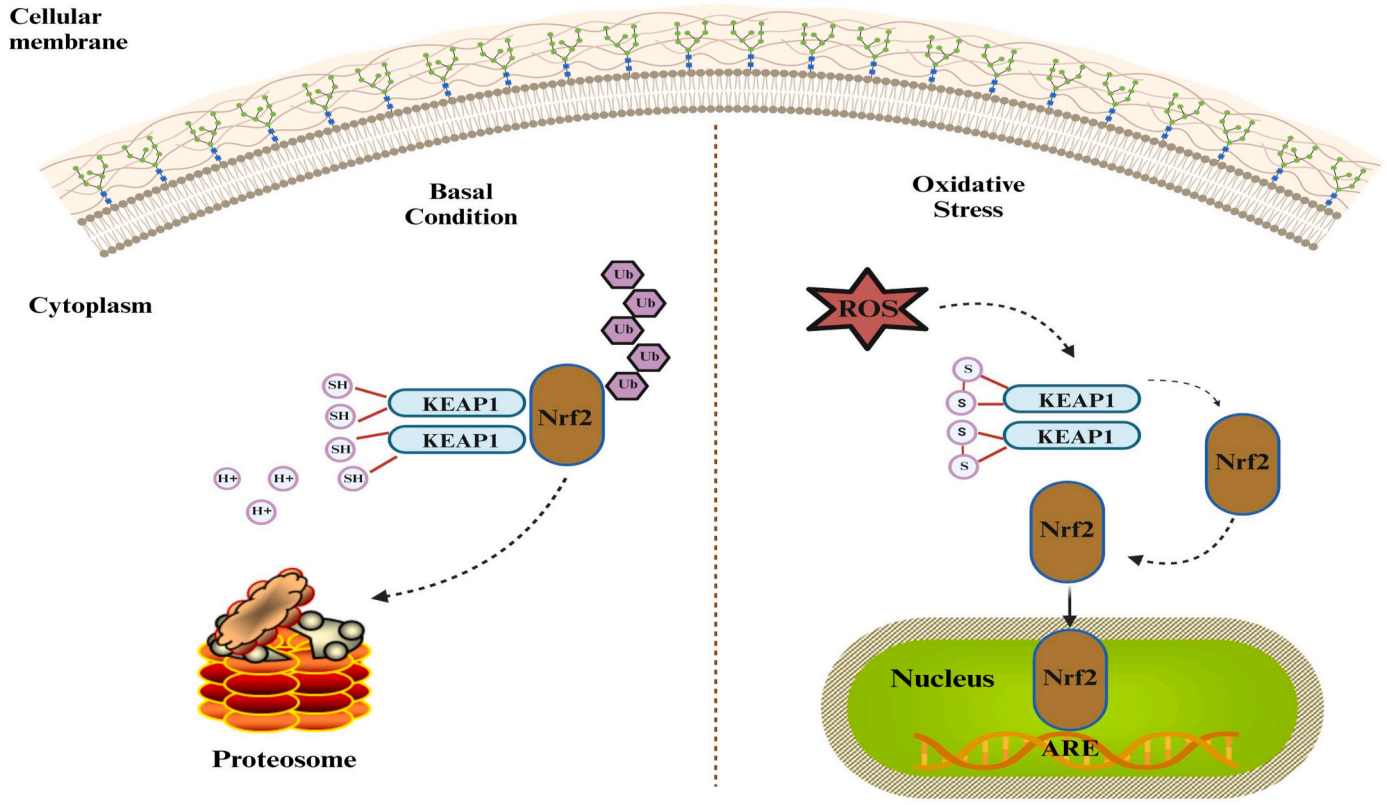


Fig. 2. Schematic depiction of the Nrf2/KEAP1 interaction under basal condition and response to oxidative stress. Under basal condition KEAP1 is tightly bound to Nrf2 promoting its polyubiquitination and proteasomal degradation. In response to oxidative stress the Nrf2-KEAP1 complex is disrupted, and Nrf2 can translocate to the nucleus to regulate the transcriptional activation of the antioxidant and cytoprotective genes. ROS: Reactive Oxygen Species; GSH: Glutathione; Nrf2: Nuclear factor erythroid 2–related factor 2; KEAP1: Kelch-like ECH-associated protein 1.

studies on allogenic hematopoietic stem cell transplanted patients indicate that high expression of Nrf2 in cytotoxic $CD8^+$ T-cells might be protective against graft-versus-host disease [54]. Also, a study on patients with childhood rheumatism (JIA) showed that Nrf2 activation of helper T cells ($CD4^+$ T cells) downregulated oxidative stress markers, altered the metabolic phenotype and reduced secretion of IFNγ [55]. This is also the case in sepsis, where 4-octyl itaconate-induced Nrf2 activation was found to negatively regulate programmed death-ligand 1 (PD-L1) as well as inhibit the production of inflammatory and oxidative stress-related factors, subsequently inhibiting organ injury and thus suggesting an Nrf2-mediated anti-inflammatory mechanism in a mouse model of sepsis [56]. Additionally, another study revealed that Nrf2 activation resolves chronic inflammation in lupus via the repolarization of macrophages and reduction of the IFNγ signature [57]. While less understood, many studies have also suggested a pathogenic role of tumor necrotic factor α (TNFα) in myocardial inflammation in DCM disease development [58]. Recently, activation of Nrf2 signaling pathways was found to reduce complications of DCM including oxidative stress and inflammation, suggesting that oxidative stress-induced inflammation may drive DCM pathogenesis [59]. Moreover, oxidative stress evoked inflammation was reported to be a critical factor in the advancement of neurodegenerative disorders, including AD and Parkinson's disease (PD). Patients with these diseases exhibit heightened levels of proinflammatory cytokines and significant activation of microglia, which are specialized macrophages that reside within the central nervous system (CNS) [60,61]. Intriguingly, knockout of Nrf2 in a mouse model of AD (APP/PS1 transgenic (AT) mice) exacerbated microglial activation along with upregulation of the proinflammatory cytokines interleukin-1 β (IL-1β) and interleukin 6 (IL-6), and TNFα, promoting AD pathogenesis and development [62,63].

2.2. *Nrf2 signaling-mediated metabolic reprogramming in IMIDs*

Energetic requirements of immune cells can differ depending on stressed or unstressed conditions and the resultant metabolic flux can influence their phenotype and function and therefore immune response. Nrf2, via direct and indirect mechanisms, regulates several genes involved in metabolism and affects the activity of redox-sensitive metabolic enzymes, including those situated at branching points in major metabolic pathways [64]. Because it regulates several metabolic enzymes, such as those involved in glucose metabolism, Nrf2 has been regarded as a major regulator of metabolic reprogramming, especially in cells that undergo rapid proliferation like cancer cells [65]. While Nrf2 is well known to modulate metabolism in cancer cells [66,67], several studies point to the regulatory role of Nrf2 in the metabolic processes of immune cells. A whole transcriptome analysis of a constitutively active Nrf2 mouse model revealed Nrf2-dependent activation of metabolic pathways and led to the conclusion that Nrf2 is crucial for the activation and balance between glycolysis and mitochondrial metabolism and serves as a key regulator of metabolic reprogramming in myeloid-derived suppressor cells [27]. In another study assessing the effect of Nrf2 on the proteome and metabolome of differentiated macrophages, comparing resting and simulated states, it was found that Nrf2 influences processes involved in redox, carbohydrate, and lipid metabolism as well as regulates respiration and mitochondrial fusion in activated macrophages [68]. These findings suggest that Nrf2 is central to macrophage metabolism and a regulator of the innate immune response. The metabolic pathways in T lymphocytes that result in the production of the metabolites and ATP required for cellular maintenance, proliferation and function also generate ROS which were reported as important mediators of downstream T cell receptor signaling [69–71]. A study using mature primary human and mouse T cell blasts provided evidence that T cells express a functional NADPH oxidase that is activated following T cell receptor stimulation to result in ROS generation [72]. Interestingly, naïve T cells, mainly reliant on OXPHOS for their energy needs, experience a metabolic switch from predominately OXPHOS to aerobic glycolysis following activation [73–75]. This metabolic switch has been implicated in ROS-mediated IL-2 production in T cells [69]. To ensure physiological, non-toxic levels of cellular ROS, cellular antioxidant mechanisms-controlled e.g., by Nrf2 pathway are pivotal for proper T cell activation, proliferation, and survival.

Many of the studies on Nrf2 using small molecule activators for the purposes of metabolic rewiring have been conducted in cancer research, with the goal to create metabolic imbalances to ultimately yield therapeutic benefits [76]. Promising preclinical data has led to clinical trials that take advantage of the vulnerabilities of the tumor cell metabolism, with an emphasis on patients who harbor a mutation that results in hyperactive Nrf2 pathway [77]. As metabolic rewiring of tumor cells has been exploited via Nrf2 activation for tumor suppression, perhaps more studies on Nrf2-mediated metabolic reprogramming within immune cells in tumor microenvironment should be conducted with the goal of modulation of immune responses during tumor growth and/or development.

Overall, changes in the redox and metabolic pathways have been implicated in fostering immune responses, highlighting the importance of developing a class of bioactive compounds with the potential to modulate oxidative stress and prevent/control inflammatory phenotypes. Considering the wealth of evidence supporting that Nrf2 activation is beneficial in counteracting immune response by induction of cellular stress response and antioxidant machinery, development of specific therapeutic approaches capable of inducing Nrf2 activation for diseases carrying an inflammatory component is warranted.

3. Application of Nrf2 activators in inflammatory diseases

The above-described studies clearly indicate that Nrf2 is an important mediator of redox homeostasis pathways within distinct immune cell subsets and contributes to their baseline inflammatory/regulatory states, impacting the development and progression of numerous inflammatory diseases. Henceforth, a wide range of therapeutic approaches have been developed to restore the metabolic and redox balance in immune cell subsets by targeting the Nrf2 pathway. One of the approaches employed is to use activators of Nrf2 to modulate signaling pathways associated with stress response and redox homeostasis in immune cells to reduce their ability to secrete inflammatory cytokines, thus preventing, or managing inflammatory diseases (Fig. 3). Intriguingly, many of these Nrf2 activators have shown promising therapeutic effects in preclinical studies as well as in clinical trials. In this section, we discuss several Nrf2 activators of established or potential clinical relevance, including natural Nrf2 activators such as sulforaphane (SFN), myricetin, resveratrol (RSV), mangiferin, isoastilbin (IAB), quercetin, nitro-fatty acids (NO_2–FAs), as well as synthetic Nrf2 activators like dimethyl fumarate (DMF), bardoxolone methyl (CDDO-Me) and, omaveloxolone (RTA-408) which were reported to play a role in protection against different diseases associated with inflammation (Table 1).The effect of these compounds on the phenotype and function of distinct immune cell subsets are also discussed (Fig. 4).

