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Macrophage migration inhibitory factor (MIF) contributes to the development of allergic rhinitis

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Abstract

Macrophage migration inhibitory factor (MIF) is a proinflammatory cytokine whose expression has been found to be critical to the generation of antigen-specific immune responses. Recent studies suggested that MIF played a role in the initiation and maintenance of allergic diseases. To elucidate MIF's role in the pathogenesis of allergic rhinitis (AR), we sensitized MIF-deficient gene knockout (KO) mice and wild-type (WT) mice intraperitoneally with ovalbumin (OVA) and compared their clinical symptoms and allergic responses after intranasal challenge. Antigen-induced nasal symptoms were significantly reduced in MIF KO mice compared to WT mice. Histological examination of nasal mucosa showed that the number of infiltrating eosinophils in MIF KO mice was significantly lower than that in WT mice ($P < 0.05$). The concentration of TNF-α in nasal mucosa was also significantly lower in MIF KO mice than in WT mice ($P < 0.05$). We have demonstrated that the absence of MIF affects several aspects of experimental AR. One mechanism by which these effects might be mediated is by down regulating TNF-α. The block of allergic inflammation in MIF KO mice *suggests that MIF may play a role in the allergic response.*
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1. Introduction

Macrophage migration inhibitory factor (MIF) was first discovered as a T cell derived factor that inhibited the random migration of macrophages [1,2]. More recently, MIF was re-evaluated as a proinflammatory cytokine and pituitary-derived hormone that potentiated endotoxemia [3]. Subsequent work has shown that T cells and macrophages secrete MIF in response to glucocorticoids and on activation by various proinflammatory stimuli [4]. Although MIF has been reported to be expressed primarily in T cells and macrophages, recent studies have shown that it is also expressed in eosinophils [5–7]. MIF expression has been found to be critical to the generation of an antigen-specific immune response. Anti-MIF antibodies inhibited T cell proliferation in vitro and suppressed antigen-driven T cell activation and antibody production in vivo [8].

There is a body of evidence suggesting a relationship between allergic responses and MIF. For example, sera from patients with atopic dermatitis (AD) and bronchial asthma have been shown to contain significantly elevated levels of MIF, compared to healthy controls. Moreover, the serum concentrations of MIF were correlated with disease severities in patients with AD [6,9]. In the experimental contact dermatitis model,

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MIF-deficient gene knockout (KO) mice showed significantly milder clinical symptoms compared to WT mice [11].

We previously demonstrated that the mean MIF levels in sera from allergic rhinitis (AR) patients were significantly higher than that in sera from healthy controls. Moreover, the levels were significantly correlated with the severity of the clinical symptoms [5]. These findings suggested that MIF played a role in the initiation and maintenance of AR, but its mechanism of action in the development of AR remained unclear. In this study, we used MIF KO mice to elucidate the role of MIF in the pathogenesis of AR.

2. Results

2.1. Antigen-induced nasal symptoms

To test the role of MIF in AR, we examined the early responses (nasal rubs and sneezing) to OVA challenge in MIF KO and WT mice sensitized to this antigen. Compared with control mice (sensitized with PBS alone), OVA-sensitized WT and MIF KO mice showed 2.5- and 16-fold increases, respectively, in the number of nasal rubs on day 31 ($P < 0.05$; Fig. 1A). In contrast, an increase in the number of sneezes was only seen in OVA-sensitized WT mice, both on day 29 and 31 ($P < 0.05$; Fig. 1B). Taken together, these results show that although antigen-specific early nasal responses were induced both in WT and MIF KO mice, they were significantly lower in MIF KO mice than in WT mice.

2.2. Eosinophilia in nasal mucosa

The presence of eosinophils was confirmed using Luna's staining technique [10]. Whereas substantial numbers of eosinophils were recruited after antigen challenge, to the nasal submucosa of WT mice (Fig 2A), in MIF KO mice there were only slight eosinophilic infiltrations (Fig 2B). The numbers of infiltrating eosinophils in MIF KO mice were significantly lower than that in WT mice ($P < 0.05$; Fig. 2C). There was little eosinophilic infiltration in the sham-immunized control mice (data not shown).

