

MINIREVIEW

Control of autoimmune inflammation by celastrol, a natural triterpenoid

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One sentence summary: Celastrol, a natural herbal product, has anti-arthritis activity and it is a promising candidate for further testing in patients with rheumatoid arthritis and other autoimmune diseases.

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ABSTRACT

Celastrol is a bioactive compound derived from traditional Chinese medicinal herbs of the Celastraceae family. Celastrol is known to possess anti-inflammatory and anti-oxidant activities. Our studies have highlighted the immunomodulatory attributes of celastrol in adjuvant-induced arthritis (AA), an experimental model of human rheumatoid arthritis (RA). RA is an autoimmune disease characterized by chronic inflammation of the synovial lining of the joints, leading eventually to tissue damage and deformities. Identification of the molecular targets of celastrol such as the NF- κ B pathway, MAPK pathway, JAK/STAT pathway and RANKL/OPG pathway has unraveled its strategic checkpoints in controlling arthritic inflammation and tissue damage in AA. The pathological events that are targeted and rectified by celastrol include increased production of pro-inflammatory cytokines; an imbalance between pathogenic T helper 17 and regulatory T cells; enhanced production of chemokines coupled with increased migration of immune cells into the joints; and increased release of mediators of osteoclastic bone damage. Accordingly, celastrol is a promising candidate for further testing in the clinic for RA therapy. Furthermore, the results of other preclinical studies suggest that celastrol might also be beneficial for the treatment of a few other autoimmune diseases besides arthritis.

Keywords: celastrol; inflammation; autoimmune diseases; immune modulation; natural products

INTRODUCTION

Rheumatoid arthritis (RA) is a debilitating autoimmune disease characterized by chronic inflammation of the synovial lining of the joints, damage to cartilage, and bone and deformities of the joints (Harris 1990; Scott, Smith and Kingsley 2003; Lipsky 2005). Both genetic and environmental factors are involved in the initiation and progression of this disease (Harris 1990; Lipsky 2005). RA pathogenesis involves the activation of autoreactive T

cells; increased production of pro-inflammatory cytokines; enhanced production of chemokines and increased migration of pathogenic cells into the joints; and increased production of mediators of bone remodeling leading to tissue damage in the joints (Harris 1990; Scott, Smith and Kingsley 2003; Lipsky 2005; Furst and Emery 2014). The non-steroidal anti-inflammatory drugs, the disease-modifying anti-rheumatic drugs and steroids represent the mainstream therapeutics for RA. The long-term use of conventional anti-inflammatory or immunosuppressive agents

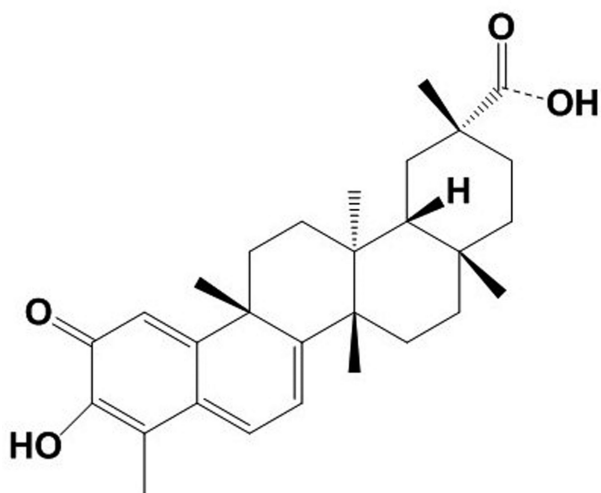


Figure 1. Structure of celastrol. Celastrol is a pentacyclic triterpenoid with a molecular formula $C_{29}H_{38}O_4$ and molecular weight 450. Celastrol is also known as tripterine/tripterin.

can have severe adverse effects (Couzin 2004; Kremers *et al.* 2004). Biologics such as anti-tumor necrosis factor- α antibody and anti-interleukin-6 (IL-6) receptor antibody, which target specific pro-inflammatory cytokines involved in the pathogenesis of arthritis, are quite effective in a proportion of RA patients but their use can predispose patients to severe infections (Lee, Yedla and Kavanaugh 2003; Garcia-Hernandez, Gonzalez-Amaro and Portales-Perez 2014). Additional drawbacks of the use of biologics include the unresponsiveness of subsets of patients, inconvenience of administration by injection and high cost (Lee and Kavanaugh 2005; Rosman, Shoenfeld and Zandman-Goddard 2013). Accordingly, there is a need for safer and effective new therapeutic products for RA.

Medicinal plants belonging to the traditional systems of medicine offer many promising herbal extracts and/or purified compounds such as *Tripterygium Wilfordii* Hook F (Thunder God Vine), *Boswellia Serrata* and celastrol (Fig. 1) for therapeutic purposes in arthritis (Abdel-Tawab, Werz and Schubert-Zsilavec 2011; Venkatesha *et al.* 2011; Liu *et al.* 2013; Lv *et al.* 2015). However, certain essential prerequisites for their effective application include: a thorough characterization of the herbal extracts, a reliable quality control of their composition and formulation, defining their mechanisms of action and satisfactorily testing them in preclinical models of human RA. Fulfillment of these criteria would then permit clinical testing of selected natural products in RA patients. Studies on celastrol have validated its anti-arthritis activity as well as defined its mechanisms of action in adjuvant-induced arthritis (AA) model of RA (Venkatesha *et al.* 2011, 2012; Cascao *et al.* 2012, 2015; Nanjundaiah *et al.* 2012; Yu, Venkatesha and Moudgil 2012). Similarly, other studies have unraveled the potential disease-modulating activity of celastrol in experimental models of other major human autoimmune diseases such as multiple sclerosis (MS) (Abdin and Hasby 2014; Wang *et al.* 2015), inflammatory bowel disease (IBD) (Pinna *et al.* 2004; Shaker, Ashamallah and Houssen 2014; Jia *et al.* 2015; Zhao *et al.* 2015) and systemic lupus erythematosus (SLE) (Li *et al.* 2005; Xu *et al.* 2007).

In this article, we have discussed RA as a prototypic autoimmune disease model to highlight the beneficial effects of celastrol against autoimmune inflammation in context of the main pathways involved in RA pathogenesis. A brief summary of the

cell signaling pathways shared by various inflammatory and autoimmune diseases and influenced by celastrol is presented in the beginning. Also included is a brief description of the effects of celastrol in models of other autoimmune diseases mentioned above.

