

Plasma endothelin-1 levels during exacerbation of atopic dermatitis

Namjil N. Tsybikov, M.D., Ph.D.,¹ Irina V. Petrisheva, M.D.,¹ Boris I. Kuznik, M.D., Ph.D.,² and Eli Magen, M.D.³

ABSTRACT

Background: Endothelin (ET) -1 was found to participate in the pathogenesis of bronchial asthma. At present, there is no information regarding the role of ET-1 in the pathophysiology of atopic dermatitis (AD).

Objective: We assessed blood ET-1 levels during the exacerbation of AD and during the 4 months postexacerbation period, to assess the relationships between blood ET-1 levels and clinical severity of AD and pruritus.

Methods: Patients with AD and during exacerbation were recruited from the dermatology department at the Chita Medical Academy (Chita, Russia). Objective Severity Scoring of Atopic Dermatitis index, itch intensity, plasma concentrations of ET-1, and serum total immunoglobulin E levels were measured at study entry and after 4 months.

Results: Eighty-two patients with AD, 35 women (45.1%) and 47 men (54.9%), mean (SD) age of 42.2 ± 11.5 years were included. Thirty healthy volunteers served as controls. The mean (SD) objective Severity Scoring of Atopic Dermatitis index score during AD exacerbation was 48.8 ± 19.4 and at the 4 months postexacerbation period was 16.1 ± 8.3 ($p < 0.01$). Mean (SD) itch score during AD exacerbation was 6.9 ± 1.9 and, at 4 months postexacerbation was 2.6 ± 0.7 ($p < 0.01$). Mean (SD) plasma levels of ET-1 in patients with AD (0.74 ± 0.45 fmol/mL) were significantly higher than in healthy subjects (0.43 ± 0.24 fmol/mL) ($p < 0.001$). Significant correlations were found between plasma ET-1 levels with the objective Severity Scoring of Atopic Dermatitis index ($r = 0.51$; $p < 0.001$), Itch severity ($r = 0.62$; $p < 0.001$), and with serum immunoglobulin E levels ($r = 0.63$; $p < 0.001$) at the exacerbation time point in patients with AD.

Conclusion: During AD exacerbation, plasma ET-1 levels were elevated and were positively correlated with AD clinical severity, itch intensity, and serum IgE levels.

(Allergy Asthma Proc 36:320–324, 2015; doi: 10.2500/aap.2015.36.3846)

Atopic dermatitis (AD) is a chronic or chronically relapsing, pruritic inflammatory skin disease.¹ The majority of patients with AD exhibit high immunoglobulin E (IgE) levels, with high levels of the T-helper (Th) 2 cytokines (interleukin [IL] 4, IL-13), which promotes immunoglobulin class switching and excessive IgE production.² Current pathophysiologic models associate AD with T-cell activation, particularly Th2/Th22 polarization, with a contribution of Th1 and Th17 components in chronic AD.³

Endothelin-1 (ET-1) was originally characterized as a potent vasoconstrictor,⁴ but it is now recognized to have many other biologic properties.⁵ ET-1 was shown to activate several inflammatory cells, including macrophages⁶ and mast cells,⁷ and it has been reported to exacerbate certain allergic reactions.^{8,9} ET-1 was also

found to be expressed in airway epithelial and endothelial cells, which play a role in the pathogenesis of bronchial asthma.¹⁰ In the airways, ET-1 has been found mainly in the bronchial epithelium,¹¹ and its increased expression was correlated with the severity of the disease.¹²

At present, there is no information regarding the role of ET-1 in the pathophysiology of AD. We hypothesized that ET may be released during the exacerbation of AD. In this observational study, we examined blood ET-1 levels during the exacerbation of AD and at the 4-month postexacerbation period. We also assessed the relationship between blood ET-1 levels and the clinical severity of AD and pruritus.