2.3. Cytokine in the nasal mucosa

As shown in Fig. 3, the levels of IL-4, IL-5 and TNF-α in the nasal mucosa of WT and MIF KO mice sensitized to OVA were markedly increased compared to those in the control mice (Fig. 3A, C, and D). Concentration of TNF-α in nasal mucosa was significantly lower in MIF KO mice than in WT mice ($P < 0.05$; Fig. 3A). The concentrations of IL-4 and IL-5 in MIF KO mice were also lower than in WT mice, although in these cases, the differences did not reach significance. The levels of IL-2 in the nasal mucosa were no different in the WT and MIF KO groups (Fig. 3B). We could not detect IFN-γ in any of the samples tested.

3. Discussion

In this study, we looked at the OVA-induced model of rhinitis in MIF KO mice and demonstrated that in

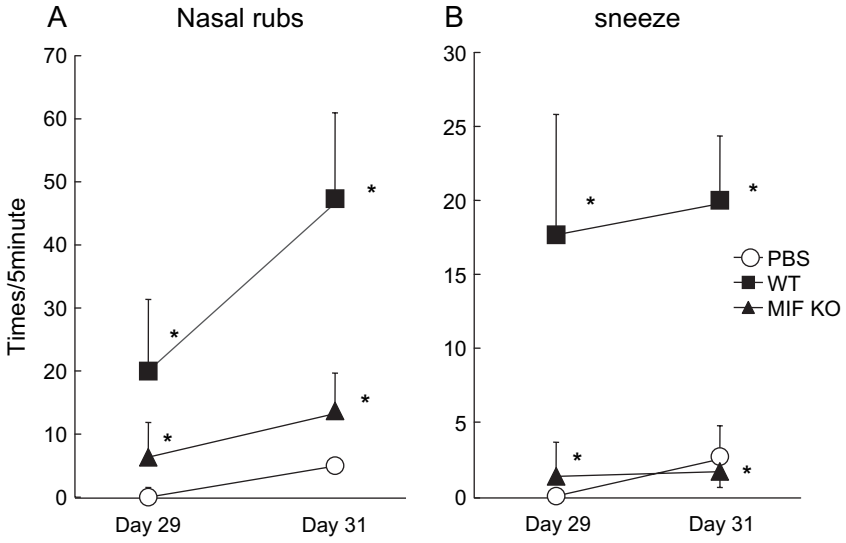

Fig. 1. Nasal symptoms after OVA antigen challenge. The allergic nasal symptoms of (A) nasal rubs and (B) sneezes were determined among all groups of mice. Compared with control mice (PBS), WT and MIF KO mice with OVA sensitization showed increase in the number of nasal rubs and sneezing. Figures given are the mean number of events seen in 5 min period, per mouse (*$P < 0.05$).

