

Interleukin-18 Is Elevated in the Sera from Patients with Atopic Dermatitis and from Atopic Dermatitis Model Mice, NC/Nga

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Key Words

IL-18 · Atopic dermatitis

Abstract

Background: Several lines of in vitro and in vivo studies have demonstrated that interleukin-18 (IL-18) shows both antiallergic and allergy-promoting activities. But its expression in allergic diseases remains unknown. **Methods:** Serum IL-18 levels from atopic dermatitis (AD) model mice, NC/Nga and control mice and from patients with AD and healthy volunteers were measured by ELISA. The relationship between IL-18 levels and serum IgE levels or clinical severity was also examined. **Results:** Serum IL-18 levels from NC/Nga mice were significantly increased compared to those from control mice. The elevation of IL-18 in the sera was observed prior to the onset and during the development of dermatitis in NC/Nga mice. In addition, IL-18 levels in the sera from patients with AD were significantly ($p < 0.05$) elevated compared to those from healthy volunteers. However, serum IL-18 levels tended to correlate negatively with serum IgE levels in patients with AD and NC/Nga mice. **Conclusion:** IL-18 is overexpressed in AD.

Introduction

Atopic dermatitis (AD) is a chronic inflammatory skin disease characterized by intensely pruritic subacute and chronic eczematous plaques [1–4]. Recent immunological analyses of the pathogenesis of the disease revealed that allergen-specific T helper type 2 (Th2) cells are found to be increased in lesional inflamed skin and are believed to play a major role both in the formation of allergic inflammation in the involved skin and in IgE elevation [1, 4, 5]. However, it has also been pointed out that in the chronic eczematous skin lesions, expression of the Th1 cytokine, interferon- γ (IFN- γ), is predominant through analysis of skin biopsies [6, 7]. An IFN- γ -inducing cytokine, interleukin-18 (IL-18), has been shown to exert innate immunity and cellular immunity through both NK activation and Th1 induction by acting on the responding cell population [8, 9]. It also has an inhibitory activity in the IgE synthesis by directly acting on B cells when given with IL-12 through its induction of endogenous synthesis of IFN- γ [10]. However, recently it has been demonstrated that IL-18 enhances eosinophil recruitment into the airways, causes basophils and mast cells to express high levels of IL-4, IL-13 and histamine, and induces naive T cells to

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develop into Th2 cells [11–13]. Thus IL-18 shows both antiallergic and allergy-promoting activities. In this study, we evaluated the serum IL-18 levels from the patients with AD and from AD model mice, NC/Nga [14, 15] to examine whether IL-18 might be associated with the immunological abnormality of AD.

Materials and Methods

Characterization of Mice Employed in This Study

All mice [specific pathogen-free (SPF) grade] were obtained from Japan SLC (Shizuoka, Japan) and housed at $23 \pm 3^\circ\text{C}$ in $55 \pm 15\%$ humidity with a 12-hour light-dark cycle (light on 7:00–19:00). Three NC/Nga mice were raised under SPF circumstances and 7 mice under conventional circumstances. The onset of skin lesions was usually observed after 7–8 weeks of age under conventional conditions [16], whereas there was no apparent skin lesion in NC/Nga mice under SPF conditions [14]. The severity of dermatitis was assessed with the following scoring system. Symptoms were evaluated by skin dryness, eruption and wounds on three parts of the body: ears, face and head and back. Symptoms were graded from – to +++ (– = no symptoms; + = mild; ++ = moderate; +++ = severe). Serum IgE levels were measured by means of an enzyme immunoassay (Yamasa Shoyu, Chiba, Japan). As controls, 3 C57/BL6 mice (8, 12, and 14 weeks of age) and 10 BALB/c mice (8–20 weeks of age) were employed. Blood was taken at the indicated week from the eye and serum was obtained by centrifugation at 3,000 rpm for 5 min at 4°C and stored at -80°C until use.

Characterization of Patients with AD

Sera were obtained from 16 adult patients with AD (age 18–34 years) and 20 age-matched healthy volunteers (age 23–45 years) and stored at -80°C until use. The severity of AD was moderate to severe, which was clinically judged by the SCORAD index [17], ranging from 13.8 to 77.5 and judged by a serum IgE level, ranging from 26 to 26,415 IU/ml. All patients met the criteria of Hanifin and Rajka [18] for AD. The study was approved by the local ethics committee, and the patients and healthy volunteers were given informed consent.