MATERIALS AND METHODS

Subjects

The subjects were recruited during the period of 2011 to 2014 from the dermatology dispensary at the Chita Medical Academy (Chita, Russia). Patients with AD, ages between 15 and 40 years, were enrolled in this study. AD was diagnosed by the criteria of Hanifin and Rajka,¹³ with a duration [mteq]1 year. The disease severity was graded by the objective Severity Scoring of Atopic Dermatitis (SCORAD) index.¹⁴ The objective

From the ¹Pathophysiology Department, Chita Medical Academy, Chita, Russia, ²Normal Physiology Department, Chita Medical Academy, Chita, Russia, and ³Clinical Immunology and Allergy Unit, Medicine B Department, Barzilai Medical Center, Ben Gurion University of the Negev, Ashkelon, Israel

The authors have no conflicts of interest to declare pertaining to this article

Address correspondence to Eli Magen, M.D., Clinical Immunology and Allergy Unit, Medicine B Department, Ben Gurion University of Negev, Zionut 21/33, Ashdod, 77456, Israel

E-mail address: allergologycom@gmail.com

Copyright © 2015, OceanSide Publications, Inc., U.S.A.

SCORAD has a range of 0 to 83 (plus an additional 10 points for severe disfiguring eczema on the face and hands). An objective SCORAD score of <15 was classified as mild, 15–40 as moderate, and >40 as severe. The subjective itch intensity was obtained by the visual analog scale, which ranges from 0 to 10, by the same dermatologist (I.V.P.). Exclusion criteria were as follows: any other chronic disease, acute infection, oral intake of any medications, ultraviolet-therapy conducted less than 2 months before the study entry, or pregnancy and lactation. A group of age- and sex-matched healthy volunteers served as controls. The study was approved by the ethics committee at the Chita Medical Academy (Chita, Russia), and all the participants gave informed consent.

There were two main reasons for choosing subjects with AD who were 15–40 years old: (1) in our region, the prevalence of asthma in children with moderate-to-severe AD ranged from 25% to 50%, and ~8% of them had food allergy, so all our subjects had AD without any other chronic disease; and (2) the children with AD experienced increased rates of infection, especially during cold seasons in Siberia, therefore, we suggested that it would be easier to find subjects with AD and without comorbidities in those who were 15–40 years old, to eliminate the potential influence of comorbidities on plasma ET-1 levels.

The subjects with AD were included during exacerbation, which was manifested by flares of eczema at ≥ 3 sites or by generalized skin lesions. Every recruited subject was assessed at two time points: (1) during the exacerbation (study entry), and (2) at the 4-month postexacerbation period, after using emollients, antihistamines, and topical steroids. None of the subjects had used oral corticosteroids and/or immunomodulatory medications during the study. Throughout the 4 weeks before the second assessment, the subjects were allowed to use emollients only, with no additional pharmacologic treatment.

Laboratory Analysis

Blood samples were collected from a cubital vein into tubes by using sterile citrate plasma tubes. Plasma was obtained by centrifugation at 3000 Relative Centrifugal Force for 15 minutes within 30 minutes of blood collection and was stored at -70°C before analysis. The sampling was performed at the same time of day for every subject. Plasma concentrations of ET-1 were measured by using commercial enzyme-linked immunosorbent assays (Biomedica Group, Vienna, Austria) in accordance with the manufacturer's instructions. Serum samples were analyzed for total IgE levels by using the immunometric assay of the Immulite 2000 system (DPC; Global Siemens Health Care, Erlangen, Germany).

Statistical Analyses

Results are presented as mean (SD). Differences in clinical and laboratory characteristics between the groups were analyzed by using χ^2 or the Fisher exact test when appropriate. Correlations among the variables were analyzed by the Pearson correlation and Spearman rank test, depending on the distribution of the data. Statistical significance was defined as two-sided p value $< .05$. All statistical analyses were performed by using the software Statistica 6 (StatSoft, Inc., Tulsa, OK).

RESULTS

The study included 82 subjects with AD, 35 women (45.1%) and 47 men (54.9%), mean (SD) age 42.2 ± 11.5 years. A group of 30 healthy volunteers, matched for age and sex, served as controls. The clinical characteristics of the subjects and controls are presented in Table 1. The mean (SD) objective SCORAD score during the exacerbation of AD was 48.8 ± 19.4 and, at the 4-month postexacerbation period, was 16.1 ± 8.3 ($p < 0.01$). The mean (SD) itch score during the exacerbation of AD was 6.9 ± 1.9 and was 2.6 ± 0.7 ($p < 0.01$) at the 4-month postexacerbation period. The mean (SD) plasma level of ET-1 in subjects affected by AD (0.74 ± 0.45 fmol/mL) was significantly higher than observed in the healthy subjects (0.43 ± 0.24 fmol/mL) ($p < 0.001$). No significant age- or sex-dependent influence on the plasma ET-1 levels was observed in the subjects with AD or in the control group (data not shown).

