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Paeoniflorin suppresses IL-6/Stat3 pathway via upregulation of Socs3 in dendritic cells in response to 1-chloro-2,4-dinitrobenze

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ABSTRACT

Mounting evidence has suggested that inflammation is associated with IL-6/Stat3 pathway in dendritic cells (DCs) and Th17 cells, which are critical for development of allergic contact dermatitis (ACD). Paeoniflorin (PF) has been clinically proved to be effective in the treatment of inflammatory skin diseases such as ACD. We have previously demonstrated the effect of PF on DCs stimulated with 1-chloro-2,4-dinitrobenze (DNCB) and naïve $CD4^+CD45RA^+$ T cells for Th17 cell differentiation. However, whether PF down-regulates IL-6/Stat3 in DCs and Th17 cells remains to be explored. In this study, we show clearly that PF markedly decreases IL-6/Stat3 in DCs stimulated with DNCB at both gene and protein levels compared with control DCs in vitro. Meanwhile, PF up-regulates suppressor of cytokine signaling 3 (Socs3). Such decreased expression of IL-6/Stat3 is abolished in DCs that were transfected with Socs3 short interfering RNA (siRNA). When mice $CD4^+CD45\ RA^+$ T cells were co-cultured with PF-treated DCs stimulated with/without DNCB, the gene expression of the Th17 cell markers such as retinoic acid-related orphan nuclear hormone receptor γt (RORγt), IL-17A, and IL-23R decreased, in accordance with the less secretions of IL-17 and IL-23 in vitro and in vivo. Finally, the suppressed Th17 differentiation induced by PF can be abolished by additional recombinant mouse IL-6. Our results suggest that the anti-inflammatory mechanisms introduced by depletion of Socs3 expression or inactivation of the negative regulator such as Socs3 may represent a promising strategy for the prevention of ACD.

1. Introduction

Allergic contact dermatitis (ACD) is the most common skin disorder, with a prevalence ranging from 15% to 25% among different occupational populations [1]. It is characterized by intensely pruritic patches, edematous erythema and occasional vesicles. The prevalence of ACD has increased worldwide rapidly, which significantly increases costs of health care and decreases life quality of patients [2]. Suppression of the inflammatory response is a fundamental approach for ACD treatment.

ACD is dendritic cell (DC) and T-cell mediated cutaneous immune response to low-molecular-weight chemicals termed haptens [3]. DCs are professional antigen-presenting cells acting as key players in the pathophysiology of ACD [4], because DCs have long been recognized first to capture and present antigen to the naïve T cells and then to induce the inflammatory response [5,6]. Furthermore, DCs by themselves produce multiple cytokines, which also regulate T cell differentiation [7]. In addition to the DCs-mediated activation, priming, and differentiation of naive T cells, DCs also participate in regulatory circuits of an immune response that has been documented in a number of studies on the ACD mouse models.

Historically, pathogenic mechanism of ACD was considered to involve a Th1-dominated or mixed Th1/Th2 response. In recent decades, the importance of Th17 cells has been recognized in ACD development [8–10]. Th17 cells are a T-cell subtype characterized by their capability toproduceIL-17, which has been associated to a wide range of cutaneous immune-mediated conditions including ACD. The link between Th17 and ACD comes from two observations. First, $IL\text{-}17^+$ T cells are predominantly infiltrated at the center of severe spongiosis of ACD. Second, an IL-17 deficient mice model has reduced contact hypersensitivity reactions, but that could be restored after wild type $CD4^+$ T cells are induced into same mice [11]. Similar to other T cell lineages, the differentiation of Th17 is orchestrated by cytokines secreted by antigen present cells,

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such as DCs [12]. Monocytes, macrophages, B-cells and DCs induce human Th17-cell differentiation through their secretion of IL-1β, IL-23 and IL-6. IL-6, which plays an important role in autoimmunity and inflammation, is essential for T helper (Th)2 and Th17 differentiation. It has been demonstrated that the reduced secretion of IL-6 in supernatants of IFN-β-treated DCs leads to a decreased differentiation of Th17-cells [13,14]. Based on these previous studies, we hypothesize that some drugs may reduce an inflammatory response of ACD by suppressing Th17 differentiation via DCs-mediated cytokines.

One of the principal regulators for cytokine signaling is the suppressor of cytokine signaling (Socs) family [15,16]. Among the Socs family members, Socs3 has been described to play the most important role in the regulation of the autoimmune response. Socs3 is an essential negative regulator of IL-6 signal transduction [17] since deletion of Socs3 results in prolonged IL-6 signaling measured by Stat3 phosphorylation. Socs3 has also been reported to play an important role in the regulation of Th17-cell differentiation through their effects on IL-6/Stat3 pathway in both innate and adaptive immune cells [15,18]. Some immunoregulatory drugs, such as IFN-β and statins, inhibit Th17-cell differentiation directly and indirectly via induction of Socs3 expression in monocytes, DCs, and B-cells. Due to their rapid induction and degradation of multiple cytokines, Socs3-mediated IL-6/Stat3-signaling pathways may represent an attractive therapeutic target for the autoimmune and inflammatory diseases.

