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Increase of macrophage migration inhibitory factor levels in lacrimal fluid of patients with severe atopic dermatitis

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Key words

Allergic conjunctivitis, atopic dermatitis, eye, MIF, serum, tears.

Short title

Increase of MIF in tears of severe atopic dermatitis

Abstract

Background and aims of the study: Atopic dermatitis is a chronic inflammatory skin disorder that frequently involves some ophthalmic features. Macrophage migration inhibitory factor (MIF) is a proinflammatory cytokine that is associated with the generation of cell-mediated immune responses. Although serum MIF levels may be elevated in severe atopic dermatitis, the quantity of MIF in regional ocular fluid remains unknown. We measured MIF levels in tears (lacrimal fluid) of patients with atopic dermatitis.

Patients and Methods: Tear samples were collected from 16 patients with atopic dermatitis, 10 patients with allergic conjunctivitis, and 15 healthy control subjects. The clinical severity of atopic dermatitis was evaluated according to the Scoring Atopic Dermatitis (SCORAD) index. The index was calculated to sum up following scores: extent criteria, intensity criteria, and subjective symptoms. Macrophage migration inhibitory factor levels were determined by a human MIF enzyme-linked immunosorbent assay. All comparisons were made two-tailed, and p-values < 0.01 were considered as statistically significant.

Results: The mean MIF concentration in lacrimal fluid collected from healthy control subjects was 0.69 ± 0.2 ng/ml. The mean tear MIF levels were 17.87 ± 6.3 ng/ml in moderate-severe atopic dermatitis (SCORAD ≥ 15, $p=0.002$), 0.93 ± 0.08 ng/ml in mild atopic dermatitis (SCORAD < 15), and 2.76 ± 0.86 ng/ml in allergic conjunctivitis ($p=0.008$).

Conclusions: A proinflammatory cytokine MIF level was elevated in tears as well as serum in cases of severe atopic dermatitis. These results suggest that MIF may play an important role in the induction or enhancement of ophthalmic features caused by severe atopic dermatitis.

Introduction

Macrophage migration inhibitory factor (MIF) was first discovered in the late 1960s; it is therefore believed to be the first lymphokine [6]. Since MIF was discovered as a part of the phenomenon, not a material, to inhibit migration of macrophages in the pre-molecular biology era, many scientists doubted its importance in the immune response. Investigations in the 1990s aimed at identifying novel systemic mediators that could regulate host inflammatory responses led to the identification of murine MIF as a product secreted by the anterior pituitary gland [2]. Upon stimulation, T cells release MIF, and MIF activity was first described as a product of cognate T cell supernatants [15]. Macrophages are also ~~now~~ identified as an important source of MIF and are known to express MIF both constitutively and upon stimulation [15]. Macrophage migration inhibitory factor is considered to act by both paracrine and autocrine stimulatory pathways to augment the activation of these cells [15]. As reported previously, MIF is essential for T cell activation and possibly contributes to maintaining Th1/Th2 imbalance [1]. Increased MIF expression has been reported in lesions from many immune/ inflammatory diseases, including psoriasis, glomerulonephritis, transplant rejection, neuro-Behçet's disease, asthma, adult respiratory distress syndrome, and inflammatory eye diseases [3, 4, 7, 11, 12, 13, 16, 17, 20, 24]. Atopic dermatitis (AD) frequently involves some ophthalmic features: blepharitis, chronic keratoconjunctivitis, keratoconus, early-onset cataract, and rarely, retinal detachment [9]. AD is a chronic inflammatory skin disorder and many reports have documented its pathogenesis in relation to genetic and immunological abnormalities as well as environmental factors [10]. Although abnormal populations of Th1 and Th2 subsets of helper T cells (Th1/Th2 imbalance) had been identified as a cause of the pathogenesis of AD [8, 13, 25], a decrease in delayed-type hypersensitivity (DTH) is considered to involve more than Th1/Th2 imbalance in AD [5]. MIF is essential for T cell activation and possibly contributes to maintaining Th1/Th2 imbalance as

described above [1]. Also, a prominent increase in systemic MIF levels was detected in patients with severe AD, and the levels decreased when the clinical symptoms improved following treatment with corticosteroid ointment [18, 19]. We hypothesized that a high concentration of MIF could exist in the regional fluid of the eye as well as in serum in cases of severe atopic dermatitis. Since Scoring Atopic Dermatitis (SCORAD) index, which is determined on the basis of several criteria concerning lesion spread and intensity as well as subjective signs, is commonly used to evaluate AD [21], AD patients were classified into two groups as moderate-severe and mild AD according to SCORAD index in the present study. We measured the MIF levels in tears (lacrimal fluid) of patients with AD and compared them to those of patients with allergic conjunctivitis (AC) and healthy people.

