International Immunopharmacology 30 (2016) 27–35

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Paeoniflorin ameliorates symptoms of experimental Sjogren's syndrome associated with down-regulating Cyr61 expression

Huidan Li [a], Xiaoxuan Sun [b], Jie Zhang [a], Yue Sun [a], Rongfen Huo [a], Haichuan Li [a,c], Tianhang Zhai [a], Baihua Shen [a], Miaojia Zhang [b,*], Ningli Li [a,**]

[a] Shanghai Institute of Immunology & Department of Immunology and Microbiology, Shanghai Jiao Tong University School of Medicine, Shanghai, China
[b] Department of Rheumatology, the First Affiliated Hospital of Nanjing Medical University Nanjing, China
[c] Maternity and Child Health Hospital, Changning, Shanghai, China

ARTICLE INFO

Article history:
Received 13 August 2015
Received in revised form 31 October 2015
Accepted 18 November 2015
Available online 26 November 2015

Keywords:
Paeoniflorin
Sjogren's syndrome
Cyr61/CCN1
Epithelial cells

ABSTRACT

Paeoniflorin (PF), an active compound extracted from Paeony root, has been used in therapy of autoimmune diseases with effective clinical efficiency and higher safety. Sjogren's syndrome (SS) is a chronic, systemic, immune-mediated inflammatory disease. In this study, we demonstrated that novel pro-inflammatory factor Cyr61/CCN1 was up-regulated in epithelial cells of salivary glands of primary SS patients and submandibular gland autoantigen-induced experimental SS mice. Blocking Cyr61 expression with special monoclonal antibody improved saliva secretion by ameliorating inflammatory infiltration and cytokines production in vivo. Furthermore, we showed that PF could alleviate inflammation by down-regulating Cyr61 expression in experimental SS mice. In conclusion, our new findings revealed for the first time that Cyr61 involves the pathogenesis of primary SS and PF alleviates SS-like symptoms associated with inhibiting Cyr61 expression, providing new insights into the potential molecular mechanism of PF in primary SS treatment.

1. Introduction

Sjogren's syndrome(SS) is a slowly progressing disease characterized with oral and ocular dryness and numerous extraglandular manifestations, such as vasculitis, nephrogenic diabetes insipidus, interstitial lung disease and other organs damage [1–3]. The autoimmune features of SS are inflammatory cells infiltration in exocrine glands (mainly the lacrimal and salivary glands) and reduction of secretory of the affected glands [4,5]. Although reported prevalence of SS varies from 0.4% to 0.7% [6,7], morbidity of SS might be more than 3%-5% and increase with aging [8]. The syndrome is classified as primary SS (pSS) or as secondary SS (sSS). In sSS patients, about 50% cases associate with systemic autoimmune rheumatic disorder, including rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and systemic sclerosis [9]. Thus, SS exert an adverse impact on human health and quality of life.

Although the pathogenesis of pSS is still unclear, the development of the disease is closely associated with genetic factors, environmental and stochastic events that involve innate and adaptive immunity [10]. It is reported that B lymphocytes, T lymphocytes, macrophages and dendritic cells are involved in the pathogenesis of pSS by direct cytotoxicity, releasing inflammatory cytokines, and producing auto-antibodies [11–13]. B cells were found to play a prominent role in driving the autoimmune disease through the secretion of various autoantibodies, in particular anti-SSA (anti-Ro) and anti-SSB (anti-La) [11]. Selective depletion of B cells led to improvement of subjective and objective parameters of disease activity of pSS [14]. Disruption of the Th1/Th2 balance has been proposed as a key event in the pathogenesis of pSS [15]. A recent report showed that Th1-cytokine IFN-γ enhances the production and release of plasminogen activation system components, causing subsequent tissue injury [16]. Macrophages, accumulating in cornea and limbus, can produce many mediators and cytokines (TNF-α, IL-1β and IL-6), which are thought to take part in the development of squamous metaplasia in pSS-associated dry eye [17].

