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Baicalin ameliorates experimental inflammatory bowel disease through polarization of macrophages to an M2 phenotype

Wei Zhu [a], Zaishun Jin [b], Jianbo Yu [b], Jun Liang [c], Qingdong Yang [d], Fujuan Li [a], Xuekui Shi [a], Xiaodong Zhu [a,*], Xiaoli Zhang [a,*]

[a] Department of Immunology, Mudanjiang Medical College, Mudanjiang, China
[b] Department of Pathology, Mudanjiang Medical College, Mudanjiang, China
[c] Department of Histoembryology, Mudanjiang Medical College, Mudanjiang, China
[d] Department of Laboratory Animals, Mudanjiang Medical College, Mudanjiang, China

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ABSTRACT

Inflammatory bowel diseases (IBDs) are chronic inflammatory disorders of the intestinal tract. Baicalin, originally isolated from the root of the Chinese herb Huangqin (*Scutellaria baicalensis* Georgi) and its main active ingredient, has a protective effect against inflammatory responses in several diseases. The present study investigated the effects of baicalin on macrophage polarization and its therapeutic role in IBD. Murine peritoneal macrophages and mice with colitis were treated with baicalin. Macrophage subset distribution, M1 and M2 macrophage-associated mRNA expression, and interferon regulatory factor 4 and 5 (IRF4 and IRF5) expression were analyzed. siRNA transfection into mouse peritoneal macrophages was utilized to suppress IRF4. Fluorescence-activated cell sorting, western blot, and real-time PCR analyses were performed. Baicalin (50 μM) limited lipopolysaccharide (LPS)-induced M1 macrophage polarization; decreased LPS-induced tumor necrosis factor α, interleukin (IL)-23, and IRF5 expression; and increased IL-10, arginase-1 (Arg-1), and IRF4 expression. siRNA-mediated IRF4 silencing significantly impaired baicalin activity. Furthermore, pretreatment with baicalin (100 mg/kg) in mice with dextran sodium sulfate (DSS)-induced colitis ameliorated the severity of colitis and significantly decreased the disease activity index (baicalin group, 3.33 ± 0.52 vs. DSS group, 5.67 ± 1.03). Baicalin (100 mg/kg) also repressed IRF5 protein expression and promoted IRF4 protein expression in the lamina propria mononuclear cells, and induced macrophage polarization to the M2 phenotype. In summary, our results showed that baicalin upregulates IRF4 protein expression and reverses LPS-induced macrophage subset redistribution. Thus, baicalin alleviates DSS-induced colitis by modulating macrophage polarization to the M2 phenotype.

1. Introduction

Macrophages, representing the major population of tissue-resident mononuclear phagocytes, play key roles in bacterial recognition and elimination as well as in the polarization of innate and adaptive immunity. They rapidly and efficiently respond to physiological changes and microbial challenges in the microenvironment, thereby promoting the return to an appropriate tissular homeostatic balance [1]. To accomplish this, macrophages can differentiate into the classic M1- or alternative M2-activated phenotypes, as appropriate. M1 macrophages are potent effector cells in inflammatory responses and are able to produce pro-inflammatory cytokines, including interleukin (IL)-23, tumor necrosis factor (TNF) α, IL-6, IL-1β, and specific chemokines, to effectively kill microorganisms. In contrast, M2 macrophages are primarily involved in tuning inflammatory responses and promoting tissue remodeling and repair through the secretion of IL-10 and transforming growth factor (TGF) β [2]. The interferon regulatory factor (IRF) 4 transcription factor specifically regulates M2 macrophage polarization in response to parasites or the fungal cell-wall component chitin [3]. In contrast, IRF5 has the dual function of activating M1 genes while repressing M2 genes by binding to similar *cis*-acting elements in gene promoters [4]. Notably, macrophage polarization is plastic, in that the switch from M1 to M2 during the inflammatory response mediates the dual role of macrophages in orchestrating the onset of inflammation and subsequently promoting healing and repair [1,5]. The two major inflammatory bowel diseases (IBDs), Crohn's disease and ulcerative colitis, are characterized by chronic recurring inflammation of the intestinal mucosa. Despite intensive research, the etiology of IBD is not completely understood; however, a prominent feature is the infiltration of highly activated macrophages into the lamina propria, which contributes to the development and perpetuation of intestinal inflammation [6]. Our previous studies showed that the proportion of M1 macrophages increased and that of M2 macrophages decreased in dextran sodium sulfate (DSS)-induced colitis, and that the transfer of M2 macrophages

