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Andrographolide alleviates imiquimod-induced psoriasis in mice via inducing autophagic proteolysis of MyD88

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ABSTRACT

Psoriasis is a chronic inflammatory skin disease with excessive activation of toll-like receptors (TLRs), which play important roles in developing psoriasis. Targeting TLR signaling remains a challenge for treating psoriasis. Here, we found that andrographolide (Andro), a small-molecule natural product, alleviated imiquimod- but not interleukin 23 (IL-23)-induced psoriasis in mice with reducing expressions of IL-23 and IL-1β in the skin. The improvement in imiquimod-induced psoriasis by Andro was not observed in microtubule-associated protein 1 light chain 3 beta (MAP1LC3B) knockout mice. Furthermore, Andro inhibited mRNA expressions of IL-23, IL-6 and IL-1β but not CD80 and CD86 in bone-marrow derived dendritic cells (BMDCs) treated with lipopolysaccharide (LPS) in a MAP1LC3B-dependent manner. In addition, Andro inhibited imiquimod-induced mRNA expressions of IL-23, IL-6, IL-1β, CD80 and CD86 in BMDCs from mice. Interestingly, Andro induced a degradation of myeloid differentiation factor 88 (MyD88) and blocked the recruitment of TNF receptor-associated factor 6 (TRAF6) to MyD88 upon LPS stimulation in BMDCs from mice. Blockade of autophagic proteolysis using NH_4Cl or MAP1LC3B$^{-/-}$ BMDCs abolished the Andro-induced MyD88 degradation. In conclusion, Andro controls activation of MyD88-dependent cytokines and alleviates psoriasis in mice via inducing autophagic proteolysis of MyD88, which could be a novel strategy to treat psoriasis.

1. Introduction

Psoriasis is an immune-mediated, genetic skin disease with a prevalence of 0.2–2%, depending on the population of different origin [1–4]. Clinical presentation of psoriasis is usually associated with red, scaly, and raised plaques, which are the consequences of a marked thickening of epidermis due to an increased proliferation of keratinocytes, an alteration of keratinocyte differentiation, and inflammatory cell infiltrates in the epidermis and dermis [3,5]. The inflammatory infiltrates are mainly composed of dendritic cells (DCs), macrophages, neutrophils and T cells in the dermis [6]. DCs in skin secret proinflammatory cytokines including interleukin 23 (IL-23), IL-6, IL-1β and tumor necrosis factor alpha (TNF-α) in developing psoriasis [2,5–9]. IL-23 polarizes naive T cells to Th17 cells [2,5,7], which contribute to keratinocyte proliferation and other hallmark features of psoriasis [1,10,11]. Targeting IL-23/Th17 axis has been proposed for treating psoriasis [7–9]. IL-1β can drive IL-17 secretion by Th17 cells [9]. IL-6 enables cutaneous T cell escape from Treg suppression and Th17 participation in inflammation [12,13]. Production of TNF-α is also elevated in psoriasis and TNF-α is considered as an activator of IL-23 synthesis in DCs [14,15]. Multiple proinflammatory cytokines are involved in developing psoriasis. It remains difficult to simultaneously control these proinflammatory cytokines in DCs.

Toll-like receptors (TLRs) are widely studied in responses of innate immune cells such as macrophages and DCs to microbial products [16]. TLR signaling involves the recruitment of adaptor proteins, such as myeloid differentiation factor 88 (MyD88) [17]. MyD88-dependent signaling includes activation of nuclear factor kappa B (NF-κB) and production of multiple proinflammatory cytokines [17]. Controlling MyD88-dependent signaling might be a potential treatment of inflammatory diseases. However, MyD88 remains difficult to target with small molecules.

Autophagy constitutes an innate defense mechanism for the sequestration and lysosomal degradation of various cytoplasmic structures, including damaged organelles and invading microorganisms [18]. There is crosstalk between autophagy and TLR signaling. On one side, autophagy can be induced by several TLR ligands and involves in innate immunity [19,20]. On the other side,

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TLR signaling can be controlled by autophagy-related proteins, aggrephagy or autophagic receptors [21]. Although it has been reported that SNPs in autophagy-related genes may contribute to higher risk of psoriasis [22], roles of autophagy in regulating TLR signaling during the development of psoriasis remain unknown.

Andrographolide (Andro) is a small molecule compound derived from andrographis (andrographis paniculata), a Chinese herbal plant [23]. Andrographis has been used for alleviation of inflammatory disorders in China, India, Japan, and Korea for a long time. Currently, it is used as a prescribed medicine for the treatment of laryngitis, diarrhea, and rheumatoid arthritis in China [23–26]. Here, for the first time, we showed that Andro alleviated imiquimod (IMQ)-induced psoriasis in mice via inhibiting the production of proinflammatory cytokines, including IL-23 and IL-1β. Consistently, Andro specifically inhibited the activation of MyD88-dependent genes in bone-marrow derived dendritic cells (BMDCs) treated with lipopolysaccharide (LPS) or IMQ. Furthermore, we found that Andro induced autophagic proteolysis of MyD88 and restricted the recruitment of TNF receptor-associated factor 6 (TRAF6) to MyD88 in BMDCs upon LPS stimulation. Therefore, control of MyD88-dependent cytokine expression by Andro could be a novel strategy to treat psoriasis.