Materials and Methods

Patients

We studied 16 patients with AD and 9 subjects with AC who visited the Departments of Dermatology and Ophthalmology, Hokkaido University Hospital, Sapporo, Japan. Atopic dermatitis is a common inflammatory disorder characterized by a chronic and relapsing course. In order to evaluate the severity of the disease as objectively as possible, the European Task Force on Atopic Dermatitis had developed a method allowing consistent assessment by means of a severity index, called Scoring Atopic Dermatitis (SCORAD) index [21]. The index should be calculated to sum up following socres: (1) extent criteria (involved surface area), (2) intensity criteria (erythema, edema/ papulation, oozing/ crusting, excoriation, and lichenification), and (3) subjective symptoms (pruritus and insomnia) [21]. We classified cases of AD as “moderate-severe” (SCORAD ≥ 15) or “mild” (SCORAD < 15) according to the SCOARD index in this study. Each patient with moderate-severe AD had atopic manifestations on the

facial skin. Allergic conjunctivitis (AC) was diagnosed by slit lamp examination according to the guidelines of diagnosis and treatment of conjunctivitis, reported elsewhere [23]. Though we collected tear samples out of pollen season (December, January, and February), five of ten AC patients had sensitivity to grass or birch pollen by interview. Most of the AC patients were considered as chronic phase of AC, and their conjunctival signs and symptoms were mild. Tear samples were collected from 9 patients with severe AD (mean age, 26.1 years; age range, 18-37 years), 7 patients with mild AD (mean age, 29.0 years; age range, 16-44 years), 10 patients with allergic conjunctivitis (AC) (mean age, 32.6 years; age range, 22-44 years), and 15 healthy volunteers (mean age, 34.6 years; age range, 23-45 years). All subjects were Japanese, and healthy volunteers with no history of AD were recruited from our colleagues as controls. Dermatologists and ophthalmologists also verified no manifestations of AD and AC in controls, when their tear/ serum samples were collected. Informed consent was obtained from every patient and control subject.

Collection of tears and sera

All of the experiments in this study followed the tenets of the Declaration of Helsinki. After informed consent was obtained, tear samples were collected from all subjects. To obtain unstimulated basal lacrimal fluid, the tear samples (10 μl) were collected with microcapillary tubes for microhematocrit (75 mm length, Funakoshi Ltd., Tokyo) at the lateral canthus of patients in the supine position without any anesthesia. After obtaining informed consent, serum samples were collected from two of severe AD patients whose tear MIF levels were quite high (> 27.2 ng/ml), exceeded 1-standard deviation (SD) from the group’s median value. Also, two subjects were chosen randomly from each group of the patients with mild AD and healthy controls to measure their serum MIF levels.

Tear samples were centrifuged immediately at 4°C to remove cells and transferred into new tubes. Tear and serum samples were stored at -80°C until further examination.

Measurement of MIF

Macrophage migration inhibitory factor levels were determined by a human MIF enzyme-linked immunosorbent assay (ELISA) (CosmoBio, Tokyo, Japan) as described previously [18]. It contains all reagents necessary for performing the assay. Statistical analysis was performed using the Mann-Whitney U test.

Results

The mean MIF level in lacrimal fluid collected from healthy control subjects was 0.69 ± 0.2 ng/ml. The mean tear MIF levels were 17.87 ± 6.3 ng/ml in cases of moderate-severe AD (SCORAD ≥ 15), 0.93 ± 0.08 ng/ml in cases of mild AD (SCORAD < 15), and 2.76 ± 0.86 ng/ml in cases of allergic conjunctivitis (AC). Tear MIF levels were significantly elevated in patients with moderate-severe AD compared to normal controls (p=0.002, Table 1). The tear MIF levels of patients with AC were also higher than those of healthy subjects (p=0.008, Table 1). However, we did not detect any significant difference in tear MIF levels between patients with mild AD (SCORAD < 15) and healthy control subjects (p=0.07, Table 1).

