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Psoriatic inflammation enhances allergic airway inflammation through IL-23/STAT3 signaling in a murine model

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ABSTRACT

Psoriasis is an autoimmune inflammatory skin disease characterized by activated IL-23/STAT3/Th17 axis. Recently psoriatic inflammation has been shown to be associated with asthma. However, no study has previously explored how psoriatic inflammation affects airway inflammation. Therefore, this study investigated the effect of imiquimod (IMQ)-induced psoriatic inflammation on cockroach extract (CE)-induced airway inflammation in murine models. Mice were subjected to topical and intranasal administration of IMQ and CE to develop psoriatic and airway inflammation respectively. Various analyses in lung/spleen related to inflammation, Th17/Th2/Th1 cell immune responses, and their signature cytokines/transcription factors were carried out. Psoriatic inflammation in allergic mice was associated with increased airway inflammation with concurrent increase in Th2/Th17 cells/signature cytokines/transcription factors. Splenic CD4+ T and CD11c+ dendritic cells in psoriatic mice had increased STAT3/RORC and IL-23 mRNA expression respectively. This led us to explore the effect of systemic IL-23/STAT3 signaling on airway inflammation. Topical application of STA-21, a small molecule STAT3 inhibitor significantly reduced airway inflammation in allergic mice having psoriatic inflammation. On the other hand, adoptive transfer of IL-23-treated splenic CD4+ T cells from allergic mice into naive recipient mice produced mixed neutrophilic/eosinophilic airway inflammation similar to allergic mice with psoriatic inflammation. Our data suggest that systemic IL-23/STAT3 axis is responsible for enhanced airway inflammation during psoriasis. The current study also suggests that only anti-asthma therapy may not be sufficient to alleviate airway inflammatory burden in asthmatics with psoriasis.

1. Introduction

Psoriasis is an immune-mediated debilitating skin disease approximately affecting 2–3% of the population [1–3]. It is a chronic skin disease, generally characterized by periods of exacerbation and remission, however it is becoming increasingly clear that psoriasis goes beyond the skin [4–6]. Psoriatic inflammation is associated with other comorbidities such as depression, cardiovascular disorders, lung infection and asthma [7–12]. For example, a recent retrospective cohort analysis on thousands of subjects suggested that those with psoriasis had a 1.38-fold increased risk of asthma [9].

The pathophysiology of psoriasis is complex and involves both skin and immune cells. Histologically, the disease is characterized by dysregulated proliferation and differentiation of keratinocytes resulting in acanthosis, parakeratosis, and hyperkeratosis in both human and mice [4–6]. Psoriatic lesions are highly infiltrated with both innate and adaptive immune cells; however IL-23, signal transducer and activator of transcription 3 (STAT3) and Th17 signature cytokines such as IL-17A, IL-22 play a central role to the formation of psoriasis skin lesions. These pro-inflammatory cytokines have been linked to the pathogenesis of psoriasis, through activation of keratinocytes and other resident cutaneous cells [5,13–15]. However, no study has previously explored how these cytokines might relate to asthmatic risk.

Abbreviations: BAL, bronchoalveolar lavage; CE, cockroach extract; i.n., intranasal; STAT3, signal transducer and activator of transcription 3; RORC, retinoic acid receptor-related orphan receptor C; IMQ, imiquimod.

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Asthma is a chronic inflammatory condition of the airways, characterized by airway eosinophilic/neutrophilic inflammation and hyperreactivity, mucus hypersecretion and remodeling [16,17]. It is well accepted that asthma results from an inappropriate Th2-dominated immune response leading to the release of IL-4, IL-5 and IL-13 which drive allergic airway responses. Real world allergens such as cockroach products which possess intrinsic serine protease activity play an important role in sensitization and subsequent development of allergic airway inflammation [18–20]. However, how these allergens interact with psoriatic inflammation is not known.

Both diseases have immune dysregulations in innate as well as adaptive compartments. IL-23/STAT3/Th17 axis is activated in systemic compartment in both human and mice during psoriasis which may impact allergic immune responses and result in increased asthma risk [13,21–24]. This view is supported by recent human studies that show increased asthma risk in patients with psoriasis [9,10]. Recent studies have also suggested involvement of IL-23/Th17 signaling in the immunopathology of asthma similar to psoriasis. For example, IL-23/IL-17A has been reported to produce neutrophilic/eosinophilic airway inflammation in murine models of asthma via regulation of Th2 and Th17 immune responses [25–28]. Based on these observations, it was hypothesized that IL-23 related signaling may be a common link between psoriasis and asthma.

Our study utilized cockroach extract (CE)-induced murine asthma model and imiquimod (IMQ)-induced psoriatic inflammation murine model, both of which are relevant to human disease pathology. CE-induced asthma model is predominantly driven by Th2 immune responses whereas IMQ-induced psoriatic inflammation model is driven by IL-23/STAT3/Th17 axis [5,13,14,18–20]. Using these models, our study shows for the first time that systemic activation of IL-23/STAT3 axis enhances allergic airway inflammation primarily by regulation of Th17 and Th2 cells and their signature cytokines/transcription factors. Our study further suggests that asthmatic inflammation in psoriatic individuals may be reduced through anti-psoriatic therapy.