Induction of Contact Hypersensitivity Reactions

Contact hypersensitivity (CHS) reactions were induced in BALB/c mice as described previously [19]. Three BALB/c mice at 7 weeks of age were sensitized topically with 100 μl of 1% oxazolone in an olive oil:acetone solution (1:4) on the shaved ventral abdomen. Five days later, the ears were challenged with 10 μl of 0.25% oxazolone and a further 2 days later sera were obtained.

Measurement of Serum Levels of IL-18

Serum concentrations of IL-18 were measured by means of ELISA assays (IL-18 ELISA kit, MBL, Nagoya, Japan). The ELISA assays could detect an active form of human and mouse IL-18.

Statistics

The nonparametric Wilcoxon signed-rank test was used to compare the significance of difference between groups.

Table 1. Characteristics of NC/Nga mice used in the study

	Dermatitis	Age weeks	Serum levels of	
			IgE, $\mu\text{g/ml}$	IL-18, pg/ml
1	–	12	<0.1	876
2	–	8	<0.1	553
3	–	14	2.2	452
4	+++	21	104.3	151
5	++	21	89.5	364
6	+	14	44.1	400
7	++	14	97.4	209
8	+	10	86.7	373
9	+++	10	102.6	582
10	+	12	54.4	938

The severity of dermatitis was assessed with the following scoring system: – = no symptoms; + = mild; ++ = moderate; +++ = severe. Serum was obtained at the indicated age and serum levels of IgE and IL-18 were measured by ELISA.

Results

Recently an AD mouse model was established by Matsuda et al. [14]. These NC/Nga mice spontaneously develop severe dermatitis which histologically mimics human AD and show a high IgE elevation with aging under conventional circumstances, whereas under SPF circumstances, neither dermatitis nor IgE elevation develops [14–16]. The characteristics of mice in the study are shown in table 1. Indeed, 7 mice that were raised under conventional circumstances developed dermatitis and showed a serum IgE elevation, whereas there was no apparent dermatitis in NC/Nga mice under SPF conditions. Even in the sera from 3 NC/Nga mice without dermatitis and from 7 NC/Nga mice with dermatitis, figure 1 demonstrates that IL-18 was greatly increased and in some mice it reached 938 pg/ml , but all IL-18 levels from 2 control mice including C57BL/6 and BALB/c were <50 pg/ml . To examine whether IL-18 levels might correlate with dermatitis, the levels of CHS-induced BALB/c mice were also measured. The challenge of oxazolone led to an increase of serum IL-18 in BALB/c mice, as was demonstrated previously [20], but the mean level was less than that from NC/Nga mice. Figure 2 shows a relationship between serum IL-18 levels and IgE. The data showed a tendency of a negative correlation ($r = -0.571$) between serum IL-18 levels and IgE if the data from 7 NC/Nga mice that developed dermatitis were analyzed, but it was not significant with the nonparametric Wil-