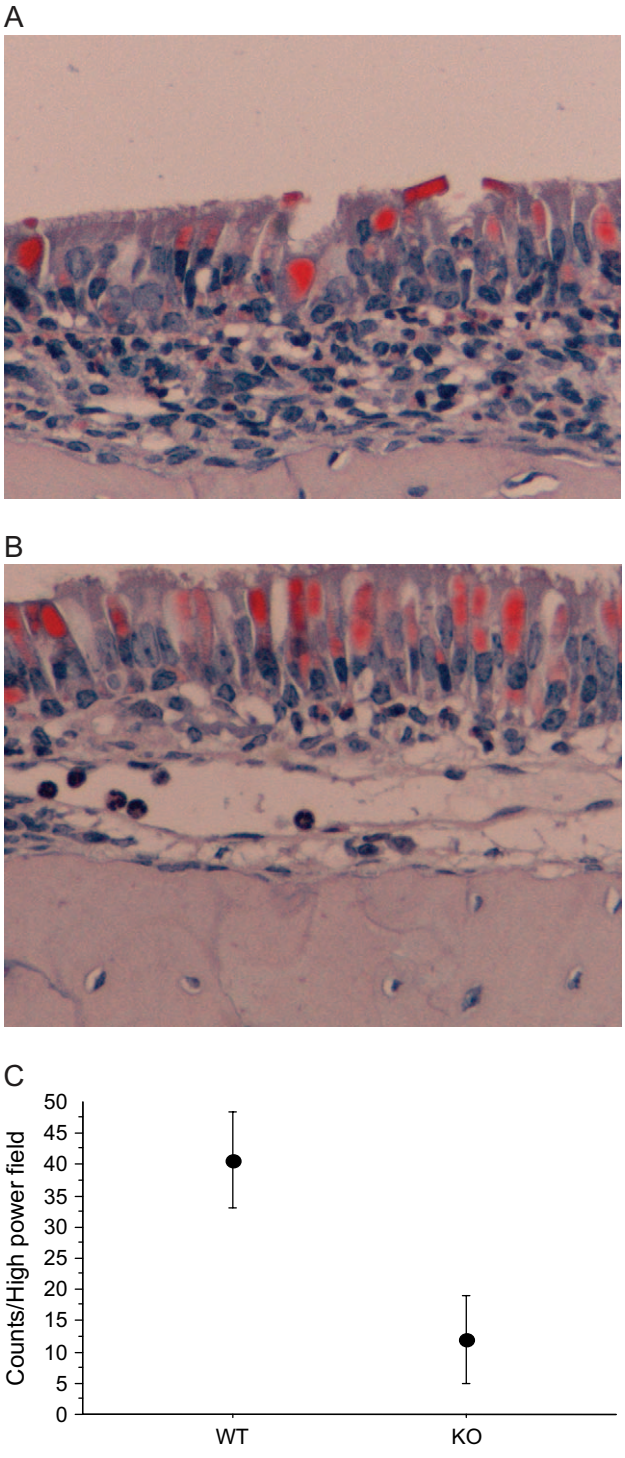

Fig. 2. Eosinophilia after antigen challenge in the nasal submucosa of OVA-sensitized WT mice (A) and MIF KO mice (B). Eosinophils were visualized using the Luna stain. The number of infiltrating eosinophils in MIF KO mice was significantly lower than that in WT mice (*$P < 0.05$; C). Original magnification, ×400.

the absence of MIF there were significant decreases in antigen-induced nasal symptoms, eosinophilia in the nasal mucosa and the concentrations of TNF-α at the sites of allergic inflammation. Although the precise mechanisms by which MIF functions in allergic inflammation are still uncertain, this and past studies have shown that the role that MIF plays in the pathogenesis of atopic diseases is likely to be in recruiting inflammatory cells, such as eosinophils and neutrophils into inflammatory sites [5–7,12]. Indeed, our study showed that eosinophil recruitment was suppressed in MIF KO mice. Eosinophils are one of the characteristic components of allergic reactions; they accumulate at inflammatory sites and release many mediators capable of initiating and maintaining allergic inflammation. Eosinophils themselves release MIF, after stimulation with phorbol myristate acetate, C5a, interleukin-5 or TNF-α, and so can further stimulate the recruitment of other eosinophils to the allergic inflammatory site [7].

AR is thought to be a T-helper type 2 (Th2)-mediated disease with a particular profile of cytokine release, which includes IL-4 and IL-5. Increasing evidence indicates that other cytokines, classically associated with Th1 cells, are also produced during the inflammatory response that characterizes human allergy [14,15]. Since the production of Th1 cytokines has generally been regarded as a feature of innate immunity, the possible association between innate immunity and allergy has attracted considerable interest.

TNF-α, one of the major Th1-type cytokines, has been implicated in allergic inflammation. This cytokine is chemotactic for granulocytes including eosinophils and is required for the production of the Th2-type cytokines such as IL-4 and IL-5 [14]. When used in a model of rhinitis, TNF-α KO mice developed significantly lower responses than WT mice, including lower antigen-specific nasal symptoms, antigen-specific IgE production, production of the Th2-type cytokines and chemokines and eosinophil infiltration in the nasal mucosa [16]. These findings indicated that TNF-α might play a key role in maintaining allergic inflammation.

MIF has been suggested to be a Th1-type cytokine, which is increased in various types of infections [17]. Monocytes, macrophages and lymphocytes constitutively express MIF, but it is rapidly released after stimulation with bacterial endotoxins and exotoxins, and cytokines. MIF induces powerful proinflammatory biological responses and has been shown to be an important effector molecule in infection [18]. A recent study has reported that MIF up-regulates the expression of Toll-like receptor 4 (TLR4), the signal-transducing molecule for the LPS receptor complex [19]. Thus, MIF may play roles in connecting innate and acquired responses.

