Review Article
The effect of icariin on immunity and its potential application

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Abstract: Icariin (ICA) is a major bioactive monomer belonging to flavonoid glycosides attracted from Epimedium, being a classic tonic agent in traditional Chinese medicine. ICA commonly presents multiple effects such as regulating sex hormones, relieving atherosclerosis and antioxidant activity, etc. Recently, more and more studies have demonstrated the application of ICA in autoimmune diseases such as rheumatoid arthritis, bronchial asthma, multiple sclerosis and systemic lupus erythematosus due to its anti-inflammatory. Additionally, ICA also has the anti-tumor activities. Multiple targets and mechanisms of ICA are reported which relates to regulate lymphocytes balance, anti-inflammatory/inflammatory cytokines, signal pathways like NF-kappaβ and Erk-p38-JNK, lymphocyte transcription factors and other targets such as TLRs, STAT and PTEN, etc. In this review, we have updated the advance in this field and these studies have suggested that ICA has a potential to treat immunological and inflammatory diseases.

Keywords: Icariin, immune regulation, inflammation, Chinese medicine

Introduction
Icariin (ICA) is one of the major bioactive compounds attracted from Epimedium which is also the most widely studied monomer [1]. Epimedium, belonging to the Berberidaceae family, in Chinese called Horny Goat Weed or Yin Yang Huo, is a classic tonic agent in traditional Chinese medicine. Epimedium, also known as epimedium, nine-leaf grass, is the aboveground part of herbaceous perennial plants such as Sagittaria Epimedi, Epimediumchinense, Epimediumwushan, or Korean Epimedium. There are more than 40 species in the world, and China is the most important distribution area of this genus. There are 27 species and 4 varieties, accounting for about 70% of the world total species [2].

At early stages, ICA is mainly used for enhancing reproductive function [3] and anti-aging. In addition, now more pharmacological studies suggest that it also possesses various therapeutic capabilities, especially for neuro-protective [4], cardio-protective [5], anti-inflammatory or anti-cancer effects [6], etc. In recent years, there has been an interest in pharmacological investigation of the immune modulator effects of ICA and its derivatives [7]. Evidence from in vitro and in vivo studies has demonstrated that the monomer has effects on regulating immunocyte, relative cytokine and multiple target mechanisms (Figure 2).

Chemical structure
ICA presents a chemical structure belonging to 8-prenyl flavonoid glycosides, which is pale yellow powder with molecular formula C₃₃H₄₀O₁₅·d (Figure 1). Its molecular weight is 676. 67 with melting point of 231–232°C. ICA can be soluble in ethyl acetate, ethanol while it is insoluble in chloroform, ether, and benzene [2].

Flavonoid glycosides are widely found in nature and are the active ingredients of various medicinal plants, which are proved to have many pharmacology activities, such as antioxidant, anti-inflammatory and anti-tumor effects, etc. It takes place at different carbon positions by hydroxyl or methoxy substitution, that is, it becomes a variety of flavonoid pigments which
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Figure 1. Chemical Structure of ICA. ICA belongs to flavonoid glycosides, of which the molecular formula is C_{33}H_{40}O_{15}. It contains sopentenyl, phenolic hydroxyl and methoxy groups which may related to antioxidant, anti-inflammatory or immune-modulatory activities. The structure suggests its material basis of potential pharmacological activities.

has different physicochemical properties. It can also be linked to glycosyl to form glycosides and has other pharmacological activities.

It is proved that in flavonoid glycosides, the number of phenolic hydroxyl groups is related to the function of antioxidant activity. The more hydrogen atoms are bound to the active radicals, the more stable the flavone radicals are formed after reaction with the active radicals, and the greater the number of hydrogen bonds is formed, the stronger the antioxidant activity will be [8]. The structure of ICA presents hydroxyl groups, suggesting its function in this aspect.

The isopentenyl-substituted compounds on the flavonoid nucleus have the activity of inhibiting tumor cells [9]. Nevertheless, flavonoids may play an anti-inflammatory or immune-modulatory role by affecting cell secretory processes, mitosis, and cell-cell interactions. It is partly related to the methoxy group structure [10]. Both structures exist in ICA, suggesting its material basis is of potential pharmacological activities.

Effects and mechanisms of immune-regulation

Lymphocytes

Th1/Th17 cells: Th17 is closely related to Th1 in development and it has been known that excessive expression of these cells is related to many inflammation diseases [11-15]. ICA can regulate lymphocytes function such as affecting Th1/Th17 or Th2 balance. In Type II Collagen-Induced Arthritis (CIA) model, ICA treatment led to the decreased ratio of CD4+ IL-17+ cells and less number of Th17 cells. Serum levels of IgG2a also reduced with alleviated arthritis score following the induced by ICA treatment, while the effects were abolished with additional IL-17 administration [16], suggesting that ICA controls CIA mainly through mediating Th17 cells in experimental autoimmune encephalomyelitis (EAE), mice administrated ICA also displayed the decreased frequencies of Th1 and Th17 cells both in the spleens and lymph node. Lower frequency of Th17 cells also was found in CNS mononuclear cells of ICA-treated mice [17]. Controversially, several studies also demonstrated that ICA may also enhance immune response. Data from these experiments demonstrated that ICA provoked the Th1-lineage development and stimulated IgG production in mice [18].