MODULATION OF CELL SIGNALING PATHWAYS BY CELASTROL

Celastrol modulates several cell signaling pathways that are associated with chronic inflammatory and autoimmune disease conditions. These pathways include the nuclear factor-kappa B (NF- κ B), mitogen-activated protein kinases (MAPK), Janus kinase (JAK)/signal transducer and activator of transcription (STAT), phosphatidylinositol-3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) and anti-oxidant defense pathways. In addition, celastrol is shown to regulate Toll-like receptor (TLR) pathway. For NF- κ B pathway, celastrol blocks I kappa B ($I\kappa$ B) kinase (IKK) activity as well as the degradation and phosphorylation of $I\kappa$ B (Lee *et al.* 2006). This in turn blocks the activation of NF- κ B and its nuclear translocation, and thereby, inhibits the expression of NF- κ B-regulated target genes (Tak and Firestein 2001; Simmonds and Foxwell 2008). For MAPK pathway, celastrol inhibits the phosphorylation of c-Jun N-terminal kinase (JNK) and extracellular signal-regulated kinase (ERK) (Jung *et al.* 2007; Venkatesha *et al.* 2011). However, its precise action on p38 MAPK is not clear. For JAK/STAT pathway, celastrol is shown to inhibit the phosphorylation of STAT3 as well as STAT3-mediated interleukin-17 (IL-17) expression, T helper 17 (Th17) differentiation and cell proliferation (Kannaiyan *et al.* 2011; Venkatesha *et al.* 2011; Rajendran *et al.* 2012; Astry *et al.* 2015). However, the effects of celastrol on JAKs and other STATs are not clear. For PI3K/Akt/mTOR pathway, celastrol has been shown to inhibit PI3K activity, Akt phosphorylation, mTOR phosphorylation, as well as the downstream effectors such as ribosomal protein S6 kinase (p70S6K), eukaryotic initiation factor 4E and ERK (Ma *et al.* 2014; Shrivastava *et al.* 2015). The modulation of these processes in turn results in the inhibition of hypoxia-inducible factor-1 (HIF-1) (Ma *et al.* 2014). In regard to TLR pathway, celastrol inhibits lipopolysaccharide (LPS)-induced angiogenesis by suppressing TLR-4-mediated NF- κ B activation (Ni *et al.* 2014). Furthermore, celastrol can inhibit TLR activation in macrophages by blocking the binding of LPS to TLR-4/myeloid differentiation factor 2 complex (Lee *et al.* 2015). This effect is thiol-dependent and involves thiol reactivity of celastrol. Taken together, celastrol interferes with multiple cell signaling pathways and induces differential actions depending on the disease processes in various inflammatory and autoimmune conditions.

CELASTROL SUPPRESSES AUTOIMMUNE ARTHRITIS

The anti-arthritis activity of celastrol/ tripterine has been the subject of various *in vivo* and *in vitro* studies (Li *et al.* 1997, 2008, 2012, 2013a,b; Venkatesha *et al.* 2011, 2012; Cascao *et al.* 2012; Nanjundaiah *et al.* 2012; Yu, Venkatesha and Moudgil 2012; Xu *et al.* 2013). Celastrol was found to effectively suppress arthritis in experimental models of human RA such as AA and collagen-induced arthritis. Various cellular and molecular mechanisms of action of celastrol have been examined using *in vitro* experiments based on splenocytes, lymph node cells (LNC) and synovial fibroblast-like cells (FLS) derived from arthritic animals as well as FLS of RA patients (RA-FLS).

Cytokine responses in arthritis and their modulation by celastrol

An imbalance of pro-inflammatory and anti-inflammatory cytokines contributes to RA pathology (Isomaki and Punnonen 1997; Brennan and McInnes 2008; Astry, Venkatesha and Moudgil 2013). Tumor necrosis factor alpha (TNF α), IL-1 β , IL-6 and IL-17 are pro-inflammatory cytokines that have extensively been studied in RA (Brennan and McInnes 2008; Astry, Venkatesha and Moudgil 2013). TNF α , IL-1 β and IL-6 are produced by cells of myeloid origin including macrophages, monocytes and fibroblasts, whereas IL-17 is predominantly produced by the CD4+ Th17 cells (Isomaki and Punnonen 1997; Brennan and McInnes 2008). Other sources of IL-17 include CD8+ T cells, $\gamma\delta$ T cells, natural killer T cells and lymphoid tissue inducer cells (Isomaki and Punnonen 1997; Stockinger and Veldhoen 2007; Brennan and McInnes 2008). These pro-inflammatory cytokines reciprocally influence the production of one another, and they can act independently or synergistically in the course of arthritis. The blocking of these pro-inflammatory cytokines has been shown to be beneficial for the therapeutic control of RA (Adorini 2003; Venkatesha et al. 2014). IL-4, IL-10 and IL-13 function mainly as anti-inflammatory cytokines and are mostly produced by Th2 cells (Isomaki and Punnonen 1997; Brennan and McInnes 2008). Apparently, the levels of anti-inflammatory cytokines are not sufficient to neutralize the deleterious effects of pro-inflammatory cytokines during the progression of RA (Isomaki and Punnonen 1997).

In our studies (Venkatesha et al. 2011, 2012; Nanjundaiah et al. 2012), celastrol altered the balance between pro-inflammatory and anti-inflammatory cytokines primarily via reduction of pro-inflammatory cytokines as tested in the draining LNC and synovial-infiltrating cells (SIC) of arthritic rats treated with celastrol and compared with control rats. We further observed that celastrol treatment inhibited IL-17 expression in the cells of the draining lymph nodes and reduced the level of IL-17 in culture supernatant of SIC (Venkatesha et al. 2011; Nanjundaiah et al. 2012). *In vitro* experiments also confirmed the IL-17-inhibitory activity of celastrol observed *in vivo*. The reduction in IL-17 expression by celastrol was further supported by inhibition of STAT3 phosphorylation and retinoid-related orphan receptor gamma t (ROR γ t), the key transcription factors involved in Th17 differentiation. Besides inhibiting Th17-derived IL-17, celastrol inhibited the expression and secretion of IL-6, which correlated with pSTAT3 inhibition. Similarly, celastrol inhibited IL-1 β and TNF α production (Cascao et al. 2012), and this effect was linked with NF- κ B-inhibitory activity of celastrol. In regard to inhibition of the release of mature IL-1 β , we observed that celastrol reduced both pro-IL-1 β as well as mature IL-1 β (Astry et al. 2015). We attribute these effects to the celastrol-mediated inhibition of NF- κ B. There is a likely additional direct effect of celastrol on the conversion of pro-IL-1 β into mature IL-1 β . In this regard, it has been reported that celastrol can downregulate inflammasome-associated caspase-1, a key enzyme involved in the conversion of pro-IL-1 β to mature IL-1 β (Cascao et al. 2012). The molecular mechanisms of some of these pathways are summarized in Fig. 2.