Importantly, MIF induces TNF-α production in various diseases, such as acute respiratory distress syndrome [20] and contact hypersensitivity [11]. Shimizu et al. [9] reported that the serum level of TNF-α decreased in parallel with that of MIF in patients with AD. In addition to these findings, this study has shown that the

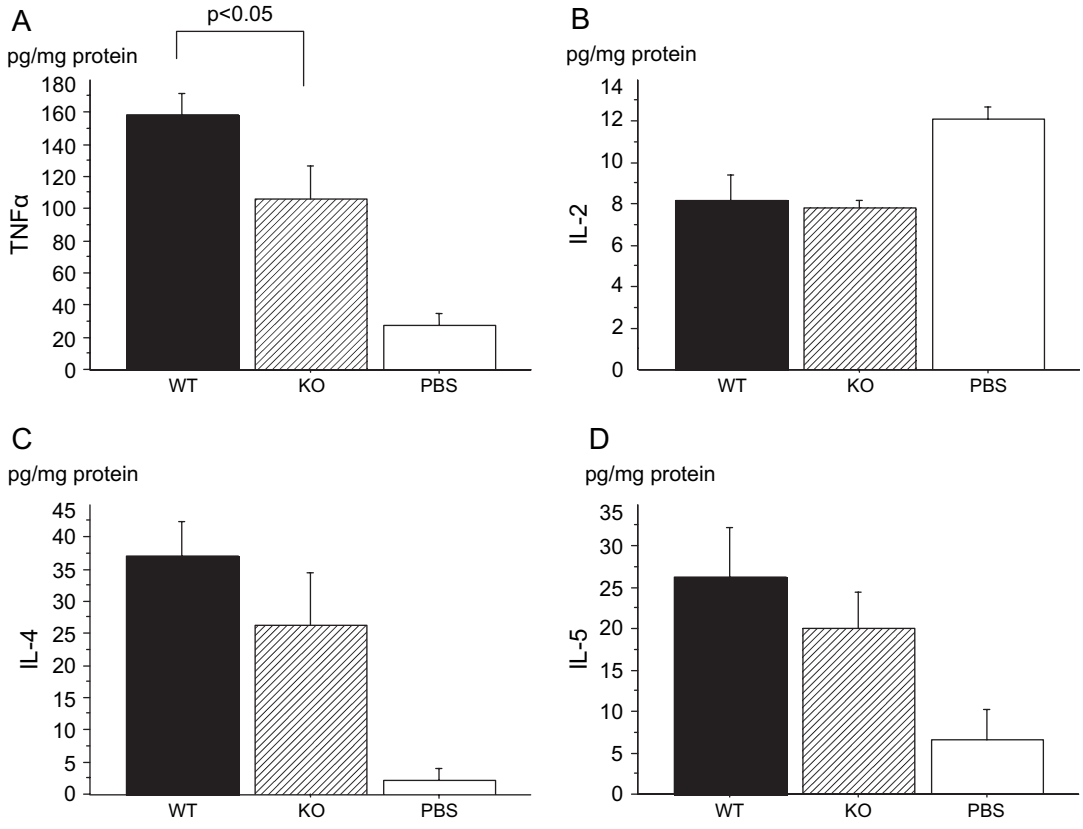

Fig. 3. Effect of MIF deficiency on cytokines in the nasal mucosa. The levels of TNF-α (A), IL-4 (C) and IL-5 (D) in the nasal mucosa of OVA-sensitized WT and MIF KO mice showed a remarkable increase compared with those in the control mice, but the levels of IL-2 (B) were no different. The concentration of TNF-α in nasal mucosa was significantly lower in MIF KO mice than in WT mice ($*P < 0.05$; A). The concentrations of IL-4 and IL-5 in MIF KO mice were also lower compared with WT mice, although the differences did not reach significance. The levels of cytokines in each sample were expressed as per mg protein.

phenotype of MIF KO mice after challenge with an allergen, such as significantly decreased nasal symptoms and eosinophil infiltration, were very similar to those in TNF-α KO mice. Moreover, we showed the concentration of TNF-α at the site of allergic inflammation was significantly depressed in these mice. These results suggest that MIF can aggravate allergic inflammation through the production of TNF-α.