3.1. *Sulforaphane*

Sulforaphane (SFN, 1-isothiocyanato-4-methylsulfinyl-butane, Scheme 1) is a bioactive organosulfur natural compound, containing an electrophilic isothiocyanate group, that is abundant in cruciferous vegetables with multifactorial beneficial effects, specifically, antioxidant and immunomodulatory activity [78]. SFN has the potential to effectively activate Nrf2, which in turn enhances the expression of key genes associated with the ARE, thus modulating redox status, and controlling immune responses in inflammatory diseases, as discussed above. Mazarakis et al. investigated the *in vitro* immunomodulatory effect of SFN on healthy human peripheral blood mononuclear cells in the presence or absence of bacterial (lipopolysaccharide) and viral (imiquimod) toll-like receptor (TLRs) stimulations [79].Pretreatment with

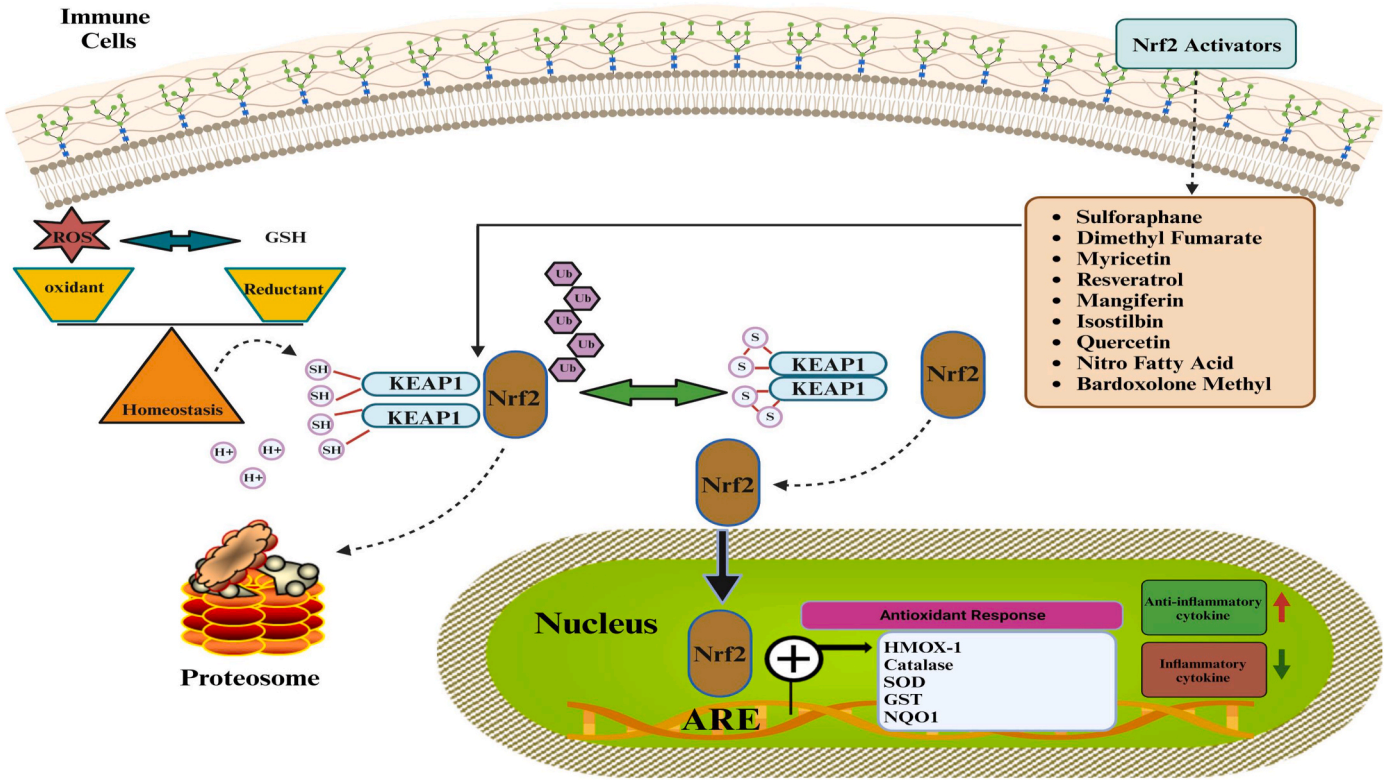


Fig. 3. Schematic diagram illustrating the application of Nrf2 activators and their role in normalizing inflammation via modulation oxidative stress. ROS: Reactive Oxygen Species; GSH: Glutathione; Nrf2: Nuclear factor erythroid 2–related factor 2; KEAP1: Kelch-like ECH-associated protein 1; HMOX-1: Heme Oxigenase-1; SOD: Superoxide Dismutase; GST: Glutathione Transferase; NQO1: NADPH Quinone Oxidoreductase1.

SFN or its metabolites like SFN-glutathione (SFN-GSH), SFN-cysteine (SFN-cys), and SFN-N-acetyl-L-cysteine (SFN-NAC) adducts significantly ameliorated the production of proinflammatory cytokines and chemokines including TNFα, IL-1β and IL-6, interleukin 10 (IL-10) and RANTES from the LPS-stimulated PBMCs and specifically reduced the frequency of $CD14^{+}$ monocytes while increasing immature monocyte-derived dendritic cells (moDCs). Also, *in vitro* investigation using a human monocyte cell line (THP-1) demonstrated that the observed changes in the monocyte phenotype and function towards the immature moDCs were mediated by SFN-induced Nrf2-ARE activity in the THP1 line [79]. In another *in vitro* study, SFN, was shown to exert protective effects against LPS-induced immune responses via inhibiting the differentiation of immature to mature moDCs through down-regulation of CD80, CD83, CD86, HLA-DR and PD-L1 markers, subsequently modulating Th2 proliferative responses and reducing anti-inflammatory cytokines while increasing the profile of regulatory cytokines [80]. Furthermore, SFN treatment *in vitro* was found to suppress NF-kB p65 and modulate IL-23 and IL-12 production from LPS-stimulated DCs by inducing the expression of the Nrf2-dependent gene heme oxygenase-1 (HO-1) to improve Th17/Th1 mediated autoimmune responses [81]. In an animal study, oral administration of SFN reduced differentiation of autoreactive Th17/Th1 cells and reduced the production of IL-17 and IFNγ *in vivo,* conferring protection against autoimmune encephalomyelitis [81]. Also, in the mouse model of pulmonary hyperoxia, oral administration of SFN led to induction of the antioxidative defenses and anti-inflammatory pathways and prevented pulmonary injury [82]. While oral supplementation of SFN in experimental models of pulmonary diseases have shown favorable effects, evidence for using SFN orally from clinical trials has demonstrated variable and conflicting results. For example, dietary SFN supplementation in healthy human subjects was found to have no effect on the expression of antioxidant genes or protection against neutrophilic airway inflammation [83]. A randomized, double-blind, placebo-controlled trial demonstrated that SFN administered orally with broccoli sprout homogenate failed to stimulate the expression of Nrf2 target genes or influence levels of other antioxidant genes such as glutathione S-transferase mu1(*GSTM1*), *HO-1,* and NADPH dehydrogenase quinone 1 (*NQO-1*) or markers of inflammation in the nasal epithelial cells and peripheral blood of patients with COPD [83].

SFN has been reported to display anti-inflammatory activity via induction of the Nrf2-mediated antioxidant response that has been shown to effectively detoxify ROS. An opposing conclusion was reached regarding the anti-inflammatory effect of SFN, whereby it was proposed that SFN inhibited inflammation by promoting basal ROS production in the lymphocytes. Checker et al. proposed that the SFN induces Nrf2 activation in T cells via phosphoinositide-3-kinase/Akt (PI3K/Akt) -mediated inhibition of the GSK3β pathway and increased ROS with decreased GSH/GSSG ratio [84]. Furthermore, Liang et al. also reported SFN-mediated increases in ROS, followed by glutathione (GSH) depletion which together mitigate the inflammatory response of primary human T cells [85]. Moreover, treatment with thiol-containing antioxidants significantly decreased measured ROS signals and abrogated the immunosuppressive effect of SFN in T cells [85]. It must be kept in mind, however, that SFN reacts directly with thiols, which may lead to GSH depletion without the involvement of ROS and vice versa, and that treatment with thiol antioxidants may deplete SFN, thus modulating its bioavailability, without the involvement of ROS scavenging.

Taken together, there exists strong experimental evidence in the preclinical models that modulating the redox equilibrium within distinct immune cells, using SFN, can be beneficial for ameliorating inflammation. More research is needed, however, to decipher the exact mechanisms beyond the SFN effects observed and the relationship between cellular oxidants and redox status, and the pro- and anti-inflammatory pathways.

Table 1
Evidence of Effect of Nrf2 Activators on the phenotype and function of distinct immune cells.