Treg cells: Regulatory T cells (Treg) are critical mediators of immune homeostasis and hold significant promise in the quest in autoimmunity and infection, while Foxp3 is a representative phenotype of Treg. Treg cells consist of diverse lymphocyte populations that include CD4+ cells, CD8+ cells, and other minor T cell population [19]. Treg population includes thymus-derived CD4+CD25+ natural Treg cells, Tr1 cells producing IL-10, Th3 cells producing TGF-beta [20] and another important cells type like iTreg cells [21], which all have a function of counterbalancing immune hyperactivity. In ovalbumin (OVA)-induced asthma model mice, IICA treatments resulted in a significant suppression on IL-17 as well as a notable increase in Foxp3 mRNA expression in isolated spleen CD4+ T cell, which suggests ICA regulates Th17/Treg balance [7].

NK cells: Natural killer cells are a type of cytotoxic lymphocyte critical to the innate immune system. The role of NK cells played is analogous to that of cytotoxic T cells in the vertebrate adaptive immune response. NK cells provide rapid responses to viral-infected cells, acting at around 3 days after infection, and respond to tumor formation [22]. It is proved that lymphokine-activated killer cell (LAK) and natural killer cell (NK) can be increased by ICA in tumor
patients. ICA may also make peripheral blood mononuclear cells to present delayed proliferation [23].

**Inflammatory cytokines**

ICA exerts diversified effect on inflammatory cytokines in treatment of different diseases. In cultivation of bronchoalveolar lavage fluid (BALF) cell, ICA caused a significant suppression in interleukin-6 (IL-6), IL-17 but not in IL-10 level [7]. In rats with brain dysfunction induced by LPS, the study demonstrated that ICA significantly improved spatial learning and memory abilities with decreased TNF-alpha, IL-1beta and COX-2 expression in the hippocampus [24]. As to titanium-stimulated mice, it is observed that ICA significantly reduced tumor necrosis factor-alpha (TNF-α) secretion, IL-6 and IL-1βin the calvariae [25]. The ICA administration also inhibited the gene expression of IL-1β, IL-8, TACR1 and ICAM-1 in HaCaT cells in a dose- and time-dependent manner. The differential production of IFN-gamma-R1 and TNF-alpha-R1 also presented after the stimulation of IFN-gamma/TNF-alpha, which was notably normalized after the ICA treatment [26]. However, in another in vitro pharmacological study of T lymphocytes stimulated by concanavalin A (ConA), ICA decreased the production of IL-2, IL-4 and IL-10 but up-regulated TNF-alpha and IFN-gamma [27]. It is possible that ICA affects immune responses depending upon the microenvironments.

**Lymphocyte transcription factors**

**RORgamma:** RORγt is a key transcription factor which controls the function and development of CD4+ Th17 and CD8+ Tc17 cells. RORγt enhances the function of Th17 cells by increasing the secretion of cytokines or chemokines such as GM-CSF and IL-17 [28]. In ovalbumin (OVA)-induced asthma model, ICA decreased the number of CD4+ RORγt+ T cells and presented a significant decrease in RORγt [7].

**T-bet:** T-box factor expressed in T cells (T-bet), is conceptualized not only a ‘master regulator’ of Th1 cells but also a broad, conserved regulator in immune responses [29]. Asthmatic rats administrated by ICA showed decreased GATA-3 mRNA expression and T-bet in pulmonary tissue and relieved by regulating the imbalance of Th1/Th2.

**TLRs**

Toll-like receptors (TLRs), as the important pattern recognition receptors in innate immunity, are also proved to be targets of ICA [30]. Ana-1 murine macrophages stimulated with ICA induced a notable expression of TLR9 which was dose-dependent. Several molecules, such as, IL-6 and TNF-alpha, which are playing an important role in downstream signaling pathway of TLR9, were obviously up-regulated by ICA [31]. On the other hand, ICA treatment also decreased the expression of toll-like receptor 4 (TLR4) of human PBMCs [32].
**STAT**

The signal transducer and activator of transcription (STAT) family are involved in regulating cellular proliferation, apoptosis, angiogenesis and the immune system response [33]. In CIA mice, ICA inhibited STAT3 activation in T cells and STAT3 inhibitor resulted in decreased IL-17 production and alleviated rheumatoid arthritis [16]. As to Inflammatory bowel disease (IBD), ICA treatment inhibited the phosphorylations of STAT1 and STAT3 in CD4(+) T cells, both are the crucial transcription factors for Th1 and Th17 respectively [34].