In conclusion, we have demonstrated that MIF has properties that exert specific effects on the allergic response in experimental AR, some of which may be mediated by the suppression of TNF-α production. By demonstrating that the absence of MIF in KO mice blocked allergic inflammation, we have shown that MIF has an important role in the allergic response.

4. Materials and methods

4.1. Mice

A MIF deficient mouse strain (bred onto a BALB/c background) was established in our laboratory, by targeted disruption of the MIF gene, as described previously [21]. *To achieve a pure strain of the BALB/c background on MIF KO mice, backcrossing was performed more than 10 times*. BALB/c wild-type mice were purchased from Japan Clea (Shizuoka, Japan). All mice were maintained under specific pathogen-free conditions. All animal procedures were conducted according to the guidelines of the Hokkaido University Institutional Animal Care and Use Committee, using an approved protocol. Adult male mice, at 6–8 weeks of age, were used in all experiment, and each experimental group consisted of six mice.

4.2. Sensitization of mice

Sensitization of mice was performed as previously described but with a slight modification [22] (Fig. 4). In brief, MIF KO and WT mice were immunized by intraperitoneal injection of 1 μg of OVA (Grade V; Sigma Chemical Co, St. Louis MO) with 1 mg of aluminium hydroxide (Kanto Chemical Co, Tokyo Japan) on days 1, 8, 15 and 22. Sham immunizations for control mice followed the same protocol, but used

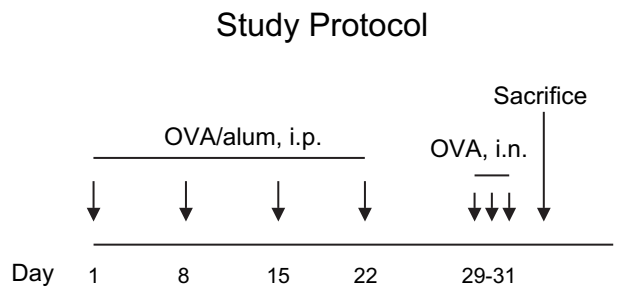

Fig. 4. Experimental design used to investigate the role of MIF in the pathogenesis of AR. MIF KO and WT mice were immunized by means of an intraperitoneal injection of OVA with aluminium hydroxide on days 1, 8, 15 and 22. The OVA challenge was administered into both nasal cavities on days 29, 30, and 31.

PBS instead of OVA. Mice were challenged with 20 μl PBS containing 500 μg of OVA in both nasal cavities on days 29, 30, and 31. Sham-challenged mice received 20 μl PBS in the same manner.

4.3. Clinical symptoms

Nasal symptoms were evaluated by counting the number of sneezes and nasal rubs (nose rubs with paws or on cage) for 5 min after the last intranasal challenge. The frequency of sneezes and nasal rubs was counted by investigators, who were blind to the treatment, watching video recordings.

4.4. Histological examination

Four hours after the final nasal challenge on day 31, the mice were sacrificed by cervical dislocation. Their heads were removed and fixed in 10% formalin for 24 h, then decalcified in 2.5% EDTA−2Na solution for 10 days. The heads were embedded in paraffin and coronal nasal sections were made and stained with Luna solution [10]. The numbers of eosinophils infiltrating the nasal mucosa in the sections were counted under the microscope.

4.5. Quantification of cytokines in nasal tissue extracts

Soon after the sacrifice, the heads of mice were removed and the whole mucosal tissues of each nose were collected under a stereomicroscope. These samples were homogenized in 0.5 ml PBS containing 0.1% Tween20, using an all-glass homogenizer, frozen in liquid N_2, thawed at 37 °C and then centrifuged for 5 min at 13,000 *g*. The protein concentration in the supernatants was determined with BCA Protein Assay Kit (PIERCE, Rockford IL) and they were stored at −80 °C until further processing. Concentrations of cytokines in the extracts were measured using the Cytometric Bead Array for mouse Th1/Th2 cytokines (BD Biosciences Pharmingen, San Diego CA), which measured five cytokines (IL-2, IL-4, IL-5, IFN-γ and TNF-α) at the same time. The levels of cytokines in each sample were compensated by the protein concentrations, the contents per mg protein were calculated.

4.6. Statistical analysis

Mann−Whitney *U*-test was used to test for correlation between the variables, with *P* values of less than 0.05 considered significant.

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