Compound	Experimental Models	Dosage	Effect/Mechanisms	References
Sulforaphane (N)	PBMC	10 μM and 50 μM in cell culture medium	(a) Reduces pro-inflammatory cytokines interleukin (IL)-6, IL-1β, and chemokines monocyte chemoattractant protein (MCP)-1 from LPS Stimulated PBMCs. (b) Reduces the proportion of natural killer (NK) cells and monocytes while increasing the proportions of dendritic cells (DCs), T cells and B cells.	[79]
	THP-1	10 μM and 50 μM in cell culture medium	Increases Nrf2-ARE activity in THP-1.	[79]
	moDCs and THP-1	10 μM in cell culture medium	Exerts protective effect against LPS-induced inflammation via the modulation of moDCs/T cells towards a regulatory profile.	[80]
	Mice model of EAE	Oral administration	Suppressed Il23a and Il12b expression *in vivo* and silenced Th17/Th1 responses *in vivo* and improves EAE.	[81]
	DC	0.3 μM in cell culture medium	Inhibits TLR4-induced IL-12 and IL-23 production, and severely suppressed Th1 and Th17 development of T cells primed by SFN-treated DCs.	[81]
	Mice model of acute lung injury	9 μmol of pure SFN in 10 μL PBS or vehicle (PBS) by oral gavage	Alleviated acute lung injury via inducing expression of transcript associated to mitochondrial metabolisms and ARE.	[82]
	RAW 264.7 monocyte/ macrophages	20 μM in cell culture medium	Suppressed mitogen induced T and B-cell proliferation, cytokine secretion, depleted GSH/GSSG ratio in lymphocytes and induced phosphorylation of PI3K/AKT leading to inactivation of GSK3β.	[84]
	Swiss-male mice	5 mg/kg; intra-peritoneal administration	Inhibited homeostatic proliferation of T-cells in mice.	[84]
	Primary Human T-cells	10 μM in cell culture medium	Exerts TH17 prone immunosuppressive effects on untransformed human T-cells by decreasing GSH and accumulation of ROS.	[85]
Dimethyl Fumarate (S)	PBMC of Multiple sclerosis patients	10 μM in cell culture medium	Reduces Memory T Cells and Shifts the Balance between Th1/ Th17 and Th2.	[86]
	PBMC of Multiple sclerosis patients	20 μM in cell culture medium	Mediates Tc17 suppression via inhibition of PI3K-AKT, STAT5 or reactive oxygen species.	[87]
	PBMC of relapsing-remitting multiple sclerosis (RRMS) patients	Oral administration Week1: 120mg/ daily, Week2: (morning-240mg; evening 120 mg), Week3–4:240 mg twice/daily	Exerts immunomodulatory effects on $CD4^+$ as well as $CD8^+$ T cells via altering metabolic profile and antioxidative capacity.	[89]
Myricetin (N)	Mouse bone-marrow-derived macrophages (BMDMs)	2.5–10 μg/mL in culture medium	Suppressed murine T lymphocyte activation	[91]
	Mice model of prediabetes	200 mg/kg; intragastrical administration	Alleviated the immunosuppressive effect in prediabetes induced by HFD	[92]
Resveratrol (N)	Mice model of high-fat diet–induced obese (DIO)	0.03% and 0.06 %; oral administration	Relieved oxidative stress, inhibit inflammatory genes expression, and increase Tregs number via aryl hydrocarbon receptor activation inhibited by HFD in DIO mice	[97]
	Mice model of high-fat diet–induced obese (DIO)	0.03% and 0.06 %; oral administration	Activated the Nrf2 signaling pathway-mediated antioxidant enzyme expression to alleviate inflammation by protecting against oxidative damage and T-lymphocyte subset-related chronic inflammatory response	[98]
	Mice model of high-fat diet–induced obesity	0.4% Resveratrol (4 g/kg diet); oral administration	alleviates obesity-induced skeletal muscle inflammation via decreasing M1 macrophage polarization and increasing the regulatory T cell population	[99]
	Human PBMC carrying Ala16Val-SOD2 genotypes	10 μM and 30 μM in cell culture medium	Mitigated the production of inflammatory cytokines in A-allele cells and increased the expression of SIRT1 in all SOD2 genotype cells	[100]
	PBMC of healthy subjects	1000 mg/day for 28 day; Oral administration	Decreased plasma levels of the proinflammatory cytokines TNF-α and MCP-1, while increased the plasma antioxidant activity compared with the corresponding antioxidant baseline activity	[101]
Mangiferin (N)	Mouse bone-marrow-derived macrophages (BMDMs) model of pyroptosis	10, 50, or 100 μg/mL in cell culture medium	Inhibited NF-κB pathway, suppressing inflammatory caspase-mediated pyroptosis cascades, and reducing GSDMD cleavage in LPS-induced BMDMs.	[106]
	Mice model of leukemia	40, 80, and 120 mg/kg of mangiferin in DMSO	Increased CD3 T-cell and CD19 B cell population and elevated survival rate of leukemia mice.	[107]
	Ovalbumin-Induced Asthmatic mouse Model	(50 mg/kg, 100 mg/kg, and 200 mg/kg); oral administration	Attenuated the imbalance of Th1/Th2 cells ratio by diminishing the abnormal mRNA levels of Th1 cytokines (IFN-γ and IL-12) and Th2 cytokines (IL-4, IL-5 and IL-13).	[108]
	Cyclophosphamide-induced immunotoxicity mouse model	10 and 20 mg/kg; intraperitoneal administration	Exerted immune protective role mediated through the inhibition of reactive intermediate-induced oxidative stress in lymphocytes, neutrophils and macrophages.	[109]
	cGVHD mouse model	50-200 μmol/L in cell culture medium	Promoted Bregs level in murine splenic MNCs, activated Nrf2 antioxidant signaling, and inhibited proinflammatory cytokine expression.	[110]
Isostilbin (N)	Mice model of colitis	50–100 mg/kg; intraperitoneal administration	Induced regulatory NK1.1− $CD4^+$ NKG2D + T cells, as well as elevated level of TGF β and IL-10 via PI3K,STAT3 and MAPK signaling pathway	[112]
	Mice model of psoriasis	4 and 10 mg/kg astilbin ointment in DMSO	Reduced IL-17 producing T-cell, and production of pro-inflammatory cytokines by downregulating MyD88.	[113]
	Mice model of Alzheimer's disease	intra-gastrically administration 40 mg/kg	Ameliorated the oxidative stress via increasing the expression of superoxide dismutase 1, catalase, heme oxygenase-1 and -2, as well as nuclear level of Nrf2	[114]

(*continued on next page*)

Table 1 (*continued*)

Compound	Experimental Models	Dosage	Effect/Mechanisms	References
Quercetin (N)	Mice Model of Experimental allergic encephalomyelitis (EAE)	50 or 100 μg/day; intraperitoneal administration	Blocked IL-12-induced tyrosine phosphorylation of the JAK/STAT signaling cascade, subsequently decreasing T cell proliferation of activated T cells and Th1 differentiation	[129]
Nitro-fatty Acids (N)	Pancreatic endothelial MS-1 cells and peritoneal RAW 264.7 macrophages	1 μM OA-NO2 in cell culture medium	Reduces production of TNF-α, IL-1β, and TGF-β from LPS stimulated macrophages and endothelial cell (EC) mediated inflammatory response via modulating STAT, MAPK and NF-κB pathways.	[138]
	NF-κB-luciferase transgenic mice	0.5 μmol/kg of; OA-NO2 intravenous administration	Reduced vascular inflammation via disrupting LPS induced activation of the NF-κB pathway	[139]
	RAW 264.7 monocyte/ macrophages	(0.1, 0.25, 0.5, or 1.0 μM) of; OA-NO2 in culture medium	Decreased the production of TNF-α, IL-1β, and IL-6 as well as •NO and superoxide anion (O2•–) production from the LPS-stimulated RAW 264.7 macrophages.	[140]
	Mice model of allergic contact dermatitis (ACD)	0.2mg/mouse OA-NO2(100μl/injection); Subcutaneous administration	Inhibited pathways associated with inflammatory cell infiltration and the production of inflammatory cytokines in the skin and promoted anti-inflammatory response by enhancing Treg activity and induced transcription of (ARE)-regulated genes including HMOX-1 which are Nrf-2 target genes.	[141]
	Mice model of Psoriasis	0.2mg/mouse OA-NO2(100 μl); Oral administration	Suppressed production of cutaneous inflammatory cytokines like IL-1β, IL-6, and IL-17A	[142]
	CD3/CD28-stimulated human T lymphoblast	5 and 10 in cell culture medium	Reduced IL-2 and IFN-γ production	[143]
Bardoxolone Methyl (S)	PBMC and Monocytes of Burn Patients	50 nM in cell culture medium	Reduced monocyte chemoattractant protein-1 (MCP-1/CCL2) cytokine production via activating Nrf2 pathway	[146]
	PBMC and Monocytes of Septic Shock Patients	20 nM in cell culture medium	Activated the expression of Nrf2 target genes like NAPDH quinone dehydrogenase 1 (NQO1) and glutamate-cysteine ligase modifier (GCLM) and suppressed the production of O2•–	[147]
	Adult male Sprague–Dawley (SD) rats (7 weeks old)	0.5 nmol/kg/day over 7 days	Ameliorated microglia activation and monocyte infiltration in the frontoparietal cortex (FPC) which in turn was accompanied by the reduction in MCP-1, TNF-α expressions and p38 mitogen-activated protein kinase (p38 MAPK) phosphorylation	[148]

(N): Natural; (S): Synthetic; IL-1: Interleukin (IL)-1; IL-2: Interleukin (IL)-2; IL-4: Interleukin (IL)-4; IL-6: Interleukin (IL)-6; IL-10: Interleukin (IL)-10; IL-12: Interleukin (IL)-12; IL-17: Interleukin (IL)-17; IL-23: Interleukin (IL)-23; MCP1: Monocyte chemoattractant protein 1; Igγ: Ig gamma; (TNF)-α: tumor necrosis factor; IFNγ: Interferon γ; Peripheral blood mononuclear cells; LPS: Lipopolysaccharide; THP-1: Monocyte-like cell line; moDCs: Monocyte-derived dendritic cells; DC: dendritic cells; SMCs: Skeletal muscle cells; Tregs: Regulatory T cells; Bregs: Regulatory B cells.

3.2. Dimethyl fumarate

Dimethyl fumarate (DMF, Scheme 1) is another clinically relevant electrophilic Nrf2 activator, derived from the TCA cycle metabolite, fumaric acid, with immunomodulatory properties that has been successfully used in immune-mediated diseases. Regarding the role of DMF in modulating the redox homeostasis in immune cells, Wu et al. demonstrated that compared to placebo 4–6 month and 18–26-month treatment with DMF (trade name Tecfidera™) diminished the proportion and activation of memory T cells, while shifting Th1/Th17 inflammatory response to a Th2-mediated anti-inflammatory response in relapsing-remitting multiple sclerosis (RRMS) patients [86]. This favorable effect of DMF was also observed *in vitro* where DMF treatment was found to decrease ROS levels, and proliferation of activated T cells [86]. Indeed, experimental evidence from the studies mentioned above suggests that DMF-induced removal of ROS in immune cells may decrease susceptibility towards inflammatory diseases. However, contrasting results from other studies on experimental models suggested that favorable effects of DMF treatment ameliorating autoimmune diseases result from the increased production of ROS in immune cell subsets. A pilot study described that DMF administration in multiple sclerosis patients displays a temporal reduction in the frequency of proinflammatory Tc17 cells toward the end of the treatment. Notably, it has been proposed that DMF treatment altered transcriptional signatures via increasing the levels of ROS, resulting in decreased interleukin-17 (IL-17) production in human and murine Tc17 cell, as supplementation with GSH and other antioxidants like N-acetyl-L-cysteine (NAC) or Trolox, a water-soluble vitamin E analog, reversed DMF-mediated effects in Tc17 cells *in vitro* [87].

Although there are numerous studies depicting DMF-induced immunological changes by modulating oxidative stress, the role of metabolic rewiring in the effects observed is less understood. Some studies, albeit in MS patients, showed that the DMF administration induces metabolic alteration to control the phenotype and function of T cells. Bhargava et al. depicted that DMF treatment alters lipid metabolism that significantly corelated with the changes in the proportion of the $CD8^+$ effector memory and $CD8^+$ naïve T cells [88]. Additionally, evidence from clinical trials has demonstrated that oral administration of DMF exerts metabolic alterations in $CD4^+$ as well as $CD8^+$ T cells, accompanied by an increase in the measured ROS levels and mitochondrial dysfunction, resulting in T cell apoptosis [89]. Moreover, *in vitro* investigation further indicated that DMF can elevate intracellular ROS in $CD4^+$ as well as $CD8^+$ T cells, promoting secretion of proinflammatory cytokines like IFN γ and GM-CSF, which can be restored via GSH supplementation, but not Nrf2 inhibition [89]. Beyond T cells, DMF has been implicated in modulating innate immune cells and has been shown reported to increase production of ROS in monocytes of RRMS patients [90].

Research continues to delve into the development of a new formulation of DMF to be used in clinical trials in immune-mediated diseases. Similar to SFN, DMF as an electrophile may directly interact with GSH and other thiols, thus deciphering the exact mechanisms of ROS involvement in the effects observed requires further studies.