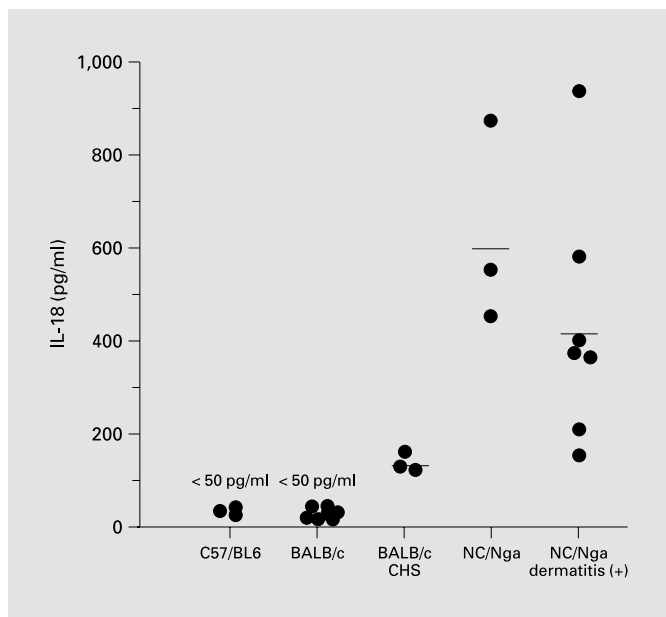


Fig. 1. Serum IL-18 levels are increased in the sera from NC/Nga mice with and without dermatitis. IL-18 levels in the sera from 3 C57BL/6, 10 BALB/c and 3 CHS-induced BALB/c mice, 3 NC/Nga mice without dermatitis (1–3 in table 1) and from 7 NC/Nga mice with dermatitis (4–10 in table 1) were measured by ELISA.

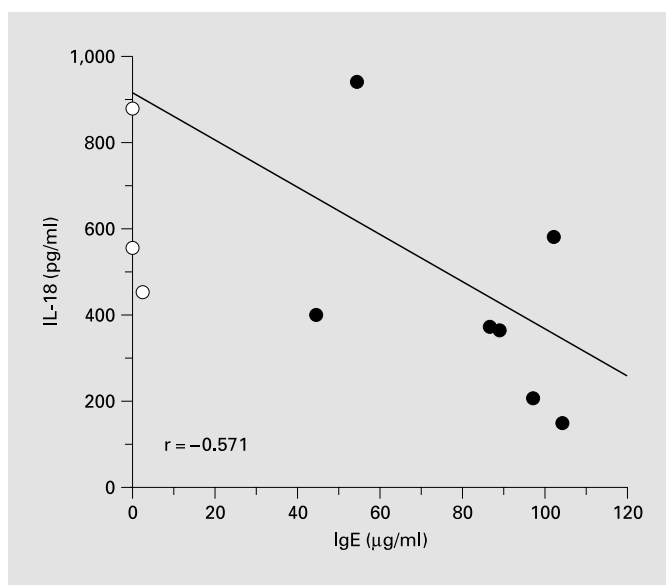


Fig. 2. Relationship of serum IL-18 levels and IgE in NC/Nga mice. The IL-18 levels showed the tendency of a negative correlation with IgE but statistical analysis showed no significance ($r = -0.571$, $p = 0.1$), when the data from 7 mice with dermatitis (●) were analyzed by using the nonparametric Wilcoxon signed-rank test.

coxon signed-rank test. Similarly, serum IL-18 levels did not show a significant negative correlation ($r = -0.578$) with IgE levels when analyzed from 10 NC/Nga mice.

Subsequently, IL-18 concentrations in the sera from patients with AD and healthy volunteers were measured. Figure 3a shows that the serum IL-18 levels were increased in the patients with AD whereas they were less than 200 pg/ml in the sera from normal controls. The average level of serum IL-18 in patients with AD was 437 pg/ml, which was significantly higher than that

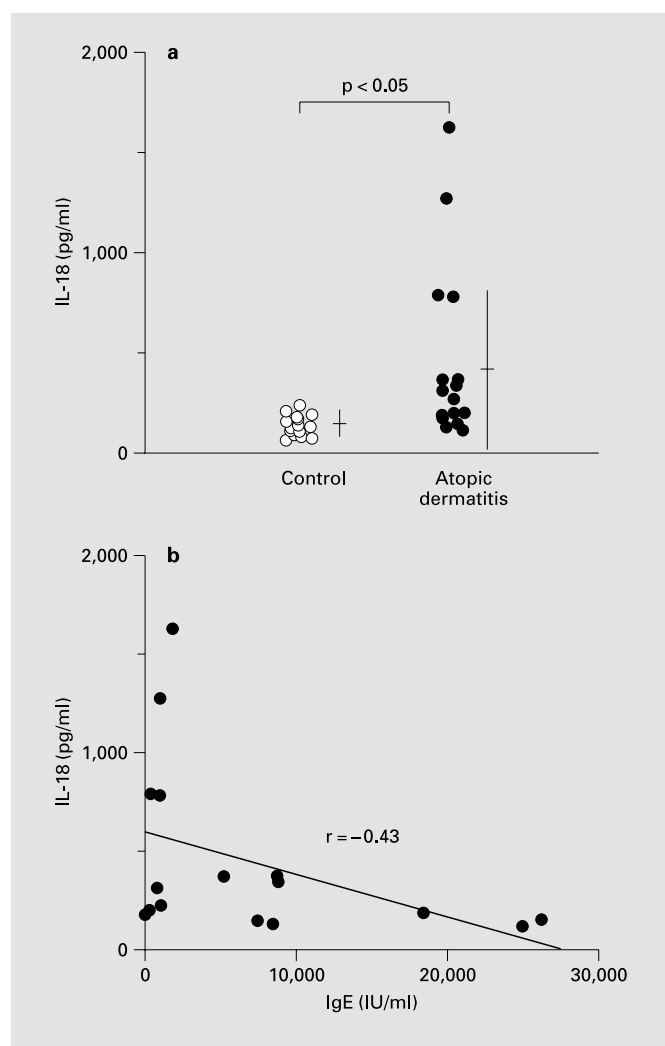


Fig. 3. Serum IL-18 levels were increased in the sera from patients with AD. **a** IL-18 levels in the sera from 20 healthy age-matched volunteers and 16 patients with AD. **b** Relationship of IL-18 levels and IgE. The IL-18 levels showed the tendency of a negative correlation with IgE but statistical analysis showed no significance.

(146 pg/ml) of the controls ($p < 0.05$) and IL-18 levels from 9 out of 16 patients were higher than 200 pg/ml. Figure 3b shows a relationship of serum IL-18 levels and IgE. IL-18 tended to show a negative correlation with serum IgE but statistically no significance was found. There was little association between the severity of dermatitis (SCORAD indexes) and IL-18 levels.

Discussion

These results indicate that IL-18 is overexpressed in the both AD patients and model mice and suggest that it might play some role in the development of dermatitis, although the precise mechanisms through which IL-18 is excessively produced and how IL-18 is associated with the development of dermatitis are unknown at the present time. Experiments using NC/Nga mice by neutralization of IL-18 through administration of anti-IL-18 antibody or through backcrossing to IL-18 gene-deficient mice will reveal the functional role of IL-18 in the process of AD.

Immune abnormalities are thought to be central to the pathogenesis of AD [1, 2]. Activated lymphocytes, mostly CD4 helper cells, and mast cells, dermal Langerhans cells, and phenotypically abnormal epidermal Langerhans cells with hyperstimulatory antigen-presenting activity have been identified in lesional and uninvolved skin [1]. Especially, Th2 cells have been shown to play a major role in the development of AD [1–5], but the molecular basis causing this preferential differentiation of Th2 cells in AD remains unknown. Moreover, the evidence that in the chronic eczematous skin lesions, expression of the Th1

cytokine, IFN- γ , is predominant makes the understanding of immune abnormality of AD more complicated [6]. It is speculated that the development of skin lesions results from sequential activation of Th2 and Th1 cells and that IL-12 from skin-invading cells such as macrophages or eosinophils is a candidate molecule leading to activation of Th1 cells in this process [7].

Recently, IL-18 in the absence of IL-12 has been demonstrated to exert allergy-promoting activities such as induction of naive T cells to develop into Th2 cells, and of basophils and mast cells to express high levels of IL-4, IL-13 and histamine [12, 13]. In addition, transgenic mice in which caspase-1 is overexpressed in keratinocytes spontaneously develop dermatitis and show high serum IL-18 and IgE [13, 21]. The caspase-1-transgenic mice by crossing with IL-18 gene-deficient mice are shown to have reduced serum IgE levels, indicating that IL-18 is responsible for inducing IgE in caspase-1-transgenic mice [13]. In NC/Nga mice serum IL-18 levels were elevated prior to the onset of dermatitis and remained high during its development. Thus NC/Nga mice are considered to represent an appropriate model to evaluate whether IL-18 is also responsible for the initial induction of Th2 differentiation and IgE synthesis in AD. Further studies are necessary to reveal the role of IL-18 in the immune abnormality in AD. Finally it should be pointed out that even if IL-18 might be an important molecule for the preferential Th2 differentiation in AD, other factor(s) that exist under conventional circumstances may be essential for the development of AD, since NC/Nga mice, when raised under SPF circumstances, do not develop dermatitis and an increase in IgE.

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