

## EGCG at the Interface of IDO, Nrf2, and Treg cells

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### Short Communication

Arthritis and related conditions have been recognized as the third largest contributor to direct health care expenditure, affecting nearly 48 million people in USA [1]. More importantly, the economic and social burden of arthritis is expected to grow, as the number of people with arthritis is expected to reach 67 million in the USA by 2030 [1,2]. Among various arthritic conditions, rheumatoid arthritis (RA) is the most common inflammatory arthritis worldwide. It is a chronic, progressive autoimmune disease characterized by cellular infiltration and proliferation of synovium, leading to progressive destruction of articular bone and cartilage [3]. As RA tends to be progressive in nature, the current treatments, including conventional therapies and biologics, mainly aim to slow clinical progression and relieve patient symptoms. However, the limited efficacy and side-effects associated with these therapies underscore the need for new, effective, and safer approaches to treating RA.

Green tea is a widely consumed beverage throughout the world, with a wide spectrum of proven health benefits. Epigallocatechin-3-gallate (EGCG) is the most abundant and most biologically active catechin found in green tea [4]. Emerging evidence has documented that EGCG possesses much of the health promoting properties ascribed to green tea, including anti-inflammatory, immunomodulatory and antioxidant effects. In 1999, Haqqi and colleagues first reported the disease modulating effects of green tea polyphenols (GTPs) on RA in a murine collagen-induced arthritis (CIA) model. They reported that GTPs administration significantly reduced the incidence and lowered the disease severity of arthritis, associated with a marked reduction in IFN- $\gamma$ , TNF- $\alpha$ , and cyclooxygenase (COX)-2, as well as total IgG and type II collagen-specific IgG (Ab) in the arthritic joints [5]. Following this, the therapeutic effects of green tea or EGCG have been consistently reported in a series of experiments using either CIA or Adjuvant-induced arthritis (AIA) animal models [6-12]. Interestingly, in a prospective cohort study of 31,336 subjects, Mikuls et al. reported that consuming >3 cups tea per day significantly reduced the risk of RA development [13].

More recently, Min and colleagues induced CIA in DBA/1J mice and fed these mice EGCG 10 mg/kg nine times over three weeks [3]. In agreement with earlier findings, their results again confirmed the therapeutic efficacy of EGCG on RA. Of relevance to this commentary, this work has contributed additional mechanistic insights on how EGCG might be functioning at the molecular level.

Although EGCG has documented effects on both the adaptive and innate arms of the immune system, a substantial degree of research has focused on the impact of EGCG on T cell function, in the context of arthritis. Of various T cell subsets, regulatory T cells (Treg) play a vital

role in maintaining immune tolerance and suppressing autoimmunity. Several studies have demonstrated that EGCG administration can increase Treg cell frequencies both *in vitro* and *in vivo*, accompanied by reduced T cell responses [10-12,14,15]. However, how EGCG modulates Treg cell function is unclear. Wong et al. proposed that EGCG could function as a DNA methyltransferase (DNMT) inhibitor to induce Foxp3 expression in naive CD4<sup>+</sup> T cells, hence enhancing Treg cell formation and function *via* an epigenetic mechanism [14]. An alternative mechanism has also been proposed by Wu and colleagues, whereby EGCG induced Treg cell differentiation was mediated by its dampening of IL-6 signaling, including reduced soluble IL-6R, membrane gp130, and IL-6-induced phosphorylation of STAT3 in naive CD4<sup>+</sup> T cells [16]. Min et al. also reported the increased frequency of Treg cells in the draining lymph nodes (dLNs) from EGCG-fed CIA mice. They also demonstrated a novel mechanism addressing how EGCG administration might lead to an increase in the number of Treg cells [3]. First, they found EGCG could enhance indoleamine-2, 3-dioxygenase (IDO) expression by CD11b<sup>+</sup> DCs, and these CD11b<sup>+</sup>IDO<sup>+</sup> DCs were functionally active. Second, using an *in vitro* coculture system, they reported that splenic CD11b<sup>+</sup>IDO<sup>+</sup> DCs from EGCG-fed mice were more potent in differentiating CD4<sup>+</sup>CD25<sup>-</sup> T cells into Treg cells. Third, to address whether these effects are IDO-dependent, an IDO inhibitor, 1-MT, was added to the CII antigen-stimulated coculture system. Indeed, the increase in the proportion of Treg cells from EGCG-fed mice was significantly abrogated. This finding was further supported by their *in vivo* studies, where 1-MT treated EGCG-fed mice displayed similar disease severity as the vehicle-fed control CIA mice. These studies suggest that EGCG induces IDO expression in CD11b<sup>+</sup> DCs, and these CD11b<sup>+</sup> IDO<sup>+</sup> DCs in turn generate Treg cells from CD4<sup>+</sup>CD25<sup>-</sup> T cells, *via* an IDO-dependent pathway. Taken together, these recent reports have advanced novel mechanisms explaining how EGCG modulates Treg cells in CIA.