It is now well known that tissue cells play very important roles in immunity and inflammation by releasing anti-inflammatory factors, inflammatory cytokines, chemokines, etc. [18,19]. Moreover, extracellular matrix (ECM) secreted by tissue cells, is emerging as an essential partner modulating the course of inflammation [20,21]. In pSS, as tissue cells, salivary gland epithelial cells play a role in the initiation and perpetuation of local autoimmune responses [22]. Increasing evidence

* Correspondence to: M. Zhang, Department of Rheumatology, The First Affiliated Hospital of Nanjing Medical University Nanjing, 300 Guangzhou Road, Nanjing 210029, China.
** Correspondence to: N. Li, Shanghai Institute of Immunology & Department of Immunology and Microbiology, Shanghai Jiao Tong University School of Medicine, No. 280, South Chong-Qing Road, Huangpu District, Shanghai 200025, China.
E-mail addresses: miaojia_zhang@163.com (M. Zhang), ninglixiaoxue57@163.com (N. Li).

http://dx.doi.org/10.1016/j.intimp.2015.11.023
1567-5769/

suggests that epithelial cells can present autoantigens, differentiate T lymphocytes, secrete cytokines (BAFF; IL-21; type I IFN; adiponectin) and other factors [23,24]. BAFF (B-cell activating factor of the TNF family) is expressed not only by macrophages, but also epithelial cells [25]. As B cells activator, down-regulation of BAFF ameliorates inflammation and acts as a therapeutic target of pSS [26]. However, whether there are new pro-inflammatory factors involves in pSS pathogenesis remains unknown.

Given that pSS is recognized in recent decade, it is important to pursuit the efficacy of pSS treatment for reducing the complications and mortality of pSS patients. By now, the treatment for pSS is mainly symptomatic and palliative. Biological therapies such as the anti-CD20 mAb Rituximab and anti-CD22 humanized immunoglobulin G1 Epratuzumab have proven to be effective in pSS treatment [27]. However, high rates of adverse drug reactions and serious toxic effects have been observed.

Paeoniflorin (PF) is one of the principal bioactive components extracted from the dried roots of *Paeonia lactiflora* Pall which has been recognized as a valuable traditional Chinese medicine for over 1500 years [28]. Series of recent studies has demonstrated that PF has strong immunosuppressive and immunomodulatory activities by suppressing T and B cells proliferation, reducing levels of inflammatory cytokines including TNF-α, IFN-γ, IL-1 and IL-17 and the expression of intercellular adhesion molecule-1, and 3-nitrotyrosine protein [29,30]. In pSS treatment, PF is found to increase the expression of AQP-5, ameliorate inflammation and normalize autoantibody profiles and thus rescue salivary gland function and delay the onset of pSS in NOD mice model [31]. However, whether PF could ameliorate symptoms of pSS by down-regulating other molecules remains unknown.

Cyr61,also named CCN1, is a component of ECM, and mainly secreted by tissue cells such as endothelial cells, fibroblasts and smooth muscle cells [21]. Its function has been well established as a new pro-inflammatory factor recently [21]. Cyr61/CCN1 is found involved in the pathogenesis of autoimmune and inflammatory diseases [21,32]. In RA, Cyr61 up-regulated the cytokines production (IL-6/IL-8/IL-1β) of synovium cells, which in turn promoted Th17 differentiation, activated macrophages and recruited neutrophils respectively [19,33,34]. Moreover, Cyr61 aggravated epidermal hyperplasia and inflammation via promoting keratinocytes (KCs) activation and proliferation in psoriasis [35]. However, whether Cyr61 plays any role in the pathogenesis of pSS as well as PF could alleviate inflammation of SS-like mouse model by down-regulating pro-inflammatory factor Cyr61 has not been explored yet.