Celastrol regulates the expression of chemokines, adhesion molecules and cell migration

The migration of lymphocytes, macrophages and other pathogenic cells into an inflamed joint is controlled by

chemokines/chemokine receptors and cell adhesion molecules expressed by the vascular endothelium (Smith et al. 2001; Middleton et al. 2004; Cascao et al. 2010; Szekanecz et al. 2010; Moura et al. 2011). The resulting cellular trafficking into the joints leads to inflammation, pannus formation and tissue damage locally. The levels of pro-inflammatory chemokines and their receptors are elevated in the serum, synovial fluid and synovial tissue of patients with arthritis (Hampel et al. 2013; Zhebrun et al. 2014). Chemokines such as 'regulated on activation, normal T cell expressed and secreted' (RANTES) and monocyte chemoattractant protein 1 (MCP-1) are potent chemoattractants involved in joint inflammation in RA as well as animal models of RA (Shahrara et al. 2008; Pavkova Goldbergova et al. 2012). Accordingly, neutralizing these and other chemokines leads to inhibition of chemokine-induced chemotaxis and suppression of arthritic inflammation (Shahrara et al. 2008; Blanchetot et al. 2013). Similarly, blocking of chemokine receptors also suppressed arthritic inflammation (Mohan and Issekutz 2007; Yokoyama et al. 2014). Thus, chemokines, chemokine receptors and adhesion molecules serve as attractive targets for arthritis therapy (Barnes et al. 1998; Halloran et al. 1999; Szekanecz, Kim and Koch 2003; Chen, Oppenheim and Howard 2004). Herbal medicine products that modulate the expression of chemokines and their receptors have been shown to suppress arthritic inflammation in animal models of RA (Chen, Oppenheim and Howard 2004; Marotte et al. 2010; Venkatesha et al. 2012). The results of our study (Venkatesha et al. 2012) on testing the culture supernatant of spleen adherent cells (SAC) and serum prepared from celastrol-treated rats showed reduction in the levels of growth-related oncogene (GRO/KC) and RANTES compared to control samples from untreated arthritic rats. However, the level of macrophage inflammatory protein 1 α (MIP-1 α) was not altered under these conditions. Furthermore, the chemokine MCP-1 showed an opposite trend in SAC culture supernatant versus serum samples. The level of MCP-1 was decreased by celastrol treatment in the former, but increased in the latter samples. However, *in vitro* treatment with celastrol of antigen (Mtb)-/IL-1 β -stimulated rat synovial fibroblasts-like cells decreased the levels of GRO/KC, RANTES, MCP-1 and MIP-1 α . In our another study, the culture supernatant of synovial-infiltrating cells (SIC) of arthritic rats treated with celastrol showed reduction in the levels of both MCP-1 and RANTES (Astry et al. 2015). However, there was no significant change in the expression of chemokine receptors. Furthermore, celastrol has been shown to inhibit the expression of E-selectin, vascular cell adhesion molecule, and intercellular adhesion molecule-1 (ICAM-1) on endothelial cells (Zhang et al. 2006).

RA is characterized by synovial inflammation (Harris 1990; Scott, Smith and Kingsley 2003; Lipsky 2005). RA-FLS, along with macrophages, are integral components of the synovial lining, which in a normal joint is one to three cell thick (Noss and Brenner 2008; Bartok and Firestein 2010). RA-FLS, upon stimulation, produce increased amounts of cytokines, chemokines and matrix-degrading enzymes (matrix metalloproteinases; MMPs) that can further enhance ongoing inflammation and tissue damage. In addition, FLS in RA become resistant to apoptosis and acquire increased invasive and migratory properties (Noss and Brenner 2008; Bartok and Firestein 2010). These functional alterations contribute to hyperproliferative synovium and spreading of inflammation to unaffected joints. Thus, RA-FLS are an attractive target for controlling arthritic inflammation. In this regard, celastrol has been shown to inhibit the migration and invasiveness of IL-17-stimulated RA-FLS. This effect involved inhibition of NF- κ B-mediated expression of MMP-9 by celastrol (Li