2. Materials and methods

2.1. Mice

Eight- to ten-week-old male C57/BL6 mice were supplied by the Experimental Animal Center of Yangzhou University (Yangzhou, China). MAP1LC3B$^{-/-}$ mice were purchased from the Jackson laboratory (Bar Harbor, ME). They were maintained with free access to pellet food and water in plastic cages at 21 ± 2 °C and kept in a 12-h light–dark cycle. Animal welfare and experimental procedures were carried out strictly in accordance with the guide for the care and use of laboratory animals (Ministry of Science and Technology of China, 2006) and the related ethical regulation of our university. All efforts were made to minimize animals' suffering and to reduce the number of animals used.

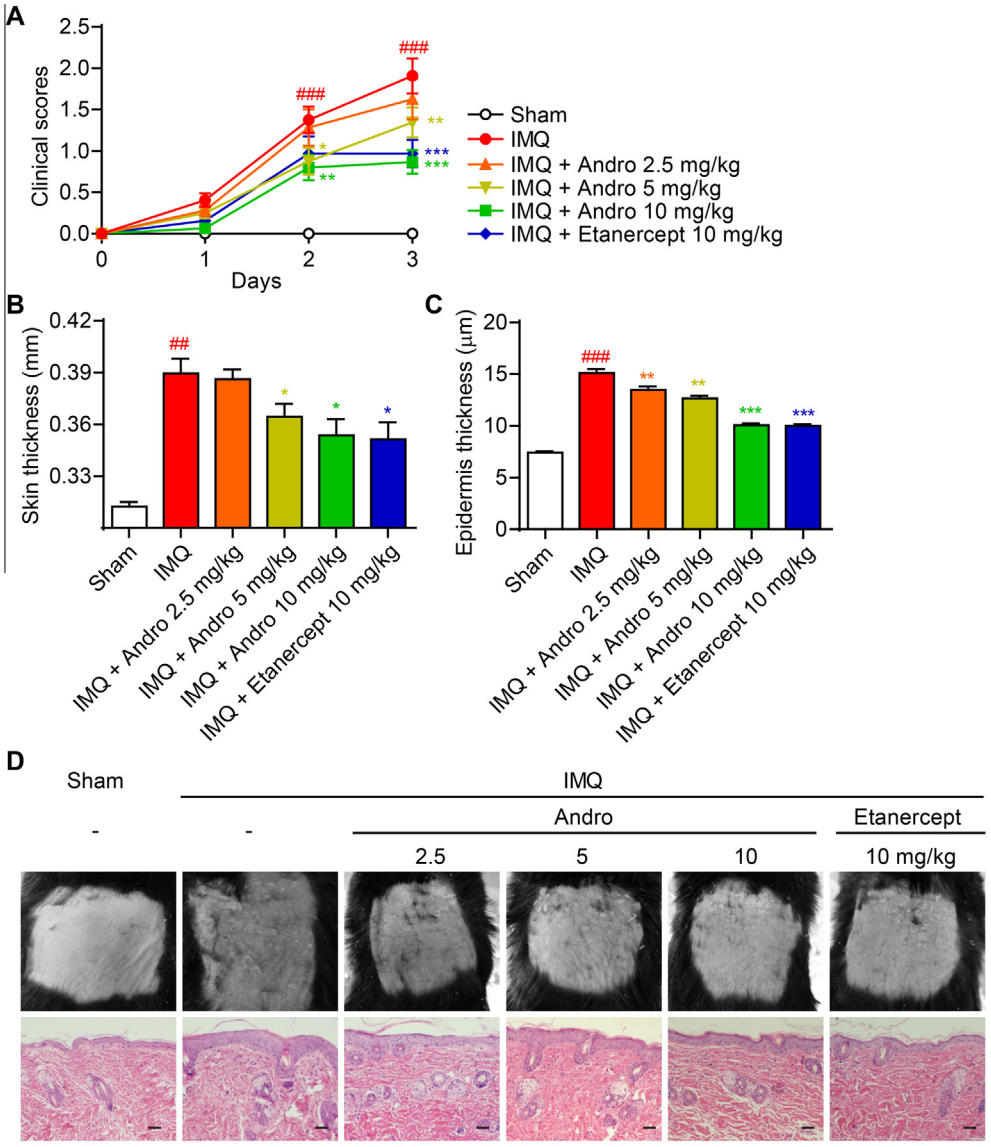

Fig. 1. Andrographolide improves IMQ-induced psoriasis in mice. (A) Clinical scores (mean ± SEM; $n = 16$ from two independent experiments). (B) Back skin thickness (mean ± SEM; $n = 8$). (C) Epidermal thickness was quantified in more than 8 fields in each back skin section (mean ± SEM; $n = 8$) $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$ versus IMQ, $^{###}P < 0.001$, $^{##}P < 0.01$ IMQ versus sham. (D) Representative photograph of back skin on day 3. Representative H&E-stained back skin sections on day 3 (scale bar, 100 μm). Data (D) are representative of at least two independent experiments.

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