3.3. Myricetin

Myricetin (Scheme 1) is a polyhydroxylated flavonoid compound abundantly distributed in numerous plants, including berries, tea, vegetable, and wine. In recent years, myricetin has been used in the treatment of inflammatory diseases because of its antioxidant properties through which it induces an immunomodulatory effect, reducing the risk of metabolic and inflammatory disorders. Ghassemi-Rad et al. demonstrated that myricetin treatment suppressed anti-CD3−and anti-CD28−induced activation of mouse T cell [91]. The hypothesis that

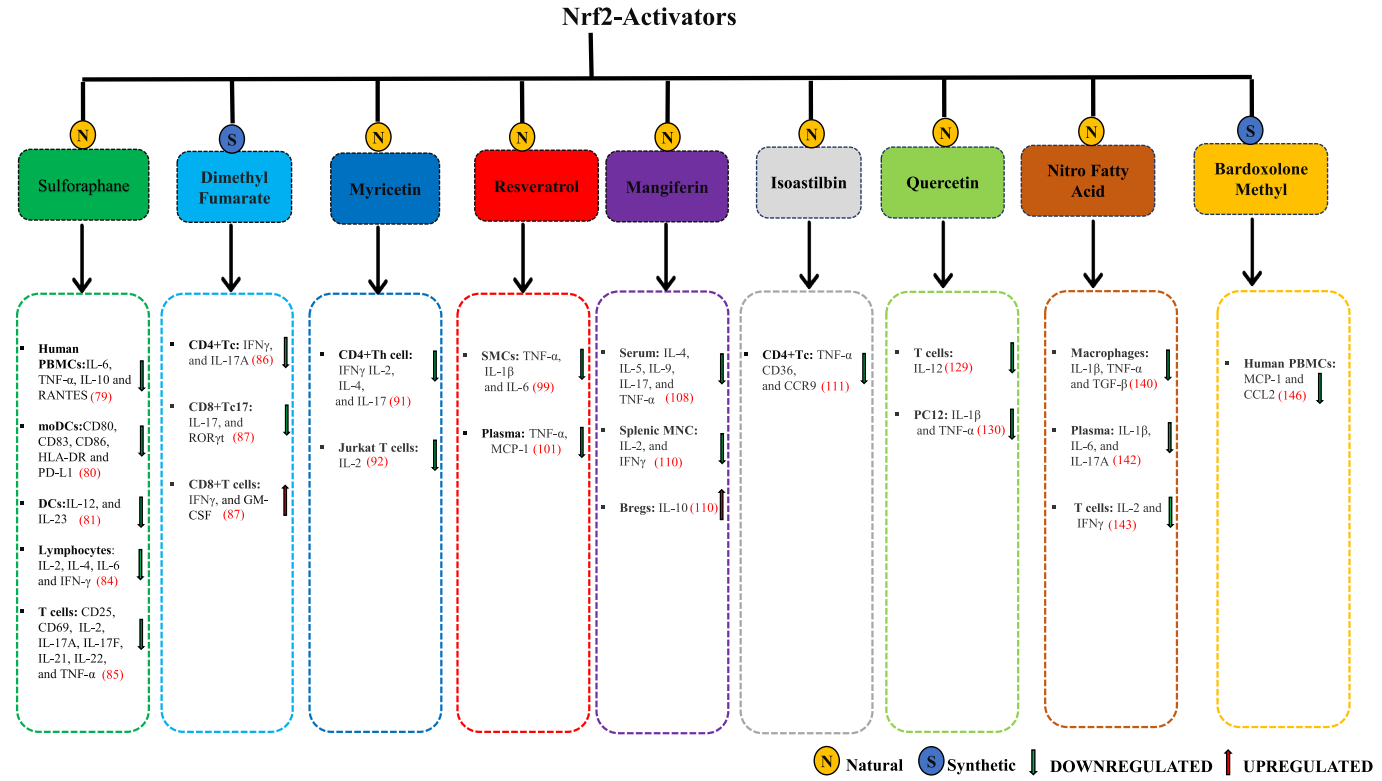
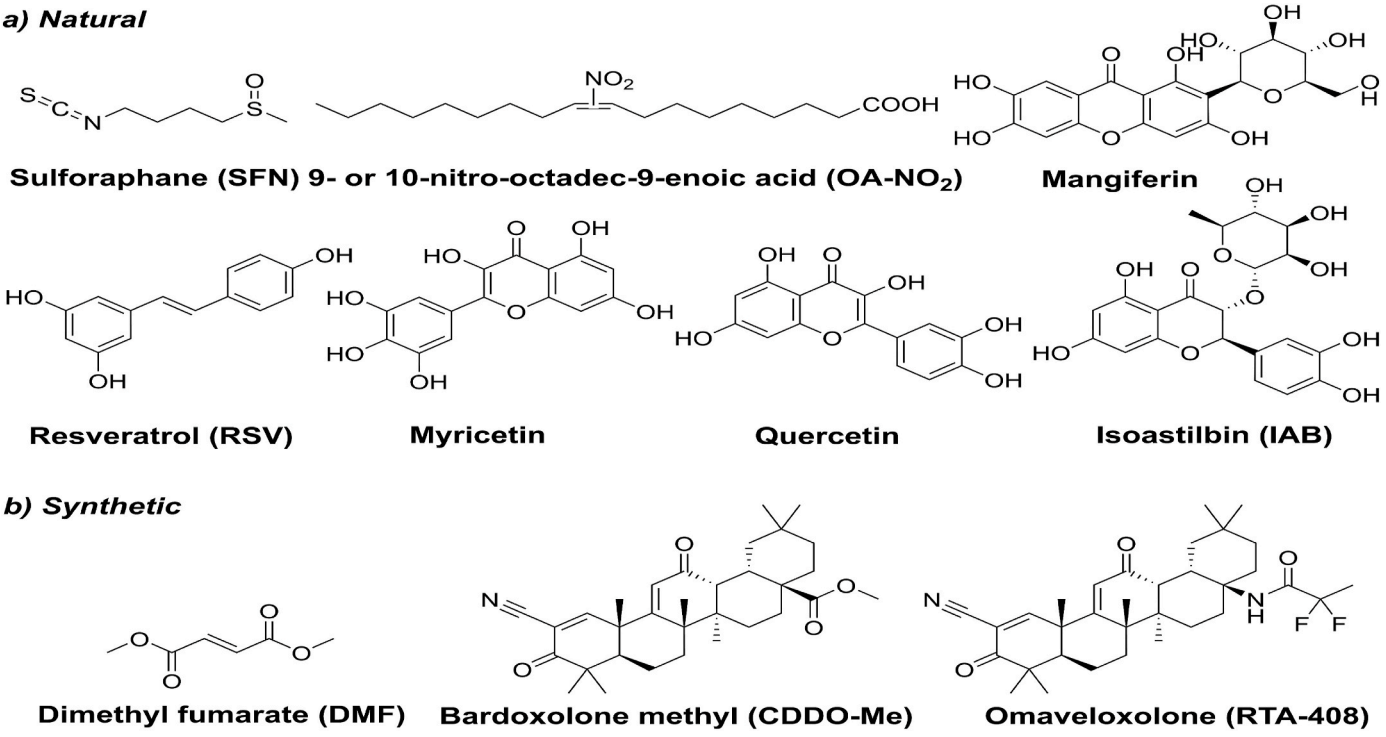


Fig. 4. Schematic depiction of the application of the Nrf2 activators and their effect on the phenotype and function of distinct immune cell subsets in different inflammatory diseases. PBMC: Peripheral blood mononuclear cells; moDCs: Monocyte-derived dendritic cells; DC: dendritic cells; SMCs: Skeletal muscle cells; MNC: Mononuclear cells; Tregs: Regulatory T cells; Bregs: Regulatory B cells; IL-1: Interleukin (IL)-1; IL-2: Interleukin (IL)-2; IL-4: Interleukin (IL)-4; IL-6: Interleukin (IL)-6; IL-10: Interleukin (IL)-10; IL-12: Interleukin (IL)-12; IL-17: Interleukin (IL)-17; IL-23: Interleukin (IL)-23; MCP1: Monocyte chemoattractant protein 1; Igγ: Ig gamma; (TNF)-α: tumor necrosis factor; IFNγ: Interferon γ.



Scheme 1. Chemical structures of Nrf2 activators discussed in this review.

immunomodulatory agents inhibit inflammation via elevation of ROS levels was supported by an Amplex Red assay, which showed that in cultured T cells, myricetin induced production of hydrogen peroxide (H_2O_2) in a dose-dependent manner, thereby suppressing the production of proinflammatory cytokines such as IFNγ, interleukin-2 (IL-2), interleukin-4 (IL-4), and IL-17 [91]. Further, in the mouse RAW 264.7 monocyte cell line, myricetin was found to reverse the glucose-induced immunosuppressive effect via restoring phagocytic and endocytic activity and increasing ROS levels without any significant cytotoxic effect [92]. Additionally, the immunosuppressive effect of myricetin genes in Jurkat human T cell line was shown by the fact that it suppressed the expression of *IL-2* and *IFNγ* related mRNA genes and increased expression of programmed cell death protein 1 [92].

While myricetin was shown to activate the Nrf2 pathway, it shows much broader biological activity [93], thus the exact role of that pathway in its immunomodulation activity is not fully understood and requires further research. For instance, in addition to the antioxidant activity, myricetin has the potential to act as a pro-oxidant due to its tendency to undergo autoxidation to form quinone intermediates [94]. Intriguingly, myricetin-derived quinones were reported to react with the cysteine residues and modify certain proteases [95]. In this context, production of quinones would be a consequence of myricetin autoxidation and such quinone may react cysteine residues in KEAP1, resulting in Nrf2 activation. Phenolic compounds, including myricetin, were shown to interfere with the Amplex Red assay, complicating the interpretation of the available experimental data on the ability of myricetin to induce H_2O_2 production [96]. Henceforth, the role of the autoxidation of myricetin and its ability to induce H_2O_2 production should be further explored.

3.4. Resveratrol

Resveratrol (RSV, Scheme 1) is a polyphenolic stilbenoid and a significant constituent of grapes, berries, peanuts, and other plants possessing numerous pharmacological benefits. It is a potent sirtuin1 (SIRT1) and Nrf2 activator that has gained substantial attention among researchers for inhibiting the progression and development of numerous inflammatory diseases. There has been increasing amount of evidence from preclinical and clinical studies that RSV ameliorates inflammation in numerous diseases via exerting an immunomodulatory effect on distinct immune cell subsets. In a high-fat diet (HFD)-induced mouse model, RSV administration for 26 weeks significantly suppressed HFD-induced oxidative stress and inflammation via restoring redox balance and shifting the Th1/Th2 response by decreasing the expression of proinflammatory genes and enhancing the abundance of Tregs by an increase in *FoxP3* expression in the peripheral blood and spleen tissue [97]. Other findings from the same research group using the HFD mouse model demonstrated that RSV supplementation maintains glucose homeostasis by activating the PI3K and SIRT1 signaling pathways. Additionally, RSV activates Nrf2-pathway-induced expression of antioxidant enzymes to mitigate oxidative stress, and, T cell-mediated inflammatory responses associated with the development of HFD-induced obesity [98]. In a separate study, RSV attenuated inflammation in the skeletal muscle of HFD-induced mouse model of obesity via inducing polarization of macrophages toward the M2 phenotype, as well as decreasing the expression of several M1 pro-inflammatory cytokines including TNFα, IL-1β, and IL-6 [99].

Modulating the redox status to mitigate inflammatory response from immune cells via Nrf2-activator treatment is an attractive therapeutic route for treating distinct inflammatory diseases. However, the therapeutic efficacy of the bioactive compounds can also be influenced by the genotype of the immune cells. Intriguingly, Capeleto et al. reported that the anti-inflammatory effect of RSV on human PBMCs is influenced by a superoxide dismutase 2 (SOD2) gene polymorphism which can either be an alanine (A) or valine (V). Treatment with RSV *in vitro* mitigated proliferation, and pro-inflammatory cytokines production from A-genotype PBMCs, while VV-cells displayed a subtle decrease in cytokine productions, suggesting the RSV effect on human PBMCs is dependent on the Ala16Val-SOD2 SNP [100]. Moreover, recent data from clinical trials indicate the immunomodulatory action of RSV on circulating immune cells in humans. The repeated doses of RSV (1000 mg/day for 28 days) induced a significant increase in the level of circulating Tregs, plasma antioxidant activity and decrease in the level of proinflammatory cytokines TNFα and MCP-1 [101].