* Corresponding authors at: Department of Immunology, Mudanjiang Medical College, No.3, Tongxiang Street, Mudanjiang, China.
E-mail addresses: xiaodongzhu@126.com (X. Zhu), xiaoli2595@126.com (X. Zhang).

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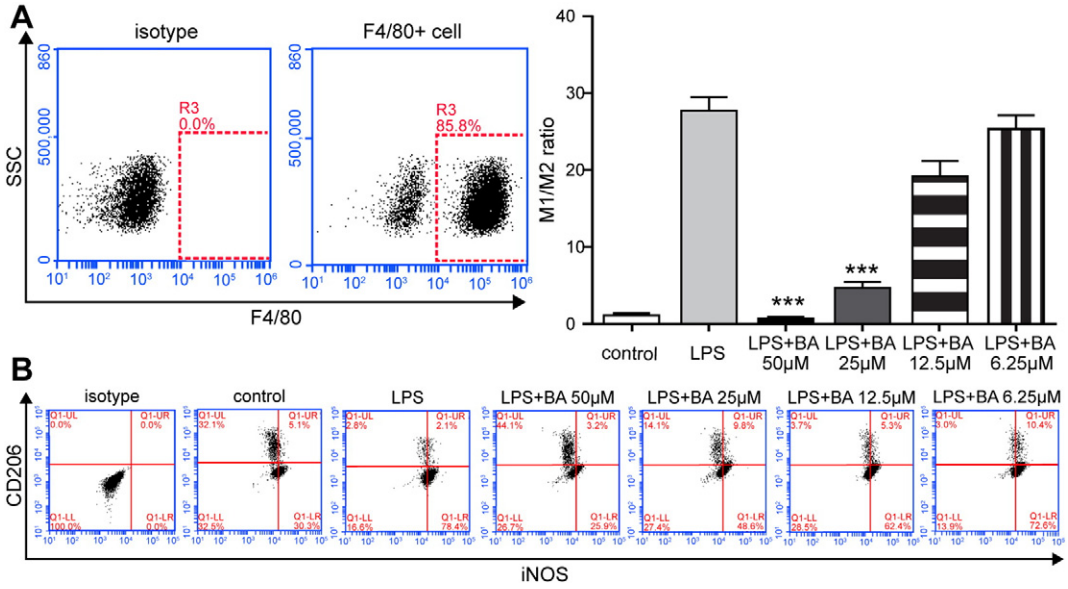

Fig. 1. Baicalin alters LPS-induced macrophage subtype distribution. Mouse peritoneal macrophages were treated with LPS (1 μg/ml) alone or with different concentrations of baicalin for 48 h followed by FACS analysis. (A) The gate is the F4/80$^+$ cells. (B) F4/80 positive cells. The data are summarized in the bar graphs. $^{\#\#\#}p < 0.001$, LPS versus control; $^{***}p < 0.001$, BA versus LPS. n = 4. Control: Cells were cultured with Dulbecco's modified Eagle's medium (DMEM); BA: baicalin.

reduced the severity of colitis by inducing IL-10 production and promoting Treg cell generation [7]. In addition, Ledesma-Soto et al. [8] found that in DSS-treated mice, extraintestinal infection with *Taenia crassiceps* significantly reduced colonic inflammation and increased arginase-1 (Arg-1) expression but decreased inducible nitric oxide synthase (iNOS) expression compared to that in uninfected mice. Furthermore, transplantation of mesenchymal stem cells has also been shown to alleviate DSS-induced colitis by recruiting macrophages to produce TGFβ. Thus, the mobilization of M2 macrophages might represent a novel approach to colitis therapy [9].