Paeoniflorin (PF), a monoterpene glucoside, is one of major bioactive components from Paeonia root. PF has been used for 2000 years as an immune modulator and an anti-allergic agent [19]. The anti-inflammatory properties of PF have been demonstrated in several animal models [20]. PF has also been clinically proved to be effective in the treatment of inflammatory skin diseases such as ACD [21]. However, PF's therapeutic mechanisms in the control of the inflammatory response have not been clearly elucidated. Previously proposed mechanisms include inhibition of Ag presentation to DCs, reduction of T cell proliferation, and modulation of cytokine production [22]. For example, studies have demonstrated that PF could inhibit Th17 differentiation and attenuate skin inflammation of psoriasis [23]. In our previous studies, we also found that PF inhibits Th17 differentiation in response to DC-mediated ACD when being challenged with 1-chloro-2,4-dinitrobenze (DNCB) [22,24]. However, it is still not clear how PF regulates the maturation of DCs, the cytokines secretion, and differentiation of Th17 cells. Based on previous studies, we hypothesize that PF inhibits Th17 cells differentiation via increasing Socs3 expression in DCs to induce IL-6 secretion. In this experimental study, we seek to verify this hypothesis. Specially, we investigate the effects of PF on the IL-6-mediated activities in DCs, such as phosphorylation level and transcriptional activity of Stat3, as well as the expression of IL-6/Stat3 signaling target genes, Socs3. This will help us to further understand the mechanisms of PF in ACD treatment.

2. Materials and methods

2.1. Regents and chemicals

1-chloro-2,4-dinitrobenze (DNCB) and dimethyl sulfoxide (DMSO) were obtained from Sigma Chemical Co. (Sigma, USA). RPMI 1640 medium, Hank's balance salt solution, penicillin, streptomycin, L-glutamin, and fetal bovine serum (FBS) were purchased from Gibco (Gibco, USA).

PF (Fig. 1) was purchased from Nanjing Zelang Biological Technology Co. Ltd. (Nanjing, China), with a purity of ≥98% as determined by HPLC.

2.2. Mice

Female BALB/c mice were purchased from the Experimental Center of Yangzhou University (Jiangsu, China) for all animal experiments. The mice were bred in the animal experimental center of Institute of Dermatology, Chinese Academy of Medical Sciences (CAMS) in Nanjing, Jiangsu. All the mice are at age of 8 weeks with body weight 18–20 g. The animals were housed with six mice per cage at 22 °C with a 12-h light-dark cycle. Water and standard diet were available ad libitum. The experiments were performed in a pathogen-free environment. All animal experiments were approved by the Animal Study Committee of the Institute of Dermatology, CAMS, according to the government guidelines for animal care.

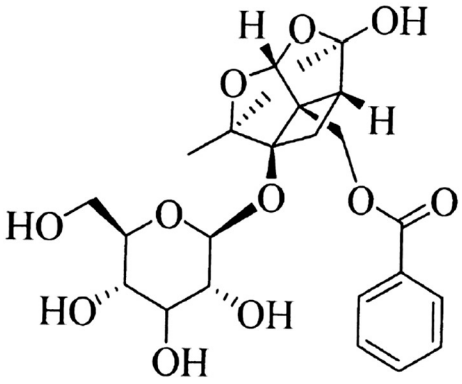

Fig. 1. The chemical structure of paeoniflorin (PF).

2.3. Cell separation and generation of bone marrow-derived DCs

Bone marrow-derived DCs were generated as fully described previously [25], with minor modifications. Briefly, bone marrow cells were obtained from femurs and tibias of normal BALB/C mice, filtered through nylon mesh, depleted of RBC with lysis buffer, and washed with PBS. Then, the cells were cultured at 5×10^6 cells/mL in RPMI 1640 containing 10% FBS (Gibco, USA) and 200 U/mL rmGM-CSF (Propetech, USA). At day 2, 5 mL of medium containing 200 U/mL rmGM-CSF was added to the plates. At days 4 and 6, half of the culture supernatants were collected and centrifuged. The cell pellet was resuspended in 5 mL of fresh culture medium containing 200 U/mL rmGM-CSF. At day 8, the cells were stained for the expression of CD11c (BD Pharmingen, USA) and DCs purity (>95%) was confirmed by flow cytometry. DCs were harvested for subsequent experiments.

2.4. Analysis of cytokines secretion by DC

At day 8, immature DCs (iDCs) were treated with PF at concentrations of 50, 100, 150, and 200 μg/mL for 24 h at 37 °C. Thereafter, DCs were either stimulated with DNCB (Sigma, USA) for 48 h or left untreated as a control group. DNCB was solubilized with non-cytotoxic concentration (≤0.02%) of dimethyl sulfoxide (DMSO, Sigma, USA).

Supernatants were collected. The supernatantIL-6 levels were measured using ProcartaPlexMul-plex (eBioscience Inc., San Diego, USA) with Luminex 200 according to the manufacturer's instructions.

2.5. Quantitative RT-PCR

The collected DCs were stimulated with DNCB (or without DNCB) in the absence or presence of PF (200 μg/mL) and DNCB for 0 h, 6 h, 12 h, 18 h, and 24 h prior to RNA isolation with an RNeasy kit (Qiagen). For the Stat3 inhibition, DCs were pretreated with 20 mM fludarabine (FLUD) for 1 h. The RNA extraction with TRIzol (Invitrogen, Carlsbad, CA, USA) was performed strictly according to the manufacturer's instructions. SYBR-Green qPCR was used to detect the relative mRNA expression levels of Socs3 and Stat3.β-actin was used as an internal control (Fermentas, Waltham, MA, USA).

The qPCR results used 2-ΔΔCT to represent the relative mRNA expression levels of the target genes. Primers used for measuring