Significant correlations were found among plasma ET-1 levels with the objective SCORAD ($r = 0.56$; $p < 0.001$) (Fig. 1), itch severity ($r = 0.62$; $p < 0.001$) (Fig. 2), and serum IgE levels ($r = 0.63$; $p < 0.001$) (Fig. 3) at the exacerbation time point in subjects with AD. No significant correlations among these parameters were observed at the 4-month postexacerbation period in subjects with AD and in the control group. During the 3 months' active treatment period, 5 subjects (6.1%) experienced AD exacerbation; during the last 4 weeks, 7 additional subjects (8.5%) had flare while they were using only emollients. For these subjects, the ET-1 levels still positively correlated ($r = 0.65$, $p = 0.021$) with the objective SCORAD at the second assessment.

DISCUSSION

In this study, we determined the plasma ET-1 levels in subjects with AD and in healthy controls. The study demonstrated that, during the exacerbation of AD, plasma ET-1 levels were elevated and normalized at the 4-month postexacerbation period. There are other findings in this study: (1) during exacerbation, plasma levels of ET-1 were positively correlated with the AD clinical severity and itch intensity, and (2) plasma lev-

Table 1. Demographic and laboratory data of the study population

	AD, <i>n</i> = 82	Healthy Controls, <i>n</i> = 30	<i>p</i> Value
Age, mean (SD), y	24.5 ± 7.9	24.9 ± 6.2	0.803
Female, no. (%)	37 (45.1)	14 (46.7)	1
Duration of AD, mean (SD), y	17.1 ± 11.2	0	<0.001
Body mass index, mean (SD), kg/m ²	25.2 ± 2.2	25.4 ± 1.9	0.684
Objective SCORAD, mean (SD)			
Exacerbation (baseline)	48.8 ± 19.4	0	<0.001
At end of 4-mo postexacerbation period	16.1 ± 8.3*	0	<0.001
Clinical severity of itch, mean (SD)			
Baseline exacerbation	6.9 ± 1.9	0	<0.001
At end of 4-mo postexacerbation period	2.6 ± 0.7*	0	<0.001
Plasma ET-1, mean (SD), fmol/mL			
Baseline exacerbation, mean (SD)	0.74 ± 0.45	0.43 ± 0.24	<0.001
At end of 4-mo postexacerbation period	0.49 ± 0.24*	0.41 ± 0.21	0.109
Serum IgE, mean (SD), kU/L			
Baseline exacerbation, mean (SD)	355.6 ± 331.2	49.2 ± 27.9	<0.001
At end of 4-mo postexacerbation period	281.5 ± 194.7	45.7 ± 32.1	<0.001

**p* < 0.001 (exacerbation vs. remission time point).

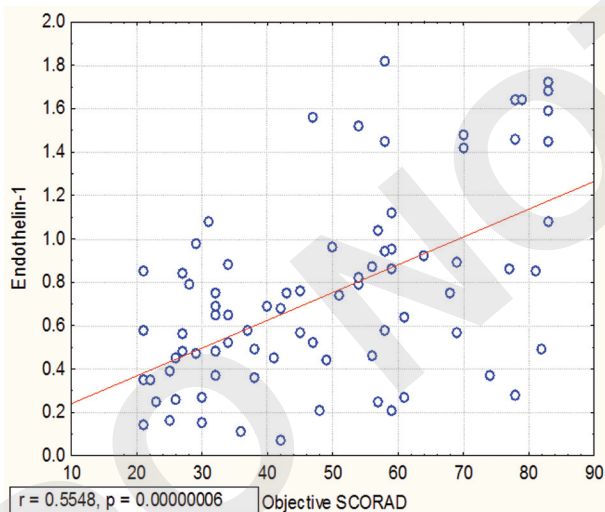


Figure 1. Correlations between plasma ET-1 levels with the objective SCORAD at the exacerbation time point in patients with AD. Endothelin-1 (fmol/mL).