Baicalin (7-glucuronic acid, 5,6-dihydroxyflavone; molecular weight = 446.36) is a flavonoid compound originally isolated from the root of the Chinese herb Huangqin (*Scutellaria baicalensis* Georgi). Baicalin is clinically proven to be safe and is used as an anti-inflammatory drug in traditional Chinese medicine [10,11]. Recent studies have shown that baicalin significantly inhibits lipopolysaccharide (LPS)-induced IL-6, IL-8, and TNFα production by down-regulating phospho-IκB kinase (IKK) α/β and phospho-nuclear factor (NF)-κB p65 expression in HBE16 airway epithelial cells [12]. Baicalin also inhibited the toll-like receptor (TLR) 4 signaling pathway in the peripheral blood mononuclear cells in a rat model of LPS-induced fever [13] and was shown to regulate the immune balance and alleviate ulcerative colitis-induced inflammation by promoting proliferation of CD4$^+$CD29$^+$ cells and modulating immunosuppressive pathways in humans [14]. Furthermore, Abbasi et al. [15] showed that baicalin modulates the erythroid differentiation of CD133$^+$ hematopoietic stem cells as peroxisome proliferator-activated receptor (PPAR) γ agonists. Based on these observations, we hypothesized that baicalin might activate macrophages, alter macrophage subtype distribution, and help alleviate IBD. To determine the role of baicalin in the alleviation of the IBD phenotype, we examined relative IRF4 and IRF5 expression and the consequence of IRF4 siRNA silencing, macrophage M1/M2 status, and disease phenotype in mouse peritoneal macrophages/lamina propria mononuclear cells (LPMCs) and an LPS-induced mouse model of IBD.

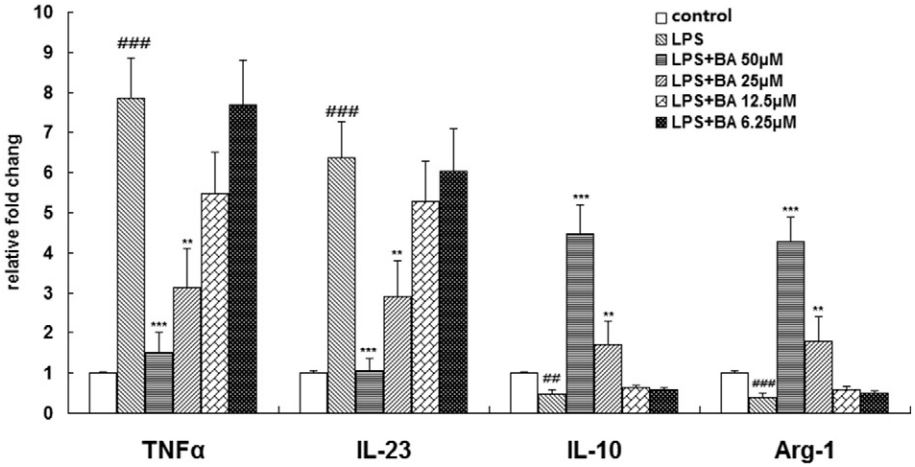

Fig. 2. Baicalin influences LPS-induced macrophage subtype-associated mRNA expression as detected by real time PCR. Mouse peritoneal macrophages were treated with LPS (1 μg/ml) alone or with different concentrations of baicalin for 24 h followed by real time PCR analysis. $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, LPS versus control; $^{**}p < 0.01$, $^{***}p < 0.001$, BA versus LPS. n = 4. Control: cells were cultured with Dulbecco's modified Eagle's medium (DMEM); BA: baicalin.