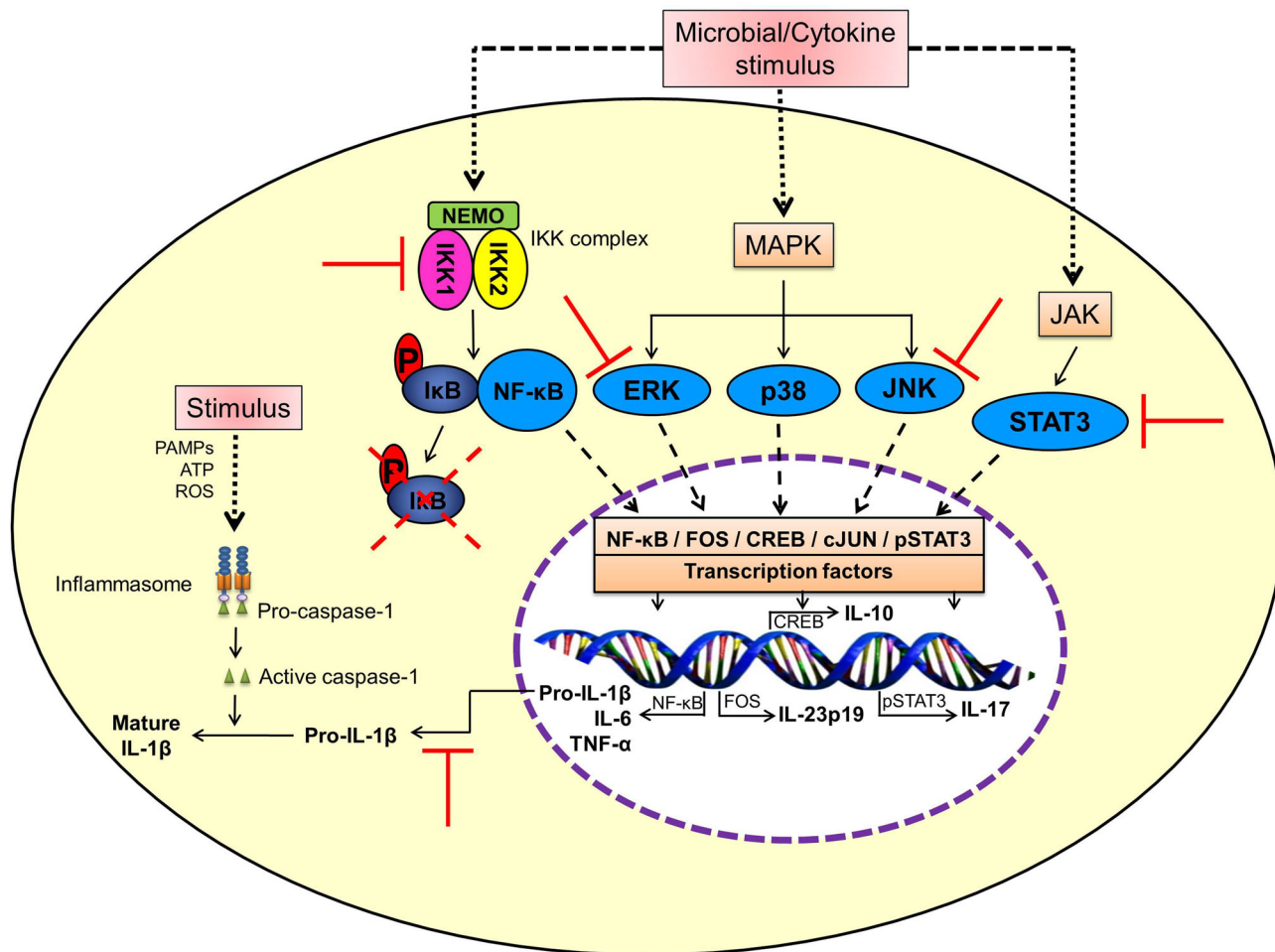


Figure 2. Celastrol inhibits NF- κ B and other cell signaling pathways associated with inflammation. Celastrol inhibits NF- κ B activation via inhibition of IKK activity as well as degradation and phosphorylation of I κ B α ; inhibits the phosphorylation of ERK, JNK and STAT3, which regulate other nuclear transcription factors; and inhibits the production of pro-inflammatory cytokines (e.g. TNF α , IL-1 β , IL-6, IL-17). The effect of celastrol on mature IL-1 β production is primarily owing to reduction of pro-IL-1 β production via inhibition of NF- κ B. (CREB—cAMP response element-binding protein; ERK—extracellular signal-regulated kinase; IKK—I κ B kinase; JNK—c-Jun N-terminal kinase; MAPK—mitogen-activated protein kinase; NEMO—NF- κ B essential modulator; NF- κ B—nuclear factor-kappa B; P—phosphorylated; PAMPs—pathogen-associated molecular patterns; STAT3—signal transducer and activator of transcription 3).

et al. 2012). In another study, celastrol inhibited hypoxia-induced migration and invasion of RA-FLS, which was attributable in part to suppression of HIF-1 α -mediated expression of CXC chemokine receptor 4 (Li et al. 2013b). Another study revealed that celastrol inhibited proliferation of these cells by inducing DNA damage, cell cycle arrest and apoptosis (Xu et al. 2013). Celastrol has also been reported to have an anti-proliferative effect on T cells and cancer cells (Chiang et al. 2014; Astry et al. 2015). Besides its effects on RA-FLS, celastrol has also been shown to reduce the cellular infiltrate into the synovium; the number of sub-lining CD68+ macrophages, which are considered as biomarkers of therapeutic response to RA treatment; and the joint damage in AA (Cascão et al. 2015). A similar reduction in infiltration of CD68+ macrophages in the synovium was also observed following treatment with conventional drugs and biologics such as methotrexate and Anakinra (Dolhain et al. 1998; Cunnane et al. 2001; Smith et al. 2001; Thurlings et al. 2008). Furthermore, we have observed reduction in Th17 cells in SIC of arthritic rats treated with celastrol (Astry et al. 2015).

Th17/T regulatory cell balance in arthritis and its control by celastrol

The major T cell subsets involved in RA include Th1, Th2 and Th17, and T regulatory (Treg) (Mosmann and Sad 1996; Stockinger and Veldhoen 2007). These subsets are identified on the basis of specific transcription factors expressed and the type of cytokines produced by them (Mosmann and Sad 1996; Stockinger and Veldhoen 2007). Th1 cells are identified by the transcription factor T-bet and the production of IFN γ , whereas, Th2 cells express the transcription factor GATA3 and produce IL-4, an anti-inflammatory cytokine. The Th17 subset is characterized by the transcription factor ROR γ t and the production of pro-inflammatory cytokine IL-17 (Mosmann and Sad 1996; Stockinger and Veldhoen 2007; Roelvelde et al. 2013; Astry et al. 2015; Venkatesha and Moudgil 2015). On the other hand, Treg cells express both high level of CD25 on cell surface and the transcription factor fork-head box protein 3 (Foxp3) intracellularly, and produce immunomodulatory cytokines IL-10 and TGF β (Gol-Ara et al. 2012).

It was believed that activated Th1 cells play a role in the initiation and progression of inflammation, whereas Th2 cells have an anti-inflammatory effect and thereby control inflammation. The identification of Th17 and Treg cells has expanded our understanding of the cellular immune events in various autoimmune diseases, including RA (Bettelli et al. 2006; Noack and Miossec 2014). Th17 cells produce IL-17, which is critical for the inflammatory cell infiltration into the synovium, synovial hyperplasia, angiogenesis, and cartilage and bone damage, and hence this subset has become an attractive therapeutic target for the treatment of RA and some other autoimmune diseases (Roeleveld et al. 2013; Astry, Venkatesha and Moudgil 2015). Following activation, naïve T cells differentiate into Th17 cells in response to IL-6 and/or IL-1 β and TGF β . The IL-6 signaling activates STAT3 and induces the Th17 lineage-determining transcription factor ROR γ t (Stockinger and Veldhoen 2007). Suppressor of cytokine signaling 3 (SOCS3) is a negative regulator of Th17 differentiation. In the presence of IL-6, TGF β inhibits SOCS3 and promotes Th17 differentiation (Qin et al. 2009). TGF β signaling is essential for Treg differentiation. TGF β enables Treg formation from naïve cells in the absence of IL-6, but in the presence of IL-2 (Chen et al. 2003; Peng et al. 2004). TGF β -induced SMAD (a word derived from Sma and Mad (Mothers against decapentaplegic)) and SMAD-independent signals are involved in the development of both Th17 and Treg cells (Fantini et al. 2004; Lu et al. 2010). In addition, IL-2 and STAT5 are involved in the generation, maintenance and function of Treg (Mahmud, Manlove and Farrar 2013).