Green tea has well documented anti-oxidant properties. In several murine models of nephritis, the improvement in renal function and histology with EGCG administration was associated with the restoration of Nuclear Factor Erythroid 2-Like 2 (Nrf-2) signaling [17-19]. Nrf-2 is a transcription factor that plays a major role in cellular defense against oxidative stress by inducing inactivate reactive oxygen species, such as Heme oxygenase-1 (HO-1). Nrf2 has been documented to be activated in the joint tissue from both arthritic mice and RA patients. Importantly, mice deficient in Nrf2 displayed more severe cartilage injury and more oxidative damage [20,21]. In patients with RA, Nrf2 activity strongly inversely correlates with RA disease activity [22]. In agreement with these findings, Min's results showed that EGCG treatment significantly increased pNrf2 activity and the expression of HO-1, an Nrf2 target gene, in the CIA model of arthritis [3].

These observations lead to an important question: is there any link between EGCG induced immunoregulation and the EGCG induced antioxidant response? Indeed, emerging evidence indicates that Nrf2, the key modulator in the antioxidant pathway, also plays a role in modulating immune responses. Perhaps the clearest demonstration of this is the observation that mice deficient in Nrf2 are susceptible to various inflammatory disorders including asthma and colitis [23,24]. Moreover, CD4<sup>+</sup> T cells isolated from Nrf2 deficient mice produce increased amounts of IFN- $\gamma$  [21], while the activation of Nrf2 in T cells inhibits IFN- $\gamma$  secretion [25]. Using siRNA blockade, two independent studies provide further support for the role of Nrf2 in EGCG-induced immunoregulation. EGCG can inhibit Transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1) induced epithelial-mesenchymal transition (EMT), and this effect was completely blocked by siRNA-mediated knockdown of Nrf2, indicating that EGCG prevents TGF- $\beta$ 1 induced EMT via the Nrf2-mediated suppression of TGF- $\beta$ 1 signaling [26]. Similarly, silencing Nrf2 increased the expression of pro-inflammatory genes and decreased antioxidant gene expression in coplanar PCB 126-stimulated, EGCG-treated vascular endothelial cells [27].

In Min and colleagues' work, they also explored whether the molecule IDO was related to the activation of Nrf2 [3]. They treated EGCG-fed CIA mice with the IDO inhibitor, 1-MT, and found 1-MT treated EGCG-fed CIA mice had comparable disease severity as the vehicle-fed CIA mice, as alluded to above. In this study, they also examined the pNrf2, Nrf2 and HO-1 levels in the joint homogenates from EGCG-fed CIA mice, 1-MT treated EGCG-fed CIA mice as well as the vehicle-fed CIA mice. The 1-MT treated EGCG-fed CIA mice exhibited similar levels of all three molecules as compared to the vehicle-fed CIA mice, implicating an IDO-dependent mechanism for the enhanced Nrf2 expression. More recently, yet another novel link between the anti-oxidant and anti-inflammatory effects of EGCG has been reported. Zeng et al. reported that EGCG down-regulated NLRP3 inflammasome expression in a contrast-induced nephropathy murine model, which was completely blocked by protoporphyrin IX zinc (II), an HO-1 blocker, indicating that antioxidant pathway molecule, HO-1, links EGCG's therapeutic effects with inflammation pathway signaling via NLRP3 [28].

In summary, the recent works by Min et al. and others offer several novel perspectives on the role of EGCG in arthritis, autoimmunity, and inflammation. Besides validating the salubrious effects of EGCG on arthritis, these studies tie together several disparate molecular pathways including the IDO pathway, the Nrf2 dependent antioxidant pathway, and Treg cell mediated immunoregulation. It appears very likely that the interplay of these different cellular and molecular pathways also lies at the heart of how EGCG might modulate other autoimmune and inflammatory diseases, though this needs to be formally tested in future studies.

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