Recently, studies also showed that the use of RSV may lead to metabolic alterations that, in turn, might be linked to immunological changes. Craveiro et al. demonstrated that at low dose (20 μM) RSV induces alterations in the bioenergetic homeostasis of TCR-stimulated $CD4^+$T cells, characterized by decreased glycolysis, increased glutamine consumption, and a shift to OXPHOS, resulting in enhanced secretion of IFN-γ from both naïve and memory $CD4^+$T cells [102]. Other published study also indicates that treatment with RSV results in metabolic reprogramming of staphylococcal enterotoxin-B (SEB)-activated immune cells via modulating miR-100 and mTOR signaling pathways in T-cells [103].

Overall, the high safety profile of RSV coupled with its immunomodulatory action has made it a promising candidate to be used in clinical trials for inflammatory diseases. Due to multiple biological pathways affected by RSV, the contribution and exact role of Nrf2 activation in the effects observed requires detailed mechanistic investigation in each model/disease under study, similar to the case of myricetin, as stated above.

3.5. Mangiferin

Mangiferin (Scheme 1) is a significant constituent of the Mangifera indica Linn. (Anacardiaceae) leaves. For several years, mangiferin and its derivatives have been used in the treatment of various diseases because of their antioxidant property [104]. Mangiferin exerts its beneficial effects through multiple mechanisms to mitigate oxidative stress such as reducing the production of ROS, restoring mitochondrial membrane potential, and protecting from apoptosis [104,105]. Additionally, it also exhibits anti-inflammatory and immunomodulatory properties [106] and has shown beneficial effects across several rodent models of inflammatory diseases [107,108]. In mouse lymphocytes, neutrophils, and macrophages, mangiferin was found to decrease the cyclophosphamide-induced immunological oxidative stress via restoring catalase and superoxide dismutase activity and reducing lipid peroxidation [109]. A recent study by Qin et al. showed that mangiferin induces the Nrf2/ARE signaling pathway in murine splenic MNC, subsequently reducing cytokine production. Additionally, treatment with mangiferin increased frequency of regulatory B cells (Bregs) and expression of Bregs-associated IL-10 via activating Janus kinase 2 (JAK2)/signal transducer and activator of transcription 3 (STAT3) and extracellular signal-regulated kinase (ERK) signaling in murine splenic MNCs [110]. Mangiferin has emerged as a promising bioactive agent preventing inflammatory diseases mechanistically via reducing oxidative stress and inflammation.

3.6. Isoastilbin

Isoastilbin (IAB, Scheme 1) is the most predominant dihydroflavonol glycoside compound mostly found in Rhizoma Smilacis glabrae and Astragalus membranaceus plants. IAB is known to possess anti-inflammatory and antioxidant properties. Recent studies also provide substantial evidence for IAB exhibiting protective effect via modulating oxidative stress. Exposure to IAB suppressed TNFα and increased CCR9 and CD36 expression of $CD4^+$ T cells and downregulated effector $CD4^+$ T cells activities via CYP1B1/ROS/PPAR pathway [111]. In dextran sulfate sodium (DSS) induced colitis mice model astilbin promoted induction of regulatory $NK1.1^-$ $CD4^+$ $NKG2D^+$ T cells, as well as elevated level of TGFβ and IL-10 *ex vivo* and *in vivo* via PI3K, STAT3 and MAPK

signaling pathways thereby reducing inflammation and progression of DSS-induced colitis [112]. In the study of Xu et al. on an imiquimod (IMQ)-induced psoriasis-like murine model it was found that topical administration of astilbin mitigates psoriasis via reducing IL-17-producing T cell accumulation and psoriasis-specific cytokine expression in skin lesions. Additionally, astilbin decreased the expression of pro-inflammatory cytokines by downregulating myeloid differentiation factor 88 [113]. Intriguingly reduction in the Nrf2 level in the APP/PS1 mouse model of AD has been found to enhance inflammatory response and increase in intracellular APP, Aβ42 and Aβ40 levels suggesting Nrf2-dependent processing and accumulation of APP/Aβ, and autophagic dysfunction. IAB has been shown to activate the Nrf2 pathway to attenuate Aβ42 level and p-tau in the AD animal model [63]. Also, intra-gastrically administration 40 mg/kg of IAB (n = 12) once daily for 28 days reduced $AlCl_3$-and D-gal-induced AD-associated symptoms in Balb/c male mice via Nrf2 mediated antioxidative and anti-apoptotic mechanisms [114]. IAB ameliorated the oxidative stress via increasing the nuclear level of Nrf2 resulting in the expression of several antioxidant enzymes including superoxide dismutase 1, catalase, HO-1, and HO-2 [114].

3.7. Quercetin

Enriched in berries, red onions and other fruits and vegetables, quercetin (Scheme 1) is a plant flavanol that gives many fruits, vegetables, and flowers their colors. As most other polyphenolic compounds, quercetin can efficiently scavenge free radicals in chemical systems and promotes redox balance in cells, while its anti-inflammatory effects, mainly on leukocytes [115], may help alleviate inflammation in various conditions such as arthritis and cardiovascular disease. Quercetin has been shown to increase the expression and nuclear localization of Nrf2 and to inhibit NF-κB/TLR-4-mediated proinflammatory signaling [115,116] which together result in a protective effect against toxic insults in various cell types. It has been demonstrated to promote the expression of glutamate-cysteine ligase catalytic subunit (GCLC), to support the rate-limiting step of GSH synthesis and promote cellular redox homeostasis [117]. More recently, quercetin was implicated for its Nrf2-mediated prevention of ferroptosis [118–120], a programmed cell death pathway that is dependent on the intracellular accumulation of iron and lipid hydroperoxides. This could prove useful as ferroptotic tissue damage can attract neutrophil granulocytes which are the first immune cells recruited to the sites of acute inflammation [121]. These inflammatory immune cells have been described using the double-edged sword metaphor [122] as they are phagocytes that regulate the initial inflammatory response following trauma, but they also contribute to sustained inflammation in some conditions such as fibrosis [123], cancer [124,125], psoriasis [126] as well as inflammation initiated by ferroptotic tissue damage [127]. Therefore, Nrf2-mediated prevention of ferroptosis may thwart the paradoxical immune response that occurs during inflammation in IMIDs. Compared to other flavonoids, such as the structurally similar cyanidin, quercetin was 10-fold more potent in the inhibition of LPS-induced nitric oxide ($^{\bullet}NO$) production and induction of Nrf2-mediated HO-1 protein expression in the murine BV-2 microglial cells [128]. It was shown to block IL-12-induced tyrosine phosphorylation of the JAK/STAT signaling cascade, subsequently decreasing T cell proliferation of activated T cells and Th1 differentiation [129]. Its effect on potent inducers of inflammatory gene expression and protein secretion represents its direct anti-inflammatory impact [130], but it can also indirectly prevent inflammation via increasing PPARγ activity whose inhibition of NF-κB transcriptional activation negatively modulates expression of inflammatory genes [131,132].

3.8. Nitro-fatty acids

NO_2–FAs, are endogenous electrophilic signaling molecules generated through non-enzymatic reactions between fatty acids (FAs) and $^{\bullet}NO$-derived reactive nitrogen species such as nitrogen dioxide ($^{\bullet}NO_2$) and peroxynitrite ($OONO^-$) and are readily detected in human biological fluids such as plasma and urine [133]. It has been reported that NO_2–FAs possess multiple pharmacological activities, including anti-inflammatory effects, showing significant protection against fibrotic and inflammatory diseases [134,135]. Recent studies also provided evidence for the role NO_2–FAs such as 9- or 10-nitro-octadec-9-enoic acid (OA-NO_2; Scheme 1) exhibiting Nrf2 activation via post-translational modifications like nitro alkylation of cysteines in KEAP1 protein, thus regulating the transcriptomic signature of the ARE related genes [136]. In addition, NO_2–FAs, have been shown to be effective in regulating the heat shock response (HSR) stress signaling pathway, protecting against proteotoxic stress in a large range of inflammatory diseases [137]. NO_2–FAs, appear to affect multiple immune regulatory mechanism in broad range inflammatory mediated pathological processes including those leading to vascular and skin diseases. OA-NO_2 reduced production of essential pro-inflammatory cytokine TNF-α, IL-1β, and TGF-β from LPS-stimulated macrophages, and further reduced endothelial cell (EC) mediated inflammatory response via modulating STAT, MAPK and NF-κB pathways suggesting that it is capable of modifying vascular and endothelial immune response [138]. Villacorta et al., demonstrated that the acute intravenous administration of OA-NO_2 *in vivo* is effective in reducing vascular inflammation via disrupting LPS induced activation of the NF-κB pathway [139]. In another study, OA-NO_2 treatment caused a significant decrease in the production of TNFα, IL-1β, and IL-6 as well as $^{\bullet}NO$ and superoxide radical anion ($O_2^{\bullet-}$) production from the LPS-stimulated RAW 264.7 macrophages [140]. It also prevented fibrotic processes in an *in vivo* model of angiotensin II-induced myocardial fibrosis by modulating the infiltration of inflammatory "M1-like" or regulatory "M2-like" macrophage subsets into affected tissue [140]. The anti-inflammatory effect of OA-NO_2 were also observed upon subcutaneous administration of OA-NO_2 in a mouse model of allergic contact dermatitis (ACD), where it inhibited pathways associated with inflammatory cell infiltration and the production of inflammatory cytokines in the skin, and promoted an anti-inflammatory response by enhancing Treg activity [141]. OA-NO_2 also induced transcription of (ARE)-regulated genes including *HMOX-1* which are Nrf2 target genes [141]. Likewise, Wang et al.; demonstrated that oral administration of OA-NO_2 suppresses production of cutaneous inflammatory cytokines like IL-1β, IL-6, and IL-17A in a mouse model of psoriasis [142]. OA-NO_2 has also been shown to lower the proliferative potential and production of IL-2 and IFNγ in CD3/CD28-stimulated human T lymphoblasts [143]. Meanwhile, it also decreased transcriptional activity of nuclear factor of activated T cells (NFAT) through modulating phosphatase activity of calcineurin (CaN), hindering NFAT dephosphorylation, and nuclear localization in activated T cells. The beneficial effect of OA-NO_2 to regulate T-cell activation has been attributed to their ability to regulate CaN phosphatase via nitro alkylation of CaN on Cys372 residue [143]. NO_2–FAs and more general nitroalkenes are an emerging class of Nrf2 activators, showcasing their potential to modulate antioxidant signaling pathways to counteract different inflammatory diseases, thus supporting their translation into clinically relevant therapeutics.