Th17/Treg imbalance has been reported in RA and experimental models of this disease (Eisenstein and Williams 2009; Noack and Miossec 2014; Astry, Venkatesha and Moudgil 2015). Therefore, novel therapeutic approaches, particularly those that can reset the Th17/Treg balance in favor of immune regulation, are being sought for RA. The inhibition of Th17 is found to be effective in several models of RA and other autoimmune diseases (Roeleveld et al. 2013). Furthermore, new approaches for the manipulation of Treg including *ex vivo* expansion of these cells for adoptive transfer or the induction of Treg to treat autoimmunity are under consideration (Golshayan et al. 2009; Putnam et al. 2009; Burmester, Feist and Dornier 2014; Noack and Miossec 2014; Vivar and Van Vollenhoven 2014). In our study

on the effect of celastrol on Th17/Treg balance in AA (Astry et al. 2015), we observed that celastrol significantly decreased the frequency of CD25⁺Foxp3⁺ Treg in the joints of arthritic rats. Furthermore, celastrol inhibited Th17 differentiation, but augmented Treg differentiation of naïve mouse CD4⁺ T cells under defined cytokine environment conditions (Astry et al. 2015). Apparently, celastrol directly inhibits Th17 differentiation and promotes Treg differentiation in part by blocking the IL-6 receptor signaling pathway through the inhibition of STAT3 phosphorylation (Astry et al. 2015). Celastrol also has an effect on cytokines required for Th17 differentiation. As mentioned above, TGF β is required for the differentiation of both Th17 and Treg, whereas the presence of IL-6 and/or IL-1 β favors Th17 generation (Noack and Miossec 2014; Astry, Venkatesha and Moudgil 2015). Celastrol treatment of arthritic rats significantly reduced the levels of IL-6 and IL-1 β *in vivo* (Venkatesha et al. 2011; Astry et al. 2015). Furthermore, celastrol inhibited the expression of activation markers CD80, CD86 and MHCII on antigen-presenting cell (APC), suggesting that it might suppress T cell activation, proliferation and expansion of the Th17 pool of cells (Astry et al. 2015). This outcome was supported in part by the observation of decreased recall response of the antigen-primed T cells *in vitro* following celastrol treatment (Astry et al. 2015). The mechanism of celastrol-induced regulation of Th17/Treg balance is summarized in Fig. 3.

Modulation of osteoimmune cross-talk by celastrol

Uncontrolled arthritis leads to bone and cartilage damage. Bone remodeling is an outcome of the balance between new bone formation by osteoblasts and bone resorption by osteoclasts (Khosla 2001; Takayanagi 2007; Zhao and Ivashkiv 2011), and this balance is disturbed in RA, with bone resorption exceeding bone formation (Schett 2006; Karmakar, Kay and Gravalles 2010; Maruotti et al. 2011; Xu et al. 2012). It is increasingly being realized that there is a cross-talk between bone and the immune system at cellular and molecular levels, and this sub-field has been named as 'osteoimmunology' (Takayanagi 2007). Pro-inflammatory cytokines such as IL-17 induce the

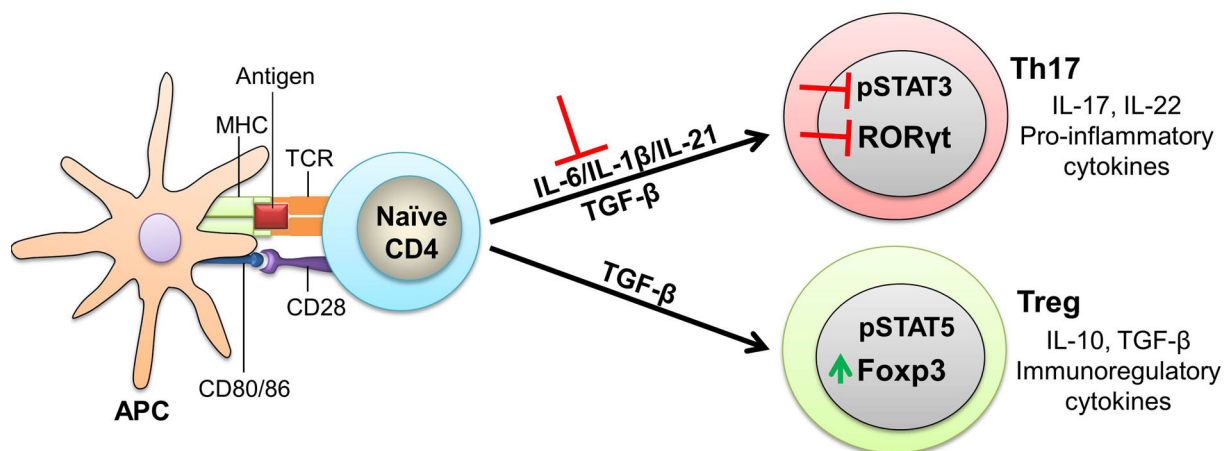


Figure 3. Celastrol inhibits Th17 differentiation, but promotes Treg differentiation. The intricacies of antigen presentation (e.g. the nature of antigen and the strength of co-stimulation) and the local cytokine environment influence the differentiation of naïve CD4⁺ into different effector CD4⁺ T cell subsets. Th17 cells secrete IL-17 and IL-22 and trigger pathogenic events in arthritis, whereas, Treg cells secrete IL-10 and TGF- β , which mediate immunoregulatory activities. For Th17 differentiation, activation of STAT3 in the presence of IL-6/IL-1 β and TGF- β induces ROR γ t. For Treg differentiation, IL-2-induced activation of STAT5 upregulates Foxp3 expression. Celastrol inhibits IL-6/IL-1 β as well as STAT3, thereby suppressing Th17 development. Apparently, celastrol-induced decrease in IL-6 provides an environment that facilitates Treg differentiation, while reducing Th17 differentiation. (IL—interleukin; STAT—signal transducer and activator of transcription; TGF—transforming growth factor; Th17—T helper 17 cell; Treg—T regulatory cell).