**Signal pathways**

**NF-kappaβ:** The NF-kappaβ is a family of transcription factors which are involved in adaptive immune functions and associated with resistance to infection [35]. The phosphorylation of c-Jun N-terminal kinases (JNK), which is the degradation of inhibitor of kappaβ, is the nuclear translocation of nuclear factor kappaβ (NF-kappaβ) p65 in LPS treated H9c2 cells. The pathway was blocked by ICA treatment. These results of studies suggested that ICA may prevent cardiomyocytes from apoptosis and inflammatory response, which may be mediated by inhibition of JNK/NFkappaβ pathway [36]. In GBC-SD cells, ICA notably inhibited both constitutive and gemcitabine-induced NF-kappaβ activity. Nevertheless, it also enhanced induced G(0)/G(1) phase arrest, caspase-3 activity, and decreased the expression of Bcl-2, Bcl-xL [37]. ICA also potentially presented the anti-tumor effect of arsenic trioxide in hepatocellular carcinoma by decreasing NF-kappaβ activity [38]. In colorectal cancer, ICA also enhanced the activity of 5-FU and suppressed tumor growth through inhibiting NF-kappaβ activity [39].

**Erk-p38-JNK:** Erk-p38-JNK plays important role in immunology functions such as affecting signals stimulating interleukin-2 (IL-2) in T lymphocytes [40-42]. It was found in B16 cell differentiation that ICA could cause cell cycle arrest at G0/G1 phase and the cell differentiation with the mechanism of inhibiting Erk1/2-p38-JNK signaling molecules [43]. In isolated human nucleus pulposus cells, ICA demonstrated significant anti-inflammatory effect, such as suppressing Erk-p38-JNK induced by IL-1bet and the activation of NF-kappaβ signaling pathways [44].

**Glucocorticoid receptor**

Glucocorticoid receptor (GR) has anti-inflammatory effect interacting with several signaling pathways such as components of the T cell receptor (TCR) signaling, PI3K, JNK and pro-inflammatory gene expression [45]. In rat model of depression induced by unpredictable chronic mild stress (CMS), oral administration of ICA for 35 days presented effect of relieving the development of depression behaviors caused by exposure to CMS with increasing mRNA expression of GR [46]. In experimental autoimmune encephalomyelitis (EAE) mice, ICA induced estrogen-like activity which modulated HPA function and increased the expression of GR of cerebral white matter [47].

**PTEN**

Phosphatase and tensin homolog (PTEN) has effect on the development and regulation of adaptive immune cells [48, 49]. These functions of PTEN are mainly related to regulating PI3K signaling pathway [50]. Cultured with ICA, ovarian cancer A2780 cells expressed increased PTEN and RECK protein expression levels [51, 52].

**PGD2**

ProstaglandinD-2 (PGD2) generated from immunologically stimulated mast cells, is the major cyclooxygenase metabolite which is thought to contribute to the pathogenesis of allergic diseases. It demonstrates various inflammatory effects [53]. In RSV-infected and OVA-induced asthma mode, BALF and PGD2 in serum were suppressed in ICA treated group [54], which shows the potential of this target in asthma by the monomer.

**Nlrp3**

The Nlrp3 plays an notable role in inflammatory responses which can adapt immunity [55]. It can support of both Th17 and Th1 responses, partially dependent on IL-18 level [56, 57]. In the hippocampus of chronic mild stress (CMS) rats, the axis of NLRP3/caspase-1/IL-1β was negatively regulated by ICA [58]. ICA also relieved renal damage of IgAN rats by inhibiting NF-kappaβ-mediated Nlrp3 activation [59].
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Conclusion

Although at early stages in traditional Chinese medicine, ICA is mainly used for enhancing reproductive function, more and more application and emerging evidence indicated that ICA is a promising compound attracted from nature herb that presents multiple immunity functions. The molecular mechanisms may include multi aspects such as the involvement of Th1/Th17 or Th2 balance, Th17/Treg regulation, NK proliferation, anti-inflammatory/inflammatory cytokines, signal pathways like NF-kappaB and Erk-p38-JNK, lymphocyte transcription factors and other targets such as TLRs, STAT and PTEN, etc. ICA has also demonstrated common immunosuppression effects on many autoimmune diseases like rheumatoid arthritis and autoimmune encephalomyelitis while it also presents anti-tumor results. Further understanding of underlying mechanism(s) of action of ICA will help us gain a comprehensive understanding of its immunity regulatory effects.

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Disclosure of conflict of interest

None.

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