3.9. Bardoxolone methyl

Bardoxolone methyl (CDDO-Me, Scheme 1) and omaveloxolone (RTA-408, Scheme 1) both are electrophilic pentacyclic semisynthetic triterpenoids derived from the natural product oleanolic acid and have been widely used in activating Nrf2 and its downstream pathway via binding to cystine residues on KEAP1 protein [144,145]. In the recent years, CDDO-Me and RTA-408 have been used in the treatment of IMIDs because of their antioxidative and anti-inflammatory properties. Eitas et al. showed that, *in vitro* treatment with CDDO-Me reduced monocyte chemoattractant protein-1 (MCP-1/CCL2) cytokine production via activating the Nrf2 pathway in burn patients PBMCs and monocytes

[146]. In PBMCs and monocyte of septic shock patients, *ex vivo* administration of CDDO-Me activated the expression of Nrf2 target genes like NAPDH quinone dehydrogenase 1 (*NQO1*) and glutamate-cysteine ligase modifier subunit (*GCLM*). Also, pretreatment with CDDO-Me suppressed the production of $O_2^{\bullet-}$ after LPS exposure in PBMCs, but not in the purified monocytes [147]. CDDO-Me ameliorated microglia activation and monocyte infiltration in the frontoparietal cortex (FPC) following status epilepticus (SE, a prolonged seizure activity) which in turn was accompanied by the reduction in MCP-1, TNF-α expression and p38 mitogen-activated protein kinase (p38 MAPK) phosphorylation [148]. Similarly, in the rat model of anterior ischemic optic neuropathy (rAION), CDDO-Me modulated Nrf2 and NFκB signaling pathways to protect retinal ganglion cell from apoptosis via regulation of NQO1 and HO-1, subsequently reducing IL-6 and Iba1 expression in macrophages and promoted microglial expression of TGF-β and Ym1 + 2 in the retina and optic nerve [149]. Moreover, CDDO ethyl amide (CDDO-EA) suppressed LPS-induced TNF-α and MCP-1 gene expression by inhibiting the NF-κB signaling pathway in L6-GLUT4myc rat myotubes [150]. Thus, the overall beneficial effects of CDDO-Me and RTA-408 are associated with the activation of Nrf2 pathways and subsequent reduction in the production of pro-inflammatory cytokines and chemokines from distinct immune cells. Importantly, RTA-408 was recently approved by the FDA for the treatment of patients with Friedreich's Ataxia, in adults and adolescents aged 16 years and older [151].

Overall, the above-described studies support the concept that Nrf2-activators can modulate oxidative stress-related immune responses endorsing the application of pharmacologically and clinically relevant activators of Nrf2 in preventing the development of inflammatory diseases via targeting the delineated signaling pathways. Also, the above-described mechanisms can be further extended to other disease settings that develop because of inappropriate immune response, including neurodegenerative and cardiovascular diseases. However, all the bioactive agents reviewed above show multiple molecular targets and pleiotropic biological activities, and thus, the role of Nrf2 activation in the effects observed needs to be experimentally verified. It is imperative that further research focuses on elucidating the effect of Nrf2-activators on gene expression and function of various cellular pathways, which may cooperatively regulate the immune response and mitigate inflammation. Additionally, contradictory results from different clinical trials using Nrf2 activators need to be addressed and explained, considering variable dosing, formulations, different biological responses between healthy vs diseased human subjects and potential involvement of additional cellular targets, which may differ between the agents used. Furthermore, long-term supplementation with Nrf2 activators for large-scale human longitudinal studies to assess their effect on inflammatory state associated disease progression is warranted.

4. Challenges in the use of conventional Nrf2 activators

While there have been advances in the drug development of Nrf2 modulators to mitigate oxidative stress and inflammation these have not been without their limitations and challenges. In this section we address the key concerns related to the solubility, specificity, delivery, and safety of the Nrf2 activators. We also discuss the challenges that encompass issues such as implication of genetic polymorphisms in response to Nrf2 therapy, and heterogeneity in treatment effect of Nrf2 activators in distinct disease.

4.1. Delivery

Studies have investigated the actions of the different modulators on the Nrf2 pathway, but the delivery of these modulators represents another issue. Accordingly, there are major obstacles in the clinical application of Nrf2 modulators including toxicity, poor solubility, low bioavailability, instability, rapid elimination and a demonstrated extensive first-pass or pre-systemic metabolism [152]. The most common manner of drug delivery, oral administration shows many advantages such as safety, tolerance, and convenience [152,153]. But it often is associated with poor and highly variable bioavailability. Inherent instabilities of many Nrf2 activators, short half-life and low solubilities under the shifting gastrointestinal environment make this manner of drug delivery particularly challenging. Additionally, poor drug permeabilities through the GI barriers and in some instances, extensive biotransformation via presystemic metabolism in the liver can drastically reduce the bioavailability of the Nrf2 activators. Perhaps an advanced approach of using nanocarriers to ensure an effective delivery of Nrf2 activators and prevent their degradation might significantly improve their stability and delivery to the target tissue, as discussed in Section 5 below.

4.2. Solubility

Most of the clinically relevant Nrf2 activators show low solubility in water and different formulations and administration approaches have been studied to address drug delivery challenges, including solid dispersions, and self-micro-emulsifying drug delivery systems (SMEDDS). Solid dispersion refers to the dispersion of active ingredients in a hydrophilic inert carrier matrix which is relatively soluble and requires no energy to break [154]. This technique can be employed to improve the dissolution rate of weakly water-soluble drugs and its advantages include enhanced drug stability, adequate porosity, and wettability [155,156]. Solid dispersion of a phospholipid complex of the Nrf2 activator quercetin exhibited improved pharmacokinetic properties and more potent protective effects in a mouse retina oxidative injury model when compared to the non-formulated quercetin [157]. Solid dispersion also has disadvantages including poor physical and chemical stability, which can result in degradation. Once the polymers used in solid dispersions absorb moisture, structural changes can occur to result in decreased solubility and instability [158–160], hindering scale-up and subsequent manufacturing potential. SMEDDS form an oil-in-water microemulsion and consist of a homogenous mixture of oils and surfactants which [161] enhance the bioavailability of poorly soluble drugs by protecting against enzymatic degradation and improving their membrane permeability across the mucosal barrier of the intestine [161]. Like solid dispersions, they can increase the solubility and dissolution rate of a drug but contribute an additional process of increased bioavailability and quick absorption via the promotion of lymphatic uptake [152,162,163]. This system has been utilized for the improvements in solubilities of Nrf2 modulators of low bioavailability [164–166], however, there are drawbacks with this technique. Despite the apparent improved dissolution rates and solubilities, animal studies have not demonstrated promising results regarding bioavailability when comparing the SMEDDS formulation of polyphenols or Nrf2 modulators to non-formulated ingredients [152,167]. Drug precipitation has been observed *in vivo* as well as limited lymphatic uptake [168–170]. SMEDDS contain relatively large amounts of surfactants such as Tweens and polyoxylglycerides, so clinical applications may be hindered by their potential toxicity and GI tract irritability [152,165].

4.3. Specificity

Another challenge in the use of Nrf2 activators is their lack of specificity leading to the modulation of numerous intracellular signaling pathways that are relevant for cell survival. Many of the activators of Nrf2 are electrophiles that target the negative regulator Keap1 [171]. The modification of Keap1 thiols represents a strategy for the irreversible stabilization of Nrf2 in the constitutive pathway while the competitive displacement of Nrf2 from its binding sites on Keap1, via the use of Nrf2 peptides or small molecules that are specific for the Keap1 Kelch domain, constitute a reversible method. This reversible method may appear to be more favorable at first glance as it avoids the

non-specific alkylation or the oxidation of reactive thiols in proteins besides Keap1 [172]. With this method, however, because (1) there are numerous Keap1-interacting proteins and (2) there are many Kelch-like proteins that contain Kelch domain structures very similar to the one in Keap1, off-target effects can be expected also for this class of Nrf2 activators. In addition, the modification of Keap1 thiols, or the irreversible method, carries with it the possibility of numerous side effects given that Keap1 has many protein partners in cells, which span a plethora of cellular processes including DNA replication, transcription, and cellular apoptosis. It may prove difficult to predict the plausible side effects while considering the limited detailed information about these Keap1-interacting proteins [172]. For example, in a phase III trial (BEACON), daily intake of 20 mg of bardoxolone methyl was found to be associated with adverse cardiovascular complication in type 2 diabetes (T2D) and stage 4 chronic kidney disease patients [173]. Since ubiquitination of Nrf2 is required for its degradation, inhibition of this Keap1-mediated pathway may prove fruitful for maintaining levels of Nrf2 and its resultant activation. The thiol-modifying agents that are currently in existence are potent inducers of the Nrf2-mediated antioxidant response, however, specificity for Keap1 is an ongoing consideration and notwithstanding the possible effects coming from the many Keap1-interacting proteins. Depending on concentration, these agents may affect multiple residues within Keap1 and other proteins [171,174] further contributing to the likelihood of unintended effects. Overall, a better understanding of the crystal structure of KEAP1 alone as well cocrystal structure with the Neh2 domain of Nrf2, and others may help in designing more selective Nrf2 activators. It needs to be emphasized, however, that having pleiotropic effects is not always a disqualifying characteristic for a drug, as many drugs in clinical use, including statins, show beneficial effects by targeting multiple biochemical pathways.

4.4. Disease heterogeneity

The activation of the endogenous Nrf2/KEAP1/ARE pathway via application of Nrf2 activators may benefit patients with certain diseases like diabetes, CVDs and neurological diseases accompanied by elevated ROS production, reduced activity of antioxidant response enzymes and chronic inflammation. A cautionary note is that while activation of Nrf2 signaling pathway is generally beneficial, the constitutive activation of Nrf2 pathway may confer differential response, for example in numerous cancers, it may induce expression of pro-survival genes, promoting cancer cell proliferation and malignant progression. Additionally, it has been suggested that Nrf2 activation that functions to restore redox balance varies across cell types, and this may affect the ability of Nrf2 activators to inhibit inflammation. Future research needs to be designed using animal models of different cancer types as well as both cancer and other diseases simultaneously to better examine where Nrf2 activation may be beneficial vs. harmful.

4.5. Patient heterogeneity

An overarching goal of the research on Nrf2 activation pathways has been to develop Nrf2 activators-based therapies to mitigate IMIDs. This has been allied with progress in conducting clinical trials with Nrf2 activators. For example, dietary supplementation of 400 mg of RSV twice a day induced significant decrease in level of $O_2^{\bullet-}$ in PBMCs and increase in plasma total antioxidant capacity in T2D patients [175]. Another pilot study in Friedreich ataxia patients designated as MOXIe, demonstrated that omaveloxolone, an Nrf2 activator, at the optimal dose level of 60 mg/day improved neurological function [176]. More recently, it has been reported from a retrospective observational study on patients with relapsing-remitting multiple sclerosis that DMF treatment *in vitro* reduced activation of inflammatory markers and iron content in patient isolated M1-polarized microglial cells [177]. Although few studies with Nrf2 activators reported beneficial effect at reducing disease complication, evidence for using Nrf2 activators from few clinical trials has demonstrated variable and conflicting results. A placebo-controlled randomized trial demonstrated that 500 mg/day supplementation of RSV for 4 weeks could not induce antioxidant and anti-inflammatory effects in nondialyzed CKD patients [178]. In addition, a placebo-controlled trial with RSV on T2D patients showed that oral administration of 40 mg/day or 500 mg/day RSV for 6-months had no beneficial effect on the metabolic pattern or concentration of C reactive-protein (CRP) of T2D patients [179]. Another, clinical trial where T2D patients were supplemented with 800 mg/day RSV for 8-weeks did not find any significant change in the metabolic parameter, inflammatory cytokines, nor circulating $CD14^+$ $CD16^+$ monocytes [180]. These apparent discrepancies across different Nrf2 activators in different IMIDs observed in clinical trials remain to be addressed. Some questions remaining to be answered are as follows: Is the heterogeneity related to the disease traits, onset, and rates of progression, or a combination of those factors a rational explanation for the limited success of Nrf2 activator-based therapeutic interventions in clinical trials across distinct IMIDs? Are there specific subgroups/endotypes of patients with a distinct outcome and therapeutic responsiveness? Also, for studies with individual Nrf2 activators in IMIDs and to assure consistency, the following questions need to be addressed: how do canonical immunological pathways differ across patient endotypes as well as across different IMIDs? What is the implication of functional polymorphisms in the Nrf2 gene across patient subgroups? While enrolling the participants, are the confounding factors like age, sex, autoantibody status, and genotype considered? Answering those questions may be important to gain benefit from the future Nrf2 clinical trials and would be transformational to clinical practice.