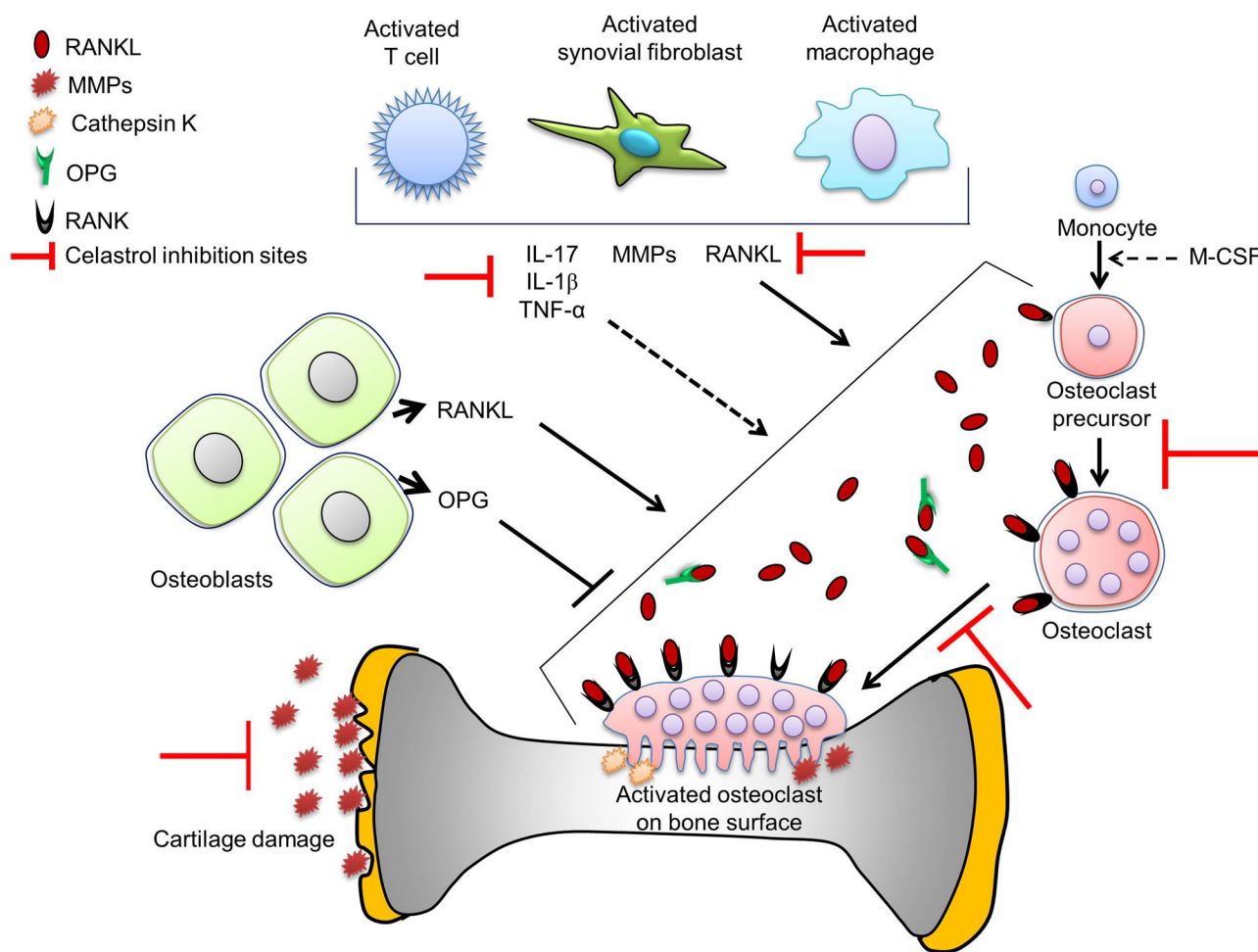


Figure 4. Celastrol inhibits osteoclastic activity and protects against joint damage. Osteoclast precursors differentiate into osteoclasts under the influence of M-CSF. The RANKL/OPG pathway is the key regulator of bone remodeling. The binding of RANKL to its receptor RANK on the surface of an osteoclast precursor promotes the proliferation and differentiation of osteoclasts. OPG, which is secreted by the osteoblasts, is a soluble decoy receptor for RANKL and it serves as a natural inhibitor of osteoclast activation. Celastrol protects against bone and cartilage damage by regulating pro-inflammatory cytokines, inhibiting RANKL production, modulating RANKL/OPG ratio and reducing the secretion of matrix-degrading enzymes such as MMPs. (M-CSF—macrophage colony-stimulating factor; MMPs—matrix metalloproteinases; OPG—osteoprotegerin; RANK—receptor activator for nuclear factor κ B; RANKL—RANK ligand).

production of other pro-inflammatory cytokines, of receptor activator of NF- κ B ligand (RANKL), and of granulocyte macrophage colony-stimulating factor (GM-CSF) (Kotake *et al.* 1999; Kolls and Linden 2004; Takayanagi 2007). RANKL produced by preosteoblastic/stromal cells binds to RANK on monocytes/macrophages (preosteoclasts) and facilitates the differentiation of preosteoclasts into mature osteoclasts (Hadjidakis and Androulakis 2006; Boyce and Xing 2008; Maruotti *et al.* 2011). GM-CSF supports the survival and proliferation of osteoclasts (Takayanagi 2007; Maruotti *et al.* 2011). On the other hand, osteoblasts produce osteoprotegerin (OPG), a decoy receptor for RANKL (Khosla 2001; Hadjidakis and Androulakis 2006; Boyce and Xing 2008; Maruotti *et al.* 2011). The RANKL/OPG ratio determines osteoclast/osteoblast activity in arthritic joints and promotes bone damage (Fig. 4) (Takayanagi 2007; Xu *et al.* 2012). Osteopontin (OPN), insulin-like growth factor (IGF)-I and tissue-degrading enzymes such as MMPs are other osteoclastogenic mediators, whereas osteocalcin (OCN) is an osteoblastic mediator that influences bone remodeling (Hill, Reynolds and Meikle 1995; Strand and Kavanaugh 2004; Franco *et al.* 2011).