2. Materials and methods

2.1. Animals

Male Balb/c mice, 8–10 weeks of age (20–25 g), free of specific pathogens, were used in the experiments. The animals were obtained from Experimental Animal Care Center, College of Pharmacy, King Saud University. The animals were kept under standard laboratory conditions of 12-h light-dark cycle and 24–26 °C ambient temperature. We used only male mice in the current study based on our earlier reports which showed robust airway inflammation in response to cockroach allergens [18–20]. All experimental animals used in this study were under a protocol approved by Animal Care and Research Committee of College of Pharmacy, King Saud University.

2.2. Imiquimod (IMQ)-induced psoriatic inflammation and cockroach extract (CE)-induced allergic airway inflammation models

Sensitization was performed according to the protocol described earlier by us [19,20]. Mice were sensitized on days 1, 2, 3, 4, and 5 with intranasal (i.n.) administrations of 50 μg whole-body German cockroach (*Blattella germanica*) extract (CE) from Greer Laboratories (Lenoir, NC) under light anesthesia. Non-sensitized control animals received only saline with the same volumes. Ten days after 1st sensitization, the mice were challenged i.n. under light anesthesia with 50 μg CE once only on days 11, 12, 13, and 14 (Fig. 1A).

2.3. IMQ-induced psoriasis-like skin inflammation in mice

Mice received a daily topical dose of 62.5 mg and 5 mg of commercially available IMQ cream (Aldara 5%; MEDA Pharma, Germany) on the shaved back and the right ear respectively for 10 consecutive days, as previously described [5,13]. Control mice were topically applied a control vehicle cream (Vaseline; Fagron; Fig. 1A).

Mice were divided into following groups: Control group (CON): mice received only vehicle cream topically on the shaved back and intranasal saline for sensitization and challenge; Sensitized and challenged group (SEN-CHAL^{CE}): mice were sensitized and challenged with CE using the same protocol described above; CE sensitized and challenged group having psoriasis-like inflammation (IMQ+SEN-CHAL^{CE}): mice received IMQ cream topically on the shaved back and the right ear for 10 consecutive days, and were sensitized and challenged with CE using the same protocol described above; IMQ treated mice (IMQ): mice received IMQ cream topically on the shaved back and the right ear for 10 consecutive days but sensitized and challenged only with saline.

To assess the role of STAT3 on allergic airway responses, a STAT3 inhibitor, STA-21 (Santa Cruz Biotech, USA) was topically applied before every IMQ or vehicle cream application at a concentration of 20 μg/mouse in acetone for 10 days as described previously [29]. CE-induced sensitization and challenge was carried out as described above (Fig. 1B). The groups were designated as STA-21+IMQ+SEN-CHAL^{CE}, or IMQ+SEN-CHAL^{CE}, or STA-21+SEN-CHAL^{CE}. Animals were sacrificed on day 15 for the collection of BAL/lung/spleen and the following analyses were carried out.

2.4. Bronchoalveolar lavage (BAL)

The mice were sacrificed by isoflurane anesthesia and the trachea was cannulated to perform BAL one day after final allergen challenge; phosphate-buffered saline was introduced into the lungs via the tracheal cannula and the total cells were counted manually in a hemocytometer chamber followed by spinning of cells onto glass slides for differential count. A differential count of at least 300 cells was made according to standard morphologic criteria on cytocentrifuged Diff-Quik (Thermo Scientific, USA) stained slides. The number of cells recovered per mouse was calculated and expressed as mean ± SE per ml for each group.

2.5. Ex vivo restimulation of splenic CD4+ T cells and adoptive transfer

Spleens were collected on day 15 from all groups. Spleens were then disrupted to create single cell suspensions, and CD4+ T cells were purified using Dynabeads® Untouched Mouse CD4+ T Cell isolation negative selection kit (Invitrogen, USA). CD4+ T cells were then stimulated with lymphocyte activator mixture [PMA (10 ng/ml)/ionomycin (400 ng/ml)/brefeldin A (5 μg/ml)] for 5 h and used for protein/mRNA expression analyses.

Lung CD4+ T cells from allergic mice were isolated on day 15 as described above and restimulated *ex vivo* with CE (100 μg/ml)+ anti-CD28 (2 μg/ml, BioLegend, USA) with/without IL-23 (20 ng/ml, BioLegend, USA) in complete RPMI 1640 (Invitrogen, USA) having 10% FCS (Gibco, USA), and 1% Pen/Strep in 24 well culture plates for 2 days and used for protein expression experiments. For adoptive transfer experiment, spleen CD4+ T cells were isolated and treated with IL-23 as described above. Naive mice were then given 3 million of these CD4+ T cells intraperitoneally. These mice were then challenged twice either with saline or CE intranasally, 6 h following the CD4+ T cells transfer, and again 24 h later. BAL