5. Recent advancement in Nrf2 activator-based strategy in the treatment of inflammatory diseases

The advent of nanotechnology to medicine has made for a promising strategy for the enhanced intracellular delivery of drugs. Nanocarriers can solve drug stability-related issues and their ability to retain their content makes for a desirable feature for successful delivery of drug to diseased tissues [181]. Nanoparticle-based drug delivery systems (nanoDDSs) have clinical appeal as they can increase drug stability and protect drugs from untimely degradation, thus improving their overall bioavailability and physiological outcomes [182]. Moreover, the controlled release of entrapped drugs using this system is common and involves an initial burst release followed by a steady, prolonged release over an extensive period [182]. Encapsulation of natural compounds, such as SFN, can significantly improve their stability [183], enabling their sustained release into cells [184], thus improving both their antineoplastic [185] as well as anti-inflammatory activities [186]. Some encapsulation systems use proteoliposomes, consisting of proteins and lipids [187,188], which simulate the lipid-protein environment of native membranes and thus provide additional stability. Zein nanoparticles, consisting of a heterogenous mixture of different peptides, have been demonstrated to improve the bioavailability of RSV, which would otherwise be highly metabolized to products of variable physiological activity, and its anti-inflammatory effects [189]. Release of RSV from this system was found to be controlled and pH-independent with its relative bioavailability 19-fold higher compared to RSV administration without the protein-based system [189]. The amphiphilic characteristic of zein protein, owing to its high percentages of hydrophobic amino acids [190], makes it insoluble in water, which ultimately contributes to the controlled release of the loaded drugs [189,191].

The size of the nanoparticle is an important contributor to the success of its transport across biological barriers [192]. For example, RSV is a potent antioxidant itself but following its nanoformulation, can easily penetrate neurons to protect them from lipid peroxidation with higher potency [192]. Higher permeation has been observed when the size of the particles was smaller and there are different targeting approaches to consider. Passive targeting approaches have been proposed, such as the

delivery of nanotherapeutics to neoplastic cells in cancer research [193]. This method exploits the distinctive characteristics inherent to the tumor microenvironment that are not normally present in healthy tissue [193]. Passive targeting also depends on the properties of the nanoparticle itself including size, shape, and surface properties [194]. Despite the validation of the capacity for nanoDDSs to passively target cancer cells in several animal models via different imaging techniques [195–197], overall passive targeting has shown heterogeneous and questionable efficacy in tumor targeting. The use of nanoparticles in the clinic is rather rare, despite their relative abundance in preclinical development [196]. Further developments in this field may increase the clinical use of nanoDDSs and offer a viable option for the efficient delivery of potent Nrf2/Keap1 modulators.

Much work with nanoDDSs that encapsulate Nrf2 modulators has been reported in the context of cancer therapy with the desired outcome of suppressing Nrf2 signaling. The anti-Nrf2 sentiment arose from the findings that Nrf2 stimulates cancer cell growth and proliferation and suppresses cancer cell apoptosis [198], as mentioned above, while also attenuating the inflammatory response of the immune system [199]. Nrf2 has been suggested to act as an anti-inflammatory regulator [56] whose activation promotes an M2-like phenotype of macrophages and suppresses macrophage [199] inflammatory response by blocking proinflammatory cytokine transcription [200,201]. Consequently, nanoDDSs have been leveraged to selectively suppress the Nrf2-mediated cytoprotection program in cancer cells and modulate macrophages' polarization from the immunosuppressive M2 phenotype to the antitumor M1 phenotype [201].This delivery system synergistically targeted both cancer cells and the tumor environment while sparing non-malignant cells.

More recently, Nrf2 activators-based nanoformulations are being explored to restore immune homeostasis in numerous inflammatory diseases. For instance, Molin et al. demonstrated that the application of SFN-loaded broccoli membrane vesicles mediated a dose-dependent reduction in the secretion of TNFα, IL-1β and IL-6 *in vitro* from the HL-60-derived macrophage-like cells stimulated with LPS [202]. Similarly, Pavez and colleagues analyzed the effect of broccoli membrane vesicles and SFN, either free or encapsulated, on the activity of human monocyte-derived M1 and M2 macrophage primary culture obtained from peripheral blood of healthy donors [203]. The pretreatment of M1 macrophages with SFN-loaded broccoli membrane vesicles (SFN-VES) significantly reduced *C. albicans*-induced secretion of TNFα, IL-6, and IL-1β compared to the control [203].

In line with this, several studies have demonstrated a protective role of RSV nanoparticles in inflammation-driven pathologies. Reduction in ROS production and lipid peroxidation using RSV nanoparticles led to the lowering of TNFα and IL-1β levels enhancing hepatoprotective effects in an animal model of CCl_4-induced hepatotoxicity [204]. Consistently, administration of RSV using galactosylated degradable nanocarriers such as poly (lactic-co-glycolic acid) (PLGA) promoted anti-inflammatory effects via lowering the production of TNFα and IL-6 and release of $^{\bullet}$NO from the LPS stimulated RAW 264.7 macrophages [205]. In another study, RSV-loaded zein/pectin nanoparticles exhibited strong anti-inflammatory effects via inhibiting the production of TNFα, IL-1β, IL-6, $^{\bullet}$NO, PGE_2 as well as inhibiting expression of TLR4, and inhibiting phosphorylation of JNK, ERK1/2, p38 and MAPK in (LPS)-treated RAW 264.7 macrophages [206]. Of importance, intraperitoneal administration of nanostructured lipid carrier (NLC) containing RSV in a rat model of middle cerebral artery occlusion (MCAO) showed a substantial reduction in infarction compared to saline controls in parallel with improved motor and cognitive function. Further, NR pretreatment ameliorated oxidative stress and reduced the level of IL-1β, IL-6, and TNF-α in the MCAO group when compared to sham [192]. In the neuroprotective assessment of RSV-based nanoparticles *in vivo*, Xiajun et al. reported that coadministration of curcumin and RSV via hydrogel/nanoparticle system mitigated expression of *NF-κB* and *TNFα* genes in a rat model of traumatic spinal cord injury [207]. Caldas et al. also reported that a combined strategy involving two different lipid nanosystems (liposomes and lipid nanoparticles) that encapsulated Omega-3 and RSV in their lipid matrix exerts a protective effect against lipid peroxidation, as well as inhibition of cyclooxygenase (COX) and $^{\bullet}$NO production in the RAW264.7 cell line [207].

Consistent with several of the RSV and SFN-based nanoformulation studies, the inhalation of solid lipid nanoparticles (SLNs) containing DMF demonstrated effective therapeutic efficacy in experimental mice models of autoimmune encephalomyelitis and pulmonary dysfunction by downregulating inflammation and increasing the influx of Tregs into the spinal cord and lung tissues [208]. This is further supported by another study where oral administration of DMF-incorporated SLNs was found to be protective against multiple sclerosis mice induced with experimental autoimmune encephalomyelitis (EAE) [209].

In summary, Nrf2 activators-based nanoformulation can emerge as a viable and superior approach in the treatment of inflammatory diseases. Further investigation into therapeutic efficacy and post-treatment protocols is needed to come to an agreement about the potential use, and safety of using Nrf2 activator-based nanoparticles in clinical trials in IMIDs. Different Nrf2 activators-based nanoparticles and their role in modulating immune responses (Fig. 5) in distinct inflammatory diseases are summarized in Table 2.

6. Conclusion and perspectives

As we elucidate the therapeutic potential of Nrf2 in the modulation of inflammatory responses in preclinical and clinical studies, the horizon of Nrf2-targeting therapies continues to expand. This review underscores Nrf2's role as a vital transcription factor with the ability to attenuate the pathogenesis of a variety of inflammatory diseases, offering a beacon of hope for new treatment strategies. However, realizing the full therapeutic promise of Nrf2 activators requires a comprehensive and multi-dimensional future research and experimental models. Additionally, the following areas require a focused attention to enhance the translational potential of Nrf2 activators:

Differential disease response. Emerging area of research in autoimmunity, neurotoxicity, chronic infection as well as cancer immunotherapy illustrates diverse phenotypes and function across immune cell subsets acting as a major barrier in designing successful therapeutic interventions [210–212]. Notably, these differences could be in part attributed to the differential Nrf2 activation states in several tissues under both normal and inflammatory conditions. However, Nrf2 signaling may also confer a differential response to promote inflammation. For example, the results of many functional studies in mouse disease models of colitis, autoimmune nephritis, and pancreatitis reflected that Nrf2 actively promotes inflammation and monocyte and macrophage infiltration into damaged epithelial as well as other tissue types [213]. In the DSS-induced mouse model of colitis, the adipocyte specific Atg7 CKO mouse exhibited an oxylipin imbalance, driven through Nrf2 mediated upregulation of epoxide hydrolase I (EPHX1). Notably, this contrasted with reduced secretion of the anti-inflammatory cytokine IL-10 from the adipose tissues which was dependent on the cytochrome P450-EPHX pathway to exacerbate intestinal inflammation, suggesting the role of autophagy-dependent regulation of anti-inflammatory oxylipins via the Nrf2 signaling [214]. Similarly, in a mouse model of autoimmune nephritis, compared to the nrf2+/+lpr/lpr female mice, the deficiency of Nrf2 was found to enhance TNF-α mediated apoptosis in the hepatocytes and splenocyte of nrf2−/−lpr/lpr mice, suggesting that Nrf2 deficiency acts as a suppressor of the autoimmune accelerating gene, lpr [215]. Additionally, in acute pancreatitis (AP) a mouse model of Nrf2 deficiency decreased inflammation and macrophage infiltration into the damaged pancreas [216]. Given this prior observation, ensuring an effective therapeutic response to arrive at better understanding of the Nrf2-mediated signaling pathways in distinct IMIDs is warranted. Perhaps a comparative analysis of how different inflammatory diseases respond to Nrf2 modulation is necessary for successfully designing,