We then focused on two cases of severe AD in which the tear MIF levels were higher than 27.2 ng/ml, because their MIF values exceeded 1-standard deviation (SD) from the group's median value. All of two had severest skin manifestations of atopic dermatitis in this study when their tears were collected. After informed consent was obtained from these patients, serum samples were drawn and the

serum MIF levels were measured. As shown in Table 2, their serum MIF levels were approximately equivalent to those in the lacrimal fluid of patients with severe AD. In contrast, although the serum MIF levels in cases of mild AD were still elevated compared to those of healthy controls, their MIF concentrations in tears were no higher than those of healthy controls (Table 2).

Discussion

In the present study, we detected high levels of MIF in the lacrimal fluid of patients with severe AD. We previously reported an increase of serum MIF levels in patients with AD [18]. Although AD frequently involves some ophthalmic features (blepharitis, chronic keratoconjunctivitis, keratoconus, early-onset cataract, and retinal detachment), how MIF behaves in the ocular fluid of patients with AD had remained unknown. It should be discussed how tear MIF levels of severe AD increase compared with AC and healthy subjeccts.

MIF is expressed and secreted in many tissues: in the brain, eye (lens, corneal epithelial cells, iris, ciliary body, and retina), ear, immune cells (thymus, spleen, lymph nodes, blood, and bone marrow, by monocytes, macrophages, T cells, B cells, dendritic cells, eosinophils, basophils, neutrophils, and mast cells), lungs, breast, endocrine systems (pituitary gland, adrenal cortex, and pacreas), liver, testis, prostate, ovaries, gastrointestinal tract, kidney, fat tissue, skin (by keratinocytes, sebaceous glands, hair follicles, endothelial cells and fibroblasts), bone, and joints [4]. This study demonstrated that tear MIF concentration is significantly higher in patients with severe AD than in controls. Patients with AC also showed significantly higher levels of MIF than healthy controls, however, there are vast differences between the averages of AD and AC groups. Since MIF levels in tears were elevated in both diseases involving immune system, one possible source of tear MIF is lymphocytes seen in conjunctival follicles.

Another possible cause of the elevation of tear MIF levels in the eyes may be the lacrimal gland, but no study has been performed if lacrimal gland expresses / secretes MIF or not. Third possible source may be peripheral blood mononuclear cells (PBMCs). As previously reported, a prominent increase in systemic MIF levels was detected in patients with severe AD and the levels decreased when the skin symptoms improved following treatment with corticosteroid ointment [18]. Furthermore, the elevated serum MIF was due to the secretion from systemic PBMC [18, 19]. We found two patients with severe AD who showed extremely high levels (in excess of 30 ng/ml) of MIF in this study. To examine how blood PBMC contributes to MIF levels in lacrimal fluid, we collected serum samples from AD patients as well as tears. Because a very high serum MIF concentration was detected in each of these two patients (Table 2) and the space of the oculi is limited, some proportion of MIF in tears may be attributable to a systemic increase of MIF. The secretion of MIF from PBMCs might contribute to the elevation of tear MIF levels more than regional inflammatory cells of eyes in severe AD. Since we did not collect blood samples from AC subjects, it is still unclear how much blood PBMC contributes to tear MIF levels in cases of AC. In the present study, AC patients did not have obvious systemic inflammation, but local. Moreover, we detected higher tear MIF levels in AC than mild AD group, suggesting that MIF may be secreted in the eye to some extent. Further studies might be requied in vernal or other etiologies of conjunctivitis, as well as treated/ untreated AC to clarify eye-derived MIF in tears.

This is the first report that MIF concentrations in tears are elevated in cases of severe AD in humans. In conclusion, MIF in regional ocular fluid may be involved in the induction or enhancement of ophthalmic features caused by severe AD.

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Table 1. Values and significance of MIF levels in tears

	MIF levels (Mean MIF ± SE)	P-values vs. normal
Normal controls	0.69 ± 0.2	-
AD- moderate to severe	17.87 ± 6.3	0.002**
AD- mild	0.93 ± 0.08	0.07
Alleregic conjunctivitis (AC)	2.76 ± 0.86	0.008**

AD: atopic dermatitis

**P<0.01 (Mann-Whitney U test, two tails)

Table 2. MIF concentrations of tears and sera

Cases	Age / Sex	Tear MIF (ng/ml)	Serum MIF (ng/ml)
1. AD- severe	20 / M	63.4	79.7
2. AD- severe	35 / M	30.1	42.0
3. AD- mild	44 / F	0.9	17.5
4. AD- mild	16 / M	0.7	12.2
5. Control	45 / F	0.8	4.7
6. Control	24 / F	1.0	3.2

AD: atopic dermatitis

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