Various therapeutic agents may differentially influence inflammation and bone damage (Joosten *et al.* 1999; Gravallesse

2002; Smolen *et al.* 2005; Fonseca *et al.* 2009). Thus, novel anti-arthritic agents that can inhibit both inflammation and bone damage in arthritis are being sought. Using the AA model, we (Nanjundaiah *et al.* 2012) and others (Cascao *et al.* 2012, 2015) have shown that celastrol effectively suppressed not only arthritic inflammation but also inflammation-induced bone and cartilage damage in rats. There was significant decrease in infiltration of immune cells into the synovium in celastrol-treated rats compared to control rats (Cascao *et al.* 2012; Nanjundaiah *et al.* 2012). Furthermore, celastrol decreased osteoclast numbers in the hind paw joints, reduced the subchondral bone loss and preserved bone volume (Nanjundaiah *et al.* 2012). Furthermore, celastrol decreased RANKL, but increased OPG, as tested in the sera and culture supernatant of SIC of celastrol-treated arthritic rats. Celastrol also caused reduction in osteoclastogenic GM-CSF. Furthermore, IGF-I and OPN were decreased in culture supernatant of SIC but not in serum. On the other hand, celastrol increased osteoblastic OCN in serum (Nanjundaiah *et al.* 2012). Additional reported effects of celastrol by other investigators include the inhibition of RANKL-induced signaling in osteoclasts, the induction of osteoclast apoptosis and the reduction in osteoclast formation in bone marrow *in vitro* and *in vivo* (Idris *et al.*

2010; Gan et al. 2015). Furthermore, celastrol inhibited the expression of MMP-9 and the invasion of synovial-like fibroblasts (Li et al. 2013a), and it decreased the expression of MMP-1, MMP-3, MMP-13 in primary human osteoarthritic chondrocytes (Ding et al. 2013). Above results suggest that celastrol is a promising agent for the concurrent control of inflammation and bone damage in arthritis. The mediators targeted by celastrol for modulating bone remodeling are shown in Fig. 4.

Microarray-based gene expression profiling reveals additional molecular targets of celastrol in arthritis

To gain an insight into the entire spectrum of celastrol-induced actions, we studied microarray-based gene expression of antigen-stimulated immune cells from the draining lymph nodes of the celastrol-treated arthritic rats, and compared the results with those of untreated arthritic rats (Yu, Venkatesha and Moudgil 2012). These cells were restimulated in culture with the disease-related antigen, mycobacterial heat-shock protein 65. The antigen-induced changes in gene expression were compared in different groups. A total of 22 523 genes were analyzed, of which, 76 genes were differentially expressed in arthritic control rats compared to 33 genes in celastrol-treated rats. The two groups shared 19 genes. Celastrol primarily regulated the genes associated with inflammatory and immune responses, including antigen processing and presentation as well as cell-/antibody-mediated immune responses. Cell proliferation, metabolism and oxidative stress represent additional pathways influenced by celastrol. Taken together, these results unraveled new potential targets and additional mechanisms of action of celastrol-mediated suppression of arthritis.

CELASTROL-INDUCED MODULATION OF IMMUNE PATHOLOGY IN MODELS OF OTHER HUMAN AUTOIMMUNE DISEASES

Studies in *in vivo/in vitro* experimental models of other human autoimmune diseases besides arthritis, such as MS, IBD, SLE, psoriasis, and type 1 diabetes (T1D), further support the potential role of celastrol in suppressing autoimmune inflammation. However, compared to arthritis, currently there are limited numbers of studies conducted in other models of autoimmunity. The results of these studies are summarized below.

Multiple sclerosis

MS is an autoimmune disease that causes demyelination of the central nervous system (CNS), which then leads to fatigue, muscle weakness and cognitive impairments (Compston and Coles 2008). Animal models of MS, such as myelin oligodendrocyte glycoprotein (MOG) peptide-induced experimental autoimmune encephalomyelitis (EAE) in C57BL/6 mice, have contributed significantly to defining the cellular and molecular mechanisms underlying the pathogenesis of this disease (Robinson et al. 2014). MS is a predominantly T cell-mediated disease (Compston and Coles 2008; Wu and Alvarez 2011). The initiation and progression of MS involve an altered balance of both pro- versus anti-inflammatory cytokines as well as pathogenic (Th17, Th1) versus protective (Th2, Treg) subsets of T cells; the trafficking of pathogenic cells into the CNS; and tissue damage therein (Compston and Coles 2008; Wu and Alvarez 2011). A few drugs are available for MS, but there is a need for new and more effective therapeutic agents. In EAE, celastrol is able to reduce

the clinical severity of the disease by targeting multiple pathways (Abdin and Hasby 2014; Wang et al. 2015). In a study in mice with MOG-induced EAE, celastrol treatment attenuated disease development, which was associated with reduction in pathogenic Th17 response in both the periphery and the target organ (Wang et al. 2015). The beneficial effect of celastrol in EAE was attributed to inhibition of dendritic cells (DC) rather than to Th17 differentiation. In another study in a relapsing-remitting EAE model in Sprague Dawley rats, celastrol treatment switched the T cell response from a predominantly Th1 (TNF α) to Th2 (IL-10) type, with an increase of IL-10 production. Moreover, NF- κ B expression and the T cell counts in the CNS tissue were reduced in celastrol-treated rats compared to controls (Abdin and Hasby 2014). Taken together, these studies demonstrate the potential of celastrol as a therapeutic for MS.

Inflammatory bowel disease

IBD, also known as colitis, involves inflammation of the intestinal tract leading to abdominal pain, recurring diarrhea, vomiting and fatigue (Garud and Peppercorn 2009; Marks et al. 2010). IBD can manifest as ulcerative colitis or Crohn's disease. The etiology of IBD is not fully defined, but it is believed to be an outcome of an interplay among genetic, environmental and lifestyle-related factors. Gut microbiota have also been invoked in IBD pathogenesis (Mukhopadhyaya et al. 2012; Hisamatsu et al. 2013). Studies on cells from biopsies of Crohn's patients and experimental models of IBD have demonstrated the efficacy of celastrol against colitis. Celastrol was tested on mucosal inflammatory biopsies from patients with Crohn's disease to determine its effects on the production of pro-inflammatory cytokines (Pinna et al. 2004). Celastrol significantly reduced the production of multiple pro-inflammatory cytokines, with most notable effect on CD33+ cells. Further analysis using a human monocytic cell line showed that celastrol inhibited LPS-induced NF- κ B translocation (Pinna et al. 2004). In dextran sulfate sodium (DSS)-induced colitis model (Jia et al. 2015), celastrol was shown to reduce the severity of colitis, and decrease cell necroptosis as evident from reduced levels of receptor-interacting protein kinase 3 and mixed lineage kinase domain-like (MLKL), which represent necroptosis factors. Moreover, the levels of pro-inflammatory mediators such as IL-1 β and IL-6 were decreased (Jia et al. 2015). Similarly, in another study in the same model (DSS-induced colitis), celastrol reduced the disease severity via downregulation of oxidative stress and inflammatory cytokines. Following celastrol administration, there was a decrease in pro-inflammatory cytokines IL-1 β , IFN γ , IL-17 and IL-23 levels, and an increase in anti-inflammatory cytokine IL-10 level (Shaker, Ashamallah and Houssem 2014). In an IL-10-deficient experimental model of Crohn's disease, it was demonstrated that celastrol treatment induced protection against colitis by upregulating autophagy via inhibition of the PI3K/Akt/mTOR signaling pathway (Zhao et al. 2015). The analysis of colon tissue of celastrol-treated mice showed decreased myeloperoxidase level, reduced neutrophil infiltration, and decreased pro-inflammatory cytokines. The above studies suggest that celastrol might be useful in alleviating the symptoms and controlling the disease pathology in IBD patients.

Systemic lupus erythematosus

SLE, also known as lupus, is a multifactorial disease that affects the kidney and several other organs. Moreover, there are multiple clinical manifestations of lupus, further indicating the

complexities of this disease (D’Cruz, Khamashta and Hughes 2007; Kiriakidou 2013; Lisnevskaja, Murphy and Isenberg 2014). SLE is characterized by defective clearance of apoptotic material, which then accumulates and then is taken up by APCs, followed by presentation of the self-antigenic epitopes to autoreactive T/B cells. In addition, there is intense polyclonal B cell activation as well as defective T cell signaling and co-stimulation (D’Cruz, Khamashta and Hughes 2007; Kiriakidou 2013; Lisnevskaja, Murphy and Isenberg 2014). Studies using the BW F1 mouse model of lupus (Xu and Wu 2002) have shown that following celastrol administration, there was a decrease in both anti-dsDNA antibodies in serum as well as excretion of protein in the urine (proteinuria), two common biomarkers of lupus. Furthermore, there was reduced expression of collagen type IV, fibronectin and tissue inhibitor of metalloproteinases 1, but increased expression of MMP-1 and 2 in the kidney. A subsequent study (Xu et al. 2007) corroborated these findings and also showed that celastrol treatment decreased renal collagen type IV presumably by suppressing local expression of TGF- β . Another study in BALB/c mice (Li et al. 2005), in which lupus was induced by injection of active chromatin, showed that tripterine (celastrol) administration reduced serum autoantibodies and total IgG, and afforded protection against glomerular injury. These effects were associated with decreased IL-10 and nitric oxide production, but without any change in IFN- γ .

Psoriasis

Psoriasis is an autoimmune condition characterized by the formation of red patches on the skin, and these patches are usually itchy and scaly in texture. There are multiple clinical manifestations of psoriasis of varying severity (Lowe, Suárez-Fariñas and Krueger 2014; Boehncke and Schön 2015). The pathogenesis of psoriasis involves a cross-talk between tissue-resident DCs and keratinocytes. The keratinocytes activate the DCs and then the DCs recruit both innate (e.g. neutrophils, macrophages and more DCs) and adaptive immune cells. The latter include Th1, Th17 and Th22 subsets of T cells. The Th22 subset is most notable because the cells of this subset produce the cytokines IL-22 and TNF- α , which play a major role in disease pathogenesis (Michalak-Stoma et al. 2013). Using the HaCaT keratinocyte cell line, it was shown that celastrol is a pro-apoptotic mediator (Zhou et al. 2011). HaCaT cells cultured in the presence of celastrol showed growth inhibition as well as signs of apoptosis, as evident by phosphatidylserine externalization, depolarization of mitochondrial membrane potential and activation of caspase-3. Furthermore, there was increased expression of B-cell lymphoma 2 (Bcl-2)-associated X protein, a pro-apoptotic factor, and decreased level of Bcl-2, an anti-apoptotic factor. Moreover, celastrol-mediated apoptosis was associated with suppression of the NF- κ B pathway. Another set of studies on HaCaT cells showed that celastrol suppressed IFN- γ -induced ICAM-1 expression leading to reduced monocyte adhesiveness (Seo et al. 2010, 2011). This effect involved induction of heme oxygenase-1 by celastrol through reactive oxygen species generation and activation of ERK and p38 MAPK. The above results suggest that celastrol might be a promising candidate for further testing in animal models of psoriasis.

Type 1 diabetes

T1D is a multifactorial disease involving an autoimmune attack on the insulin-producing β -islet cells in the pancreas.

There is infiltration of autoreactive T cells into the pancreas. These T cells target the β -islet cells with cytokines such as IFN γ , TNF α and IL-1 β (Cnop et al. 2005). This leads to an increase in blood glucose that can result in polyuria, polydipsia, blindness, kidney failure and other ailments (Atkinson, Eisenbarth and Michels 2014). Using a rat pancreatic beta-cell line RINmSF, it has been shown that celastrol treatment reduced the cytokine-induced cell death; the production of pro-inflammatory mediators such as inducible nitric oxide synthase, cyclooxygenase-2 and CC chemokine ligand 2; and the activation of NF- κ B (Ju et al. 2015). However, in another study based on the non-obese diabetic mouse model of T1D, celastrol failed to prevent the development of diabetes, although it caused transient lowering of blood glucose (Grant et al. 2013).

CONCLUSION

Based on the results of preclinical studies, we suggest that celastrol is a promising candidate for further evaluation in RA patients. If validated, celastrol might serve as a useful adjunct/alternative to conventionally used drugs, whose long-term use is associated with severe adverse effects. In this regard, *Tripterygium Wilfordii* extract has been tested in RA patients (Liu et al. 2013). In a recent study on RA therapy, *T. Wilfordii* extract was found to be as effective as methotrexate (Liu et al. 2013; Lv et al. 2015). These studies have set the stage for systematic testing of celastrol and other purified herbal compounds in RA patients in the near future. Furthermore, celastrol has potential therapeutic benefits in several other autoimmune diseases besides RA.

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