In the current study, we first found that expression Cyr61 was up-regulated in the minor labial salivary glands (MSG) tissue of pSS patients and submandibular gland (SMG) autoantigen induced experimental SS (ESS) mice. To further explore the pathogenic role of Cyr61 in ESS mice, we blocked Cyr61 expression with a neutralizing monoclonal antibody of Cyr61 in vivo and found that impaired Cyr61 expression ameliorated inflammatory cytokines production and improved saliva secretion in ESS mice, thereby supplying evidence that ECM Cyr61 is involved in the development of SS. Furthermore, we treated the ESS mice with equivalent dose PF and found that PF treatment significantly down-regulated the expression of Cyr61 in SMG and ameliorated the SS-like symptoms of ESS mice. Therefore, our new findings revealed for the first time that Cyr61 involves the pathogenesis of SS and PF alleviates SS-like symptoms associated with inhibiting Cyr61 expression, providing new insights into the potential immunomodulatory effect of PF in SS treatment.

2. Materials and methods

2.1. Human MSG specimens

Nine pSS patients (7 females and 2 males; age range, 35–62 years; mean age, 55 years) were enrolled in this study after signing an informed consent. All the patients fulfilled the 2002 revised American–European criteria for SS. The clinical laboratory data such as erythrocyte sedimentation rate, C-reactive protein, immunoglobulin, labial salivary gland biopsy and clinical characteristics (shown in Supplementary Table 1) are determined. As non-pSS controls (4 females and 2 males; age range, 42–65 years; mean age, 52 years), six patients with xerostomia and/or eye drying complaints who did not fulfill the 2002 revised criteria for pSS were recruited. None of the patients enrolled were treated with glucocorticoid and/or immunosuppressive drugs. The experiments were performed in accordance with the Declaration of Helsinki Principles. All study protocols and consent forms were approved by the Ethics committee of the First Affiliated Hospital of Nanjing Medical University.

2.2. Animals

6–8 weeks, female, C57BL/6J mice were purchased from the Shanghai Laboratory Animal Center, Chinese Academy of Science. Mice were maintained under pathogen-free conditions. All experiments were performed according to the Animal Care and Use Committee guidelines.

2.3. Drugs

PF (molecular weight 480.05) was obtained from Liwah Plant Extraction Technology Co., Ltd. (Ningbo, China). PF was dissolved in double-distilled water and filtered before use. In this study, 150 mg/kg/day of PF was delivered by intraperitoneal injection into mice for 42 days.

2.4. Establishment and treatment of ESS mice

The SS-like mouse model was induced by immunization with SMG autoantigen as previously described with some modifications [36]. Collection of the SMG autoantigen was conducted on ice from normal 6–8 weeks, female, C57BL/6J mice. The bilateral SMG was homogenized in phosphate buffered saline and centrifuged at 12,000 $\times g$ for 5 min at 4 °C. Supernatant was collected with protein concentration was determined by the bicinchoninic acid (BCA) assay (Biotime). The supernatant was dissolved in PBS, adjusted to 4 mg/ml, and emulsified in an equal volume of complete Freund's adjuvant (Sigma) to a concentration of 2 mg/ml. On days 0 and 7, mice were injected subcutaneously with 0.1 ml/mouse of the emulsion. The booster injection was administered on day 14 with half dose of autoantigen emulsified in incomplete Freund's adjuvant (Sigma). Treatment with mAb or PF was carried on the second day of boost immunization. For the antibody treatment, mice were given 200 μg/mouse control IgG (Millipore, Billerica, MA) or anti-Cyr61mAb 093G9 generated in our laboratory (i.p.) twice a week as described previously [19]; For the PF treatment, mice were handled with 150 mg/kg/day of PF or the vehicle control (H_2O) (i.p.) every day, according to previous studies [28,30]. On day 56, all mice were sacrificed and SMG specimens were collected and studied.

2.5. Salivary flow rate measurement

Stimulated total salivary flow rate (SFR) was measured before execution. The mice were anesthetized with pentobarbital sodium (0.06 g/kg body weight) by intraperitoneal injection. Then mice were injected intraperitoneally with 20 μg of sterile isoproterenol (Sigma Chemical Co.) in PBS per 100 g body weight. After 5 min intervals, saliva was collected for 10 min by a dry cotton swab. The weight of the cotton swab was measured before and after saliva collection. The amount of saliva collected was normalized to μg saliva per g body weight per 10 min.