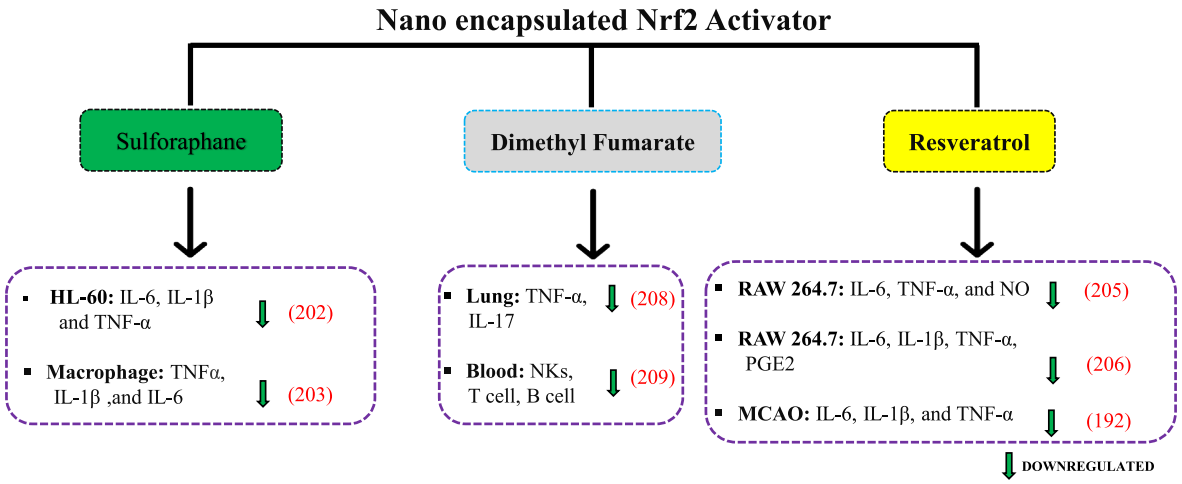


Fig. 5. Nano formulations of Nrf2 activators in modulating immune response within distinct immune cell subsets in different experimental models. IL-1β: Interleukin-1 beta; Interleukin (IL)-6; IL-10: Interleukin (IL)-10; (TNF)-α: tumor necrosis factor; PGE2: Prostaglandin E2; NO: Nitric oxide; Tregs: Regulatory T cells; HL-60: acute promyelocytic human leukemia (HL-60) cells; MCAO: Middle Cerebral Artery Occlusion; ALE: autoimmune encephalomyelitis.

Table 2
Evidence of Anti-inflammatory effect of Nrf2 Activators based nanoconjugates on distinct immune cells.

Nrf2 Activators	Nano-conjugates	Experimental Model	Mechanism of Action	References
Sulforaphane	broccoli membrane	LPS stimulated HL-60 cells	Attenuated TNF-α, IL-1β and IL-6 production	[202]
	broccoli membrane	M1 and M2 macrophages from healthy donor	Reduced C. albicans induced secretion of TNF-α, IL-6, and IL-1β	[203]
Resveratrol		animal model of CCl_4-induced hepatotoxicity	Reduced TNF-α and IL-1β enhancing hepatoprotective effects	[204]
	galactosylated nanoparticles	LPS-induced RAW 264.7	Lowered the production of TNF-α and IL-6 and release of nitric oxide (NO)	[205]
	zein-pectin core/shell nanoparticles	LPS-induced RAW 264.8	Inhibited the production of NO, PGE2, IL-1β, IL-6, TNF-α	[206]
	calcium alginate hydrogel	Rat Model of Traumatic Spinal Cord Injury	Attenuated ROS production and lipid peroxidation	[207]
Dimethyl Fumarate	Solid lipid nanoparticles (SLNs)	Mice model of EAE	Downregulated inflammation and increased the influx of Tregs	[208]
	Solid lipid nanoparticles (SLNs)	Mice model of EAE	Reduced numbers of T cells, B cells and natural killer (NK) cells in the blood	[209]

IL-1: Interleukin (IL)-1; IL-2: Interleukin (IL)-2; IL-4: Interleukin (IL)-4; IL-6: Interleukin (IL)-6; IL-10: Interleukin (IL)-10; IL-12: Interleukin (IL)-12; IL-17: Interleukin (IL)-17; IL-23: Interleukin (IL)-23; MCP1: Monocyte chemoattractant protein 1; Igγ: Ig gamma; (TNF)-α: tumor necrosis factor; IFNγ: Interferon γ; Peripheral blood mononuclear cells; LPS: Lipopolysaccharide; THP-1: Monocyte-like cell line; moDCs: Monocyte-derived dendritic cells; DC: dendritic cells; SMCs: Smooth muscle cells; Tregs: Regulatory T cells; Bregs: Regulatory B cells; EAE: Experimental autoimmune encephalomyelitis.

conducting, and interpreting Nrf2-based therapies to specific conditions. Additionally, characterization of endotypes (well-defined patient subtypes) exhibiting differing immune phenotypes and therapeutic responsiveness within distinct IMIDs [217] would help to predict Nrf2-based therapeutic responsiveness and identify disease-specific immune cell populations for designing targeted therapeutic strategies.

Single vs. multiple targets. All discussed Nrf2 activators have multiple cellular targets and show pleiotropic biological activities. While those may be beneficial, detailed understanding of the molecular targets and pathways affected is necessary.

Dosing strategies. Drugs typically exhibit dose dependent toxicity that can lead to chronic as well unpredictable side-effects [218]. Henceforth, establishing dose escalation strategies for Nrf2 activators based on relevant preclinical models and through rigorous clinical trials with appropriate sample size will be essential for their safe and effective clinical application.

Delivery approaches. The therapeutic efficacy of drugs to a large extent depends upon its absorption, distribution, metabolism, and elimination (ADME) within the physiological system [219]. In recent years advent of nanomedicine has greatly improved the drug efficacy using biodegradable, biocompatible, and well-tolerated nanoparticles. In the future it will provide an opportunity to utilize nanoDDSs in the clinical setting for the targeted delivery of Nrf2 activators in inflammatory diseases.

Long-term effects. The development of a broadly applicable therapies to manage inflammation is a challenging task [220]. It's extremely important to assess long term effects via establishing the safety and quality of the drug. Investigating the long-term effects of sustained Nrf2 activation on immune function and identification of potential risks will be crucial for ensuring patient safety.

Translational milestones. During the pre- or post-onset disease progression patients depict changes in the function and phenotype of distinct immune cell subsets [221,222]. Therefore, Nrf2 supplementation and targeted follow up studies to measure Nrf2-induced changes in combined immune cell signature and activity associated with disease progression will provide an opportunity to develop biomarkers for Nrf2 activators, to be used in the clinic by aiding in patient selection, therapeutic monitoring, and potentially in modification of the drug administration regimen over the course of treatment.

Robust clinical trials. Despite of the advancement in the field of immune therapeutics, and many randomized controlled trials (RCTs) with new drugs, a large portion of patients fail to respond or eventually relapse after treatment, demonstrating the need for further improvement in anti-inflammatory therapeutic strategies [223]. Well-designed human studies allowing multiple randomizations of patients at different stages, utilizing large retrospective datasets to predict results of

RCTs, and allowing the use of advanced statistical methodology are imperative to confirm the benefits of Nrf2 activators observed in pre-clinical models, focusing on both efficacy and safety.

Exploration of additional pharmacological agents upregulating Nrf2 signaling. There is a growing body of work investigating a range of compounds—from established drugs being repurposed to novel molecules designed to enhance Nrf2 pathways. In this context bioactive metabolites and synthetic analogs of Nrf2 activators have been found to be highly effective in mouse models of neurotoxicity [224]. For instance, DMF and its bioactive metabolite monomethylfumarate (MMF) was found to activate *in vitro* the Nrf2 pathway via promoting the S-alkylation of Keap1 and showed neuroprotective effect [224]. More recently, MMF was found to rescue human astrocytes from oxidative stress via modulating oxidative stress induced growth inhibitor 1 (OSGIN1) expression, and this effect was found to mediated by Nrf2 activation [225]. Similar effects were observed using quinone diterpene Cryptotanshinone, that was found to attenuate LPS-induced neuroinflammation via Nrf2/HO-1 signaling pathway in BV-2 microglial cells [226]. Additionally synthetic analog of SFN such as SFX-01 (Evgen Pharma developed drug) have been recently explored for subarachnoid hemorrhage, breast neoplasm and prostate cancer, and AD drug development [63,227]. Moreover, chimeric peptides such as Keap1-Keap1 peptide (KKP1) based on proteolysis-targeting chimera technology (PROTAC) are also being considered to induce KEAP1 degradation via ubiquitination. For example, KKP1 peptide activated Nrf2 downstream factors such as HO-1, and inhibited NF-Kβ, TNF-α, and IL-1β related inflammatory pathways in rat hepatic stellate (HSC-T6) cell line [228]. Furthermore, recently reported bivalent KEAP1 inhibitor, was found to instantly activate Nrf2 resulting in the maintenance of redox homeostasis, as well as the resolution of acute inflammatory response *in vivo* [229]. These studies are crucial in broadening the available arsenal of Nrf2-modulating agents and understanding their distinct mechanisms of action, safety profiles, and therapeutic windows.

Human stem cell models for Nrf2 signaling-related drug testing. The emergence of stem cell technology has opened a unique avenue for conducting high-throughput investigations into the effects of Nrf2 activators within a human context. Human-induced pluripotent stem cells (iPSCs), reprogrammed from somatic cells like skin fibroblasts, offer the advantage of almost limitless expansion in culture and the potential to differentiate into virtually all cell types. In recent years, our research group, along with others in the field, has successfully generated a wide array of cells and three-dimensional organoids derived from human iPSCs for disease modeling and drug assessment [10,61,230–233]. Importantly, the successful generation of various immune cell types, including macrophages, dendritic cells, and microglia, as well as organoids containing immune cells from human iPSCs have been well documented [234–237]. These stem cell-based models of human immune cells and organoid systems offers a robust platform for more precise modeling of human inflammation diseases, playing a pivotal role in bridging the gap between the findings from animal studies and real-world clinical scenarios. These discoveries will provide invaluable insights into the response of human Nrf2 signaling in diverse disease states. Furthermore, these human stem cell-derived immunocytes can be used to assess the therapeutic effectiveness of Nrf2 activators in various inflammation diseases within a human context.

In conclusion, the path ahead for Nrf2 research and its extensive therapeutic application in various inflammatory diseases is promising. Optimization of Nrf2 activator delivery approaches, integration of innovative drug discoveries, the use of advanced stem cell-derived human immune cell models, and well-designed clinical studies, will pave the way for Nrf2 activators to emerge as effective therapies for a spectrum of inflammatory conditions. The collective efforts of basic and clinical researchers in this dynamic field of research hold the potential to drive progress in translational medicine, ultimately leading to enhancements in patient care and outcomes in the face of inflammatory diseases.

Funding

This work was partially supported by grant R01GM112696 and 1R35GM148177 from the National Institutes of Health (to X. Bai), Advancing a Healthier Wisconsin (to X. Bai), and Neuroscience Research Center Award from Medical College of Wisconsin (to X. Bai).

CRediT authorship contribution statement

Tarun Pant: Writing – review & editing, Writing – original draft, Supervision, Software, Resources, Investigation, Formal analysis, Conceptualization. **Nnamdi Uche:** Writing – review & editing, Writing – original draft. **Matea Juric:** Writing – review & editing. **Jacek Zielonka:** Writing – review & editing. **Xiaowen Bai:** Writing – review & editing, Writing – original draft, Supervision, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Acknowledgements

All the images in the present manuscript were created using BioRender.com.

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