

Neutrophil Activation in Patients with ASA-Induced Urticaria

Sung-Jin Choi · Young-Min Ye · Gyu-Young Hur ·
Seung-Youp Shin · Jae-Ho Han · Hae-Sim Park

Received: 19 November 2007 / Accepted: 14 December 2007
© Springer Science + Business Media, LLC 2007

Abstract The pathogenic mechanism of acetyl salicylic acid (ASA)-induced urticaria (AIU) is not fully understood. We compared the levels of neutrophil activation and related cytokines in patients with ASA-intolerant acute urticaria (AIAU) and ASA-intolerant chronic urticaria (AICU). A total of 51 patients with AIAU, 88 patients with AICU, and 102 normal controls (NC) were enrolled in this study. The serum levels of myeloperoxidase (MPO), interleukin-8 (IL-8), and IL-18 were compared among the three groups. The serum levels of MPO were highest in the AIAU group, followed by the AICU and NC groups, and the serum levels of IL-18 were significantly higher in the AIAU and AICU groups than in NC group. Within the AIU groups, significant correlations were noted between the levels of MPO and IL-8, and IL-8 and IL-18. In conclusion, neutrophil activation, which was associated with the levels of IL-8 and IL-18 in the AIAU group, may be involved in the pathogenic mechanism of AIU. A role for IL-18 in the pathogenesis of AIU is also suggested.

Keywords ASA-intolerant acute urticaria · IL-8 · IL-18 · neutrophil activation

S.-J. Choi · Y.-M. Ye · G.-Y. Hur · H.-S. Park (✉)
Department of Allergy and Rheumatology,
Ajou University School of Medicine,
San-5, Wonchondong, Youngtonggu,
Suwon, Korea
e-mail: hspark@ajou.ac.kr

S.-Y. Shin
Department of Otolaryngology,
Kyunghye University School of Medicine,
Seoul, Korea

J.-H. Han
Department of Pathology,
Ajou University School of Medicine,
Suwon, Korea

Introduction

The clinical features of aspirin (acetyl salicylic acid [ASA]) hypersensitivity include aspirin-intolerant asthma (AIA), aspirin-intolerant acute or chronic urticaria/angioedema (AIAU or AICU, respectively), anaphylaxis, and rarely hypersensitivity pneumonitis [1, 2]. Among these conditions, AIA and aspirin-intolerant urticaria/angioedema (AIU) are the most prevalent. Chronic urticaria is defined as daily or almost daily symptoms lasting for more than 6 weeks [3]. There is general agreement that aspirin aggravates chronic urticaria in 20–40% of patients [4], who are classified as AICU. Patients whose cutaneous symptoms improve within 6 weeks are classified as AIAU [3]. The pathogenesis of AIA is known to involve the inhibition of cyclooxygenase by ASA or other nonsteroidal anti-inflammatory drugs, resulting in enhanced production of leukotrienes (LTs), which are potent inflammatory mediators synthesized from arachidonic acid via a sequential pathway [1, 2, 5]. However, knowledge of the pathogenic mechanism of AIU is limited, as there are no published data comparing the clinical features and pathogenic mechanisms of AIAU and AICU.

One major feature of urticaria is the release of histamine from activated mast cells, which leads to raised, superficial, erythematous wheals and intense pruritus [3]. Neutrophilic urticaria, a subtype of urticaria characterized by neutrophilic extravasation and a predominantly neutrophilic dermal infiltrate [6–8], has been observed in cases of chronic, cholinergic, and cold urticaria [9, 10]. However, no published data have demonstrated neutrophil involvement in AIU.

Myeloperoxidase (MPO) is a marker of neutrophil activation in serum, and the cytokine interleukin-8 (IL-8) causes neutrophil chemotaxis in vitro [11]. Recent data have indicated a role for the cytokine IL-18 in the

pathogenesis of several inflammatory diseases, including bronchial asthma [12], atopic dermatitis [13, 14], diabetes, and rheumatoid arthritis [15]. In addition, it has been shown that IL-18 promotes neutrophil accumulation, thereby promoting the early innate immune response [15].

To better understand the pathogenic mechanism of AIU, we compared the clinical features, including neutrophil activation status and serum cytokine levels, of AIAU patients with those of AICU patients.

Methods

Subjects

Three subject groups (51 patients with AIAU, 88 patients with AICU, and 102 normal controls [NCs]) were enrolled at Ajou University Hospital in Suwon, Korea. AIU was defined by a positive result on an ASA oral provocation test, which was performed using 500 mg of ASA (Rhonal, KunWha Pharmaceutical Co., Seoul, Korea). Those patients diagnosed with AIU were then divided into two groups, AICU and AIAU, according to the duration of urticaria. Patients whose cutaneous symptoms of ASA hypersensitivity were resolved within 6 weeks of ASA ingestion were assigned to the AIAU group, and those whose cutaneous symptoms lasted longer than 6 weeks were placed in the AICU group. NCs who were non-atopic with no personal or family history of allergic disease and no past history of ASA hypersensitivity were recruited from the general population. All subjects gave written informed consent, as required by the institutional review board of Ajou University Hospital. Skin prick tests were performed using 12 common aeroallergens (Bencard Co., Bredford, UK), and atopy was defined by one or more positive reactions. Total immunoglobulin E (IgE) was measured using a UniCAP system (Phadia, Uppsala, Sweden). The presence of rhinosinusitis was evaluated by clinical history and rhinoscopy.

ELISA for MPO, IL-8, and IL-18

The serum MPO level was measured by enzyme-linked immunosorbent assay (Bioxytech® MPO-Enzyme Immunoassay, OXIS Health Products, Inc., Portland, OR, USA), as were the serum IL-8 (Pierce Biotechnology, Inc., Rockford, IL, USA) and serum IL-18 levels (Medical & Biological Laboratories Co., Ltd., Nagoya, Japan), as per the manufacturers' guidelines.

Biopsy and Immunohistochemistry

To confirm neutrophilic infiltration into the lesions of the patients with urticaria, a skin biopsy was collected from the

site of urticaria on each patient's forearm. Tissue samples from eight patients with AIU were fixed in formalin and embedded in paraffin for immunohistochemical analysis using rabbit polyclonal antibodies against neutrophil elastase (DakoCytomation, Glostrup, Denmark) and MPO (Novocastra Laboratories Ltd., Newcastle, UK). Briefly, the sections were deparaffinized in xylene and rehydrated in a graded alcohol series; endogenous peroxidase activity was blocked by treatment with 3% hydrogen peroxide for 10 min. The sections were then treated with protein blocking solution and primary antibodies for 2 h at room temperature. After several rinses in phosphate-buffered saline (PBS), the sections were incubated with biotinylated secondary antibodies. Bound antibodies were detected by the streptavidin-biotin method using a Cap-Plus detection kit (Zymed Laboratories Inc., South San Francisco, CA, USA). Finally, the slides were rinsed in PBS, exposed to diaminobenzidine, and counterstained with Mayer's hematoxylin. All sections were evaluated by conventional light microscopy ($\times 100$, $\times 200$, and $\times 400$). The numbers of stained cells are presented as cells/mm² of tissue. Each sample was divided into fields using a grid containing 100 points (intersections of crosses, covering a surface area of 0.5 mm²); the cells were observed through an eyepiece graticulate at $\times 200$. The mean cell count was derived from the sum of four fields, as reported by Fokkens et al. [16].

Statistical Analysis

Statistical analyses were completed using SPSS version 11 (SPSS Inc., Chicago, IL, USA). A *p* value of 0.05 or less was considered to be significant. Student's *t*-test or a chi-square test was used to analyze differences between the groups. Pearson's correlation analysis was used to assess the correlation between two variables.

Results

Clinical Characteristics of the Study Subjects

The clinical characteristics of the study subjects are summarized in Table 1. There were no significant differences in mean age or sex distribution between the AIAU and AICU groups; however, mean age, sex, and total IgE level differed significantly between the AIAU group and the NC group ($p < 0.001$, $p = 0.012$, and $p < 0.001$, respectively), and between the AICU group and the NC group ($p < 0.001$, $p = 0.002$, and $p < 0.001$, respectively). There were no significant differences in the prevalence of atopy, allergic rhinitis, or allergic asthma between the AIAU and AICU groups.

Table 1 Comparison of the Clinical and Laboratory Findings Among the AIAU, AICU, and NC Groups

Parameter	AIAU (<i>n</i> =51)	AICU (<i>n</i> =88)	NC (<i>n</i> =102)	<i>p</i> value		
				AIAU vs. AICU	AIAU vs. NC	AICU vs. NC
Age (years)*	37.7±14.2	36.7±12.3	29.4±6.7	NS	<0.001	<0.001
Sex (M/total) (%)	25/51 (49%)	45/88 (51.1%)	28/102 (27.7%)	NS	0.012	0.002
Duration (months) ^a	0.6±0.6	3.08±4.4	NA	NS	NA	NA
Atopy (positive/total; %)	25/40 (62.5%)	59/86 (68.6%)	0/3 (0%)	NS	NS	0.036
Allergic rhinitis (positive/total; %)	10/16 (62.5%)	8/19 (42.1%)	NA	NS	NA	NA
Allergic asthma (positive/total; %)	0/51(0%)	1/88(1.1%)	0/102 (0%)	NS	NS	NS
Log total IgE (IU/ml)	2.1±0.5	2.3±0.4	0.5±0.3	NS	<0.001	<0.001
MPO (ng/ml)	386.4±100	150.2±40.9	129.6±8.4	0.032	0.014	NS
IL-8 (pg/ml)	27.3±4.7	22.7±3.5	18.3±1.7	NS	NS	NS
IL-18 (pg/ml)	200.8±19.3	200.5±17.7	147.8±10.2	NS	0.008	0.011

All total IgE values were log-transformed and are presented as the mean±standard deviation

All MPO, IL-8, and IL-18 values are presented as the mean±standard error

NS Not significant; NA not applicable; AIAU aspirin-intolerant acute urticaria; AICU aspirin-intolerant chronic urticaria; NC non-atopic healthy control

^a All values presented are the mean±standard deviation

Serum MPO, IL-8, and IL-18 Levels

The serum MPO level was significantly higher in the AIAU group (386.4±100 ng/ml) than in the AICU (150.2±40.9 ng/ml) and NC groups (129.6±8.4 ng/ml; $p=0.032$ and $p=0.014$, respectively); in contrast, there were no significant differences in the serum IL-8 level among the AIAU (27.3±4.7 pg/ml), AICU (22.7±3.5 pg/ml), and NC groups (18.3±1.7 pg/ml). The serum IL-18 level was significantly higher in the AIAU (200.8±19.3 pg/ml) and AICU groups (200.5±17.7 pg/ml) than in the NC group (147.8±10.2 pg/ml; $p=0.008$ and $p=0.011$, respectively; Table 1).

Comparison of Clinical and Laboratory Parameters According to the MPO Level in the AIAU and AICU Groups

We next divided the AIAU and AICU groups each into two subgroups according to the serum MPO level, using a level of 300 ng/ml, which was derived from the mean control value plus a twofold standard deviation, as the dividing line. We compared several clinical and laboratory parameters between the two groups. In the AIAU group, the patients with >300 ng/ml MPO had a significantly higher serum IL-8 level ($p=0.004$); however, no significant difference in sex, atopy, age, total IgE, or IL-18 level was found between the two AIAU subgroups. In the AICU group, the patients with >300 ng/ml MPO tended to be older ($p=0.011$), but there was no significant difference in sex, atopy, total IgE, or in the level of IL-8 or IL-18 between these two subgroups (Table II).

Correlations Among Serum MPO, IL-8, and IL-18 Levels in AIAU and AICU Patients

To evaluate the relationship between the MPO and cytokine levels, the correlations among the serum MPO, IL-8, and IL-18 levels in the AIAU and AICU groups were analyzed. There were significant correlations between the serum MPO and IL-8 levels, and between the serum IL-18 and IL-8 levels in the AIAU group ($r=0.36$, $p=0.01$; and $r=0.57$, $p=0.001$, respectively; Fig. 1). In addition, a weak correlation between the serum MPO and IL-18 levels was indicated. Among the AICU patients, significant correlations were found between the serum MPO and IL-8 levels, the serum MPO and IL-18 levels, and the serum IL-8 and IL-18 levels ($r=0.49$, $p=0.001$; $r=0.40$, $p=0.001$; and $r=0.40$, $p=0.001$, respectively; Fig. 2).

Immunohistochemistry

Figure 3 shows an abundance of elastase-positive cells around a vessel in an AICU patient, and several MPO-positive cells were present around a different vessel in the same tissue sample. There was no significant difference in the number of neutrophils between the AICU and AIAU groups (data not shown).

Discussion

In this study, no significant differences were found in several clinical parameters between AIAU and AICU patients, except for the serum MPO level, which was

Table II Comparison of the Clinical and Laboratory Parameters Between the AIAU and AICU Subgroups Divided According to Serum MPO Level (Cutoff Value, 300 ng/ml)

Parameter	AIAU Patients			AICU Patients		
	Serum MPO (ng/ml)		<i>p</i> value	Serum MPO (ng/ml)		<i>p</i> value
	<300 ^a	≥300		<300 ^a	≥300	
Age (years)	38±14.89	36.73±12.13	0.792	35.8±11.7	48.8±14.7	0.011
Sex (M/total) (%)	21/40 (52.5%)	4/11 (36.3%)	0.499	40/81 (49.4%)	5/7 (71.4%)	0.435
Atopy (positive/total; %)	24/36 (66.7%)	1/4 (25.0%)	0.139	56/79 (70.9%)	3/7 (42.9%)	0.199
Log total IgE	2.13±0.50	2.14±0.74	0.952	2.3±0.4	2.3±0.2	0.942
IL-8 (pg/ml)	18.7±2.6	58.9±17.0	0.040	19.3±3.0	68.7±27.1	0.129
IL-18 (pg/ml)	174.0±13.9	298.2±68.2	0.102	189.6±16.2	348.7±130.1	0.278

All total IgE values were log-transformed and are presented as the mean±standard deviation

All IL-8 and IL-18 values are presented as the mean±standard error

AIAU Aspirin-intolerant acute urticaria; AICU aspirin-intolerant chronic urticaria

^a The cutoff value was the mean + 2 standard deviations of the NC serum MPO level

significantly higher in the AIAU group than in the AICU group. Considering that MPO is a known marker of neutrophil activation [11, 17, 18], the pathogenic mechanism of AIAU may involve more extensive neutrophil activation than that of AICU. Although the pathogenic mechanism of AIU is not clearly understood, it may be thought of as a non-immunologic reaction in which the possible rapid depletion of prostaglandin E2 triggers skin eruptions by promoting mast cell discharge and cysteinyl LT biosynthesis [19]. Toppe et al. [20] identified 22 cases of neutrophilic urticaria by biopsy from 108 urticarial patients with acute and chronic urticaria, dermatographism, cold and cholinergic urticaria, and delayed pressure urticaria. They reported that the patients with neutrophilic urticaria had a shorter mean duration of the disease than did the other urticarial patients, and they proposed that neutrophilic urticaria may be an acute-phase urticarial reaction associated with intense inflammatory infiltration and marked upregulation of some mast cell-derived

cytokines, leukotriene B4 (LTB4), platelet activating factor, and/or chemokines [20]. These findings suggest that neutrophil activation is involved in AIU and that it may play a pathogenic role in AIAU.

The mechanism of neutrophil activation in AIU patients is not fully understood. Bochenska-Marciniak et al. [11] demonstrated that nasally induced IL-8 caused an influx of neutrophils into the nasal mucosa in vivo in patients suffering from seasonal allergic rhinitis, and several studies have shown that IL-8 is a potent chemo-attractant and activating factor for neutrophils [21, 22] and may cause bronchial hyper-responsiveness and airway neutrophil accumulation in vivo in guinea pigs [23]. It was also reported that mast cell-derived neutrophil chemotactic activity was increased during a lysine-ASA broncho-provocation test in ASA-intolerant asthmatics [24–26]. LTB4, a neutrophil chemotactic factor, is known to play a role in IL-8-induced bronchial hyper-responsiveness [23]. Considering that LTB4 could be released from mast cells in AIU patients, we speculate that LTB4 may have a role in neutrophil activation in AIU patients. In the present study, a significant correlation was found between the serum MPO and IL-8 levels in AIAU patients, suggesting that IL-8 might be directly involved in neutrophil activation and might thereby contribute to the development of AIAU.

The cytokine IL-18 has been shown to cause neutrophil chemotaxis in vitro [15]. IL-18 is a member of the IL-1 cytokine family, which enhances T cell and natural killer (NK) cell cytotoxicity and directly induces interferon- γ production in NK cells. IL-18 has been proposed to play a role in the innate immune response [15] as well as in the pathogenesis of several inflammatory diseases, including bronchial asthma [12], atopic dermatitis [13, 14], diabetes, and rheumatoid arthritis [15]. Yoshimoto et al. [27] showed that basophils and mast cells derived from bone marrow

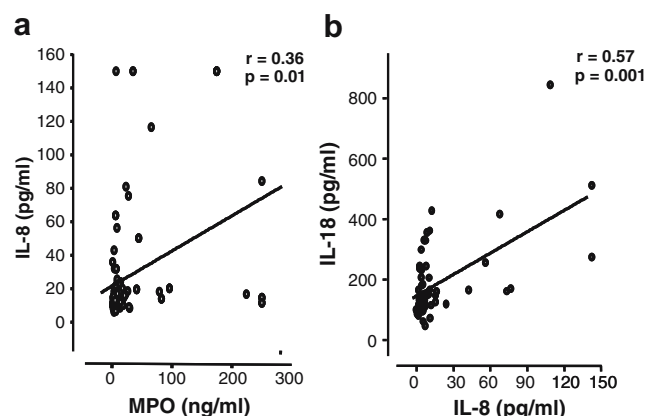
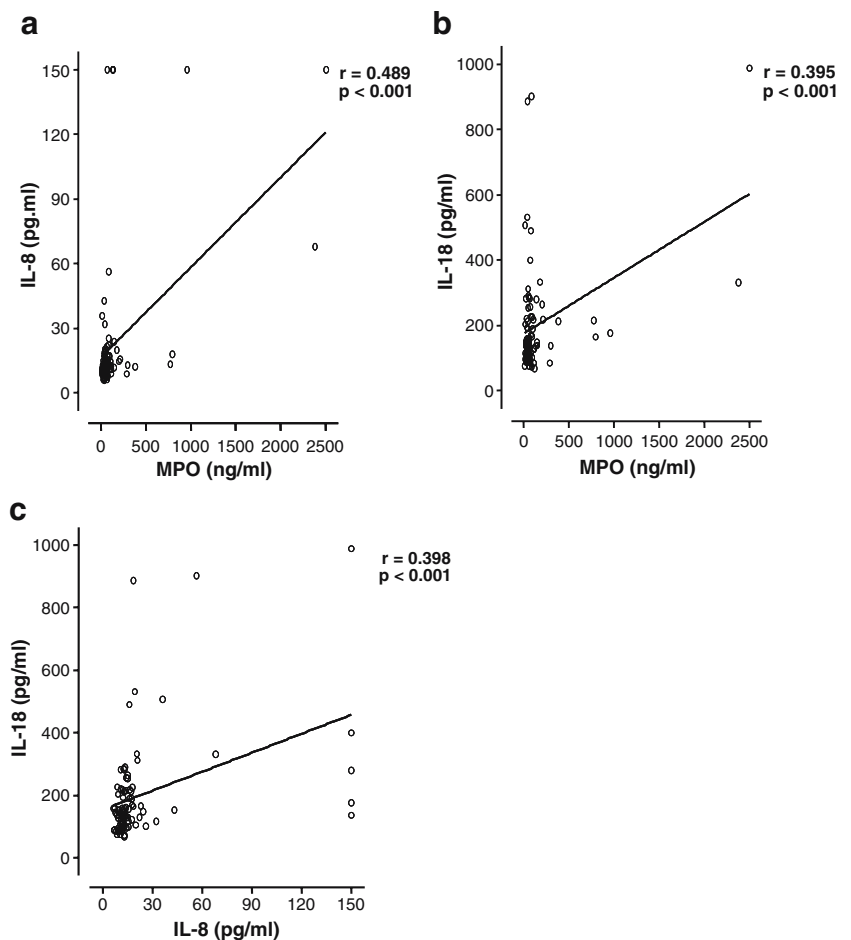


Fig. 1 Correlation between the serum levels of MPO and IL-8 (a) or IL-18 (b) in AIAU patients

Fig. 2 Correlation between the serum levels of MPO and IL-8 (a) or IL-18 (b), and IL-8 and IL-18 (c) in AICU patients



cells cultured with IL-3 for 10 days expressed IL-18R α chain and that the basophils also produced large amounts of Th2 cytokines, such as IL-5 and IL-13, and histamine in response to stimulation with IL-3 and IL-18. Moreover, transgenic mice over-expressing IL-18 in their keratinocytes spontaneously produced IgE and developed atopic dermatitis even in the absence of STAT 6 activation, which is required for Th2 cell development and IgE responses; this suggests that IL-18 can induce allergic inflammation

independent of Th2/IgE [27]. Thus, IL-18 can directly stimulate T cells and mast cells to release Th2 cytokines and histamine [28]. Furthermore, IL-18 has the capacity to recruit neutrophils by inducing neutrophil chemotaxis, similar to the function of IL-8 in humans [29]. Nevertheless, there are no published data to suggest a role for IL-18 in the pathogenesis of AIU. We found that the serum IL-18 level was significantly higher in both the AIAU and AICU groups compared with the NC group and that the IL-8 and

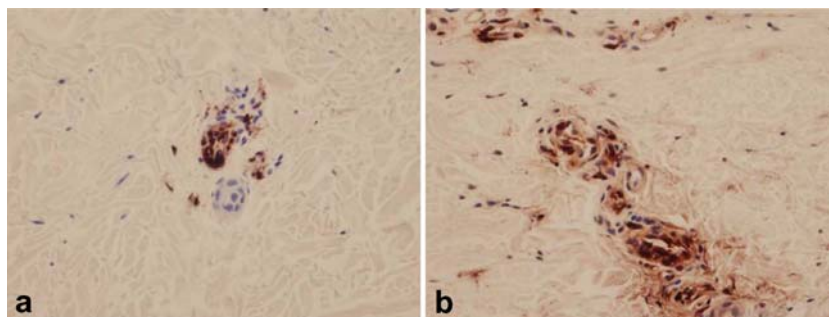


Fig. 3 Immunohistochemical staining of skin tissue from a single AIU patient. **a** Abundant cells around a vessel are positively stained with antibodies to neutrophil protein elastase. **b** MPO-positive cells are seen around a vessel in the same tissue

IL-18 levels were significantly correlated in both the AIAU and AICU groups. Although the involvement of IL-18 in AIU has not been determined in detail, we propose that IL-18 might participate in the release of histamine from mast cells and basophils and might indirectly contribute to the release of IL-8 in the pathogenesis of AIU.

In conclusion, our results demonstrate that neutrophil activation is increased in AIAU, in association with elevated levels of IL-8 and IL-18, suggesting that neutrophil activation and IL-18 are involved in the pathogenesis of AIU.

Acknowledgements This study was supported by a grant from the Korean Health 21 R&D Project, Ministry of Health and Welfare, Republic of Korea (03-PJ10-PG13-GD01-0002 and A050571).

References

- Simon RA. Adverse respiratory reactions to aspirin and nonsteroidal anti-inflammatory drugs. *Curr Allergy Asthma Rep.* 2004;4:17–24.
- Szczeklik A, Stevenson DD. Aspirin-induced asthma: advances in pathogenesis, diagnosis, and management. *J Allergy Clin Immunol.* 2003;111:913–21.
- Powell RJ, Du Toit GL, Siddique N, Leech SC, Dixon TA, Clark AT, et al. BSACI guidelines for the management of chronic urticaria and angio-oedema. *Clin Exp Allergy.* 2007;37:631–50.
- Mastalerz L, Setkiewicz M, Sanak M, Szczeklik A. Hypersensitivity to aspirin: common eicosanoid alterations in urticaria and asthma. *J Allergy Clin Immunol.* 2004;113:771–5.
- Marek LK, Joanna SM. Aspirin-exacerbated respiratory disease. *Allergy Clin Immunol Int-J World Allergy Org.* 2006;18:140–9.
- Peters MS, Winkelmann RK. Neutrophilic urticaria. *Br J Dermatol.* 1985;113:25–30.
- Winkelmann RK, Reizner GT. Diffuse dermal neutrophilia in urticaria. *Hum Pathol.* 1988;19:339–93.
- Winkelmann RK, Wilson-Jones E, Smith NP, English JS, Greaves MW. Neutrophilic urticaria. *Acta Derm Venereol (Stockh).* 1988;68:129–33.
- Winkelmann RK. Cholinergic urticaria shows neutrophilic inflammation. *Acta Derm Venereol (Stockh).* 1985;65:432–4.
- Winkelmann RK. Immunofluorescent and histologic study of cold urticaria. *Arch Dermatol Res.* 1985;278:37–40.
- Bochenska-Marciniak M, Kupczyk M, Gorski P, Kuna P. The effect of recombinant interleukin-8 on eosinophils' and neutrophils' migration in vivo and in vitro. *Allergy.* 2003;58:795–801.
- El-Mezzein RE, Matsumoto T, Nomiya H, Miike T. Increased secretion of IL-18 in vitro by peripheral blood mononuclear cells of patients with bronchial asthma and atopic dermatitis. *Clin Exp Immunol.* 2001;126:193–8.
- Novak N, Kruse S, Potreck J, Maintz L, Jenneck C, Weidinger S, et al. Single nucleotide polymorphisms of the IL18 gene are associated with atopic eczema. *J Allergy Clin Immunol.* 2005;115:828–33.
- Yoshizawa Y, Nomaguchi H, Izaki S, Kitamura K. Serum cytokine levels in atopic dermatitis. *Clin Exp Dermatol.* 2002;27:225–9.
- Leung BP, Culshaw S, Gracie JA, Hunter D, Canetti CA, Campbell C, et al. A role for IL-18 in neutrophil activation. *J Immunol.* 2001;167:2879–86.
- Fokkens WJ, Holm AF, Rijntjes E, Mudler PGH, Vroom TM. Characterization and quantization of cellular infiltrates in nasal mucosa of patients with grass pollen allergy, non-allergic patients with nasal polyps and controls. *Int Arch Allergy Appl Immunol.* 1990;93:66–72.
- Schmekel B, Karlsson SE, Linden M, Sundstrom C, Tegner H, Venge P. Myeloperoxidase in human lung lavage. I. A marker of local neutrophil activity. *Inflammation.* 1990;14:447–54.
- Tauber E, Herouy Y, Goetz M, Urbanek R, Hagel E, Koller DY. Assessment of serum myeloperoxidase in children with bronchial asthma. *Allergy.* 1999;54:177–82.
- Mastalerz L, Setkiewicz M, Sanak M, Rybarczyk H, Szczeklik A. Familial aggregation of aspirin-induced urticaria and leukotriene C4 synthase allelic variant. *Br J Dermatol.* 2006;154:256–60.
- Toppe E, Haas N, Henz BM. Neutrophilic urticaria: clinical features, histological changes and possible mechanisms. *Br J Dermatol.* 1998;138:248–53.
- Baggiolini M, Walze A, Kunkel SL. Neutrophil-activating peptide-1/interleukin 8, a novel cytokine that activates neutrophils. *J Clin Invest.* 1989;84:1045–9.
- Leonard EJ, Yoshimura T. Neutrophil attractant/activation protein-1 (NAP-1) (interleukin-8). *Am J Respir Cell Biol.* 1990;2:479–86.
- Fujimura M, Xiu Q, Tsujiura M, Tachibana H, Myou S, Matsuda T, et al. Role of leukotriene B4 in bronchial hyperresponsiveness induced by interleukin-8. *Eur Respir J.* 1998;11:306–11.
- Kim SS, Park HS, Yoon HJ, Lee YM, Lee SK, Nahm DH. Enhanced serum neutrophil chemotactic activity was noted in both early and late asthmatic responses during lysine-aspirin bronchoprovocation test in ASA-sensitive asthmatic patients. *J Korean Med Sci.* 2003;18:42–7.
- Grzelewska-Rzymowska I, Szmidi M, Rozniecki J, Grzegorzczak J. Serum neutrophil chemotactic activity (NCA) during aspirin-induced bronchoconstriction. *Allergol Immunopathol (Madr).* 1988;16:99–103.
- Ortoli C, Capsoni F, Restuccia M, Pastorello E, Riboldi PS, Zocchi M, et al. Neutrophil chemotactic factor of anaphylaxis (NCF-A) release in aspirin-induced asthma. *Clin Allergy.* 1984;14:443–52.
- Yoshimoto T, Tsutsui H, Tominaga K, Hoshino K, Okamura H, Akira S, et al. IL-18, although antiallergic when administered with IL-12, stimulates IL-4 and histamine release by basophils. *Proc Natl Acad Sci U S A.* 1999;96:13962–6.
- Konishi H, Tsutsui H, Murakami T, Yumikura-Futatsugi S, Yamanaka K, Tanaka M, et al. IL-18 contributes to the spontaneous development of atopic dermatitis-like inflammatory skin lesion independently of IgE/stat6 under specific pathogen-free conditions. *Proc Natl Acad Sci U S A.* 2002;99:11340–5.
- Wang W, Tanaka T, Okamura H, Sugita M, Higa S, Kishimoto T, et al. Interleukin-18 enhances the production of interleukin-8 by eosinophils. *Eur J Immunol.* 2001;31:1010–6.