Mode of Action of Aspirin in Experimental Autoimmune Encephalomyelitis

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Multiple sclerosis (MS) is a chronic and debilitating autoimmune disorder of the central nervous system in which the autoimmune T cells destroy myelin, thus causing lesion, damage, and neuronal dysfunction. Experimental autoimmune encephalomyelitis (EAE) is an animal model of MS that is particularly useful for testing new therapeutic approaches against MS. Aspirin (acetyl salicylic acid) is one of the oldest and widely used medicines in the world, and recently it has been shown that low-dose aspirin is capable of suppressing the disease process of EAE in mice. One of the root causes of this autoimmune disease process is the decrease and/or suppression of Foxp3-expressing anti-autoimmune regulatory T cells (Tregs) and associated increase in autoimmune T-helper 1 (Th1) and Th17 cells. Aspirin upregulates Tregs and decreases Th1 and Th17 responses. Accordingly, the suppression of Tregs abrogates the protective effect of aspirin on EAE, indicating that aspirin protects EAE via Tregs. While there are several mechanisms for the maintenance of Tregs under immune insults, aspirin increases the level of interleukin-11 (IL-11), an immunomodulatory cytokine, and IL-11 alone is sufficient to protect Tregs. Being a multifunctional molecule, aspirin stimulates the activation of cAMP-response element-binding (CREB) to promote the recruitment of CREB to the *IL-11* gene promoter and stimulate the transcription of *IL-11* in splenocytes. Therefore, it appears that low-dose aspirin protects EAE via CREB-mediated stimulation of IL-11–Treg pathway and that aspirin may have therapeutic importance in MS.

Keywords: aspirin, experimental autoimmune encephalomyelitis, regulatory T cells, Th1/Th17 cells, IL-11

Introduction

CURRENTLY, ABOUT 2.3 MILLION PEOPLE in the world are affected by multiple sclerosis (MS), the most common demyelinating disease in young adults (Martin *et al.*, 1992). Being an autoimmune disease, women are more susceptible to MS compared with men (Duquette *et al.*, 1992; Voskuhl and Palaszynski, 2001). Although the etiology of MS is not completely understood, it is believed that in MS patients, misdirected and over-reactive autoimmune T cells infiltrate the central nervous system, causing widespread inflammation, death of myelin-producing oligodendrocytes, and demyelination of neurons that ultimately drive to MS symptoms and pathology (Martin *et al.*, 1992; Miron *et al.*, 2011). Experimental autoimmune encephalomyelitis (EAE), an experimental animal model of MS, is widely used to evaluate the causes and find different treatment options for MS (Maugh, 1977; Viglietta *et al.*, 2004; Pahan, 2010). Although some drugs are available to tackle the disease process, always there is a need to find an effective, safe, and economical medicine for MS.

Aspirin, also known as acetyl salicylic acid, finds a place in the 2017 Model List of Essential Medicines of the World Health Organization as one of the most effective, safe, and

economical drugs needed for basic health care. Although aspirin has been officially healing our pains, aches, fever, etc., since August 10, 1897, Sumerians used extracts of willow tree that contains salicylic acid, the natural aspirin, as pain remedies 4000 years back. Hippocrates, the father of modern medicine, wrote about 2500 years back that willow leaves and bark had relieved pain and fevers.

In this review, we analyze mechanisms by which our old medicine friend, aspirin, suppresses the disease process of EAE in mice.

Mechanisms Leading to the Attenuation of EAE by Aspirin Treatment

Repression of autoimmune Th1 and Th17 cells by aspirin

Before the discovery of interleukin-23 (IL-23), adaptive immune responses were known to be controlled by T-helper 1 (Th1) and Th2 cells (Brahmachari and Pahan, 2008; El-behi *et al.*, 2010). Classically, Th1 cells are inflammatory and Th2 cells are anti-inflammatory. However, after the discovery of IL-23 (Oppmann *et al.*, 2000), Th17 cells came with a bang, and accordingly recent studies highlight Th17 cells as more

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active players than Th1 cells in different autoimmune disorders, including MS and EAE (Croxford *et al.*, 2012; Rostami and Ciric, 2013; Yasuda *et al.*, 2019). Th17 cells are characterized by its signature cytokine IL-17 and the transcription factor ROR γ t (Jadidi-Niaragh and Mirshafiey, 2011; Rendon and Choudhry, 2012). Aspirin treatment has been shown to suppress the expression of IL-17 and ROR γ t in myelin basic protein (MBP)-primed T cells (Mondal *et al.*, 2018). Accordingly, aspirin also reduces the abundance of CD4⁺IL-17⁺ T cells in EAE mice (Mondal *et al.*, 2018), suggesting the suppression of Th17 response by aspirin. Similarly, aspirin treatment also decreases the levels of Tbet and IFN γ , characteristic markers of Th1 cells, in MBP-primed T cells (Mondal *et al.*, 2018). In contrast, aspirin treatment increases the frequency of Th2 cells. Since Th2 cells are known to inhibit EAE and autoimmune signaling, together it appears that aspirin switches Th cells in a way to ultimately benefit EAE and MS.

Upregulation of regulatory T cells by aspirin: mechanisms behind the suppression of autoimmune T cells

As part of our life, everybody is supposed to get immune insults. However, only a few get autoimmune disorders such as MS. This is because of the fact that we are endowed with a specialized immune cell type called regulatory T cells (Tregs) to control aberrant immune responses (Hori *et al.*, 2003; Coffey and Burgering, 2004). However, it is found that during MS and EAE, there is significant reduction in the number and function of Tregs (Viglietta *et al.*, 2004; Huan *et al.*, 2005). As a result, self-reactive T cells get the opportunity to proliferate, eventually destroying the fine balance between autoimmune and anti-autoimmune T cells and causing an autoimmune attack. Therefore, understanding the mechanisms and finding drugs for the protection of Tregs under autoimmune insults is an important area of research. According to a recent study (Mondal *et al.*, 2018), aspirin could be an important molecule in maintaining the balance between immune response and immune tolerance via the upregulation and/or protection of Tregs. Aspirin treatment restored/increased the expression of Foxp3, CD25, CTLA4, and CD62, different markers of Tregs, in MBP-primed T cells. Similarly, treatment of EAE mice with low-dose aspirin also led to the protection of CD4⁺Foxp3⁺ Tregs (Mondal *et al.*, 2018).

How does aspirin upregulate Tregs? Involvement of IL-11

Although there are several mechanisms for the protection of Tregs, aspirin employs a new pathway to upregulate these cells. IL-11, an immunomodulatory cytokine of the gp130 family of molecules, is expressed by different cells, including PBMC and brain cells (Taga and Kishimoto, 1997; Xu *et al.*, 2016). IL-11 is known to reduce the production of IL-12 for inhibiting Th1 polarization (Curti *et al.*, 2001). IL-11 also inhibits the activation of NF- κ B and suppresses the expression of proinflammatory molecules in the macrophages (Trepicchio *et al.*, 1996). Interestingly, after neuro-antigen priming, the level of IL-11 goes down in T cells. However, aspirin rapidly stimulates the expression and production of IL-11 from normal T cells and restores the level IL-11 in MBP-primed T cells (Mondal *et al.*, 2018). IL-11 induction

by aspirin is not cell-specific as another study has reported an increase in IL-11 in the astrocytes by aspirin treatment (Modi *et al.*, 2013). Moreover, IL-11 plays an important role in the protection and/or stimulation of Tregs. In the absence of aspirin, IL-11 alone is sufficient to increase Foxp3 and protect Tregs in MBP-primed splenocytes (Mondal *et al.*, 2018). It has been shown that autoimmune insults are capable of reducing Foxp3 and inhibiting Tregs through the production of nitric oxide (NO) (Brahmachari and Pahan, 2009, 2010). Therefore, the suppression of NO production from the macrophages by recombinant human IL-11 (Trepicchio *et al.*, 1996) suggests that IL-11 protects Tregs in MBP-primed splenocytes by suppressing the production of NO. Accordingly, the neutralization of IL-11 abrogates aspirin-mediated upregulation of Foxp3 and protection of Tregs, indicating that aspirin requires IL-11 for defending and promoting Tregs during autoimmune insults.

Aspirin requires CREB for the upregulation of IL-11

The analysis of *IL-11* gene promoter reveals the presence of a consensus cAMP response element (CRE), which could be engaged by CRE-binding (CREB), a multifunctional transcription factor. Therefore, Mondal *et al.* (2018) investigated the possible involvement of CREB in aspirin-mediated upregulation of IL-11. Accordingly, aspirin is seen to increase the phosphorylation of CREB in splenocytes, and the siRNA knockdown of CREB abolishes the induction of IL-11 in aspirin-stimulated cells (Mondal *et al.*, 2018). Moreover, aspirin treatment leads to the recruitment of CREB to the *IL-11* gene promoter, indicating a direct involvement of CREB in the transcription of *IL-11* (Fig. 1). The mechanism of CREB

FIG. 1. Signaling pathways for the induction of IL-11 by aspirin in T cells. Aspirin induces the activation of CREB, which in turn binds to CRE present in the promoter of *IL-11* gene to stimulate *IL-11* gene transcription in T cells. CREB, cAMP-response element-binding; IL, interleukin.

activation by aspirin has been investigated in different studies. For example, aspirin rapidly induces the activation of protein kinase A (PKA) in the astrocytes (Modi *et al.*, 2013) and neurons (Rangasamy *et al.*, 2018), and the inhibition of PKA suppresses aspirin-mediated activation of CREB in these cells. Therefore, it is possible that aspirin also requires PKA for the activation of CREB in splenocytes and hence the upregulation of IL-11.

Does aspirin require the IL-11–Tregs pathway to inhibit EAE?

Since aspirin ameliorates the disease process of EAE and promotes IL-11–Tregs signaling, Mondal *et al.* (2018) investigated the role of IL-11–Treg pathway in aspirin-mediated protection of EAE. The level of IL-11 is less in the splenic sections of EAE mice compared with normal mice. However, aspirin treatment prevents the loss of IL-11 in EAE mice. Accordingly, the protective effect of aspirin vanishes when EAE mice were treated with IL-11-neutralizing antibody. Consistent with the involvement of IL-11 in the upregulation of Tregs, Mondal *et al.* (2018) have seen the downregulation of Tregs in EAE mice. The restoration of Tregs in EAE mice by aspirin treatment and the abrogation of aspirin-mediated upregulation of Tregs by IL-11-neutralizing

antibody suggest that aspirin upregulates and/or reinstates Tregs in EAE mice via IL-11. Consequently, the neutralization of Tregs by anti-CD25 antibody also abolishes the protective effect of aspirin on EAE. Therefore, aspirin involves IL-11-mediated Treg signaling pathway for the suppression of Th1/Th17 responses and the protection of EAE (Fig. 2).

Concluding Thoughts

Currently, controlling the neuroimmune symptoms of MS to a certain extent is the only possible relief from the pain and suffering due to this disease. Moreover, very few options are available for primary progressive MS. Therefore, it is exciting to learn the possibility that low-dose aspirin, a readily available popular drug, may have therapeutic efficacy in MS. If EAE results are translated, low-dose aspirin has the capability to calm down autoimmune Th1 and Th17 cells by promoting the development of Tregs through CREB-mediated transcription of IL-11. According to Budd *et al.* (1993), low-dose aspirin in slow-releasing formulation shows efficacy as an anti-platelet agent without much noticeable side effects. Although aspirin is reported to exhibit some toxic effects, aspirin suppresses the disease process of EAE at low doses, and baby-dose aspirin (81 mg) is considered to be very much safe in adults for daily use. Therefore, our old medicine friend may be

FIG. 2. Schematic presentation of aspirin-mediated immunomodulation and suppression of EAE. Aspirin promotes Tregs via IL-11 to suppress Th1 and Th17 cells, which are responsible for driving autoimmune responses in EAE and multiple sclerosis. EAE, autoimmune encephalomyelitis.

repurposed to promote Tregs via IL-11 and control the debilitating autoimmune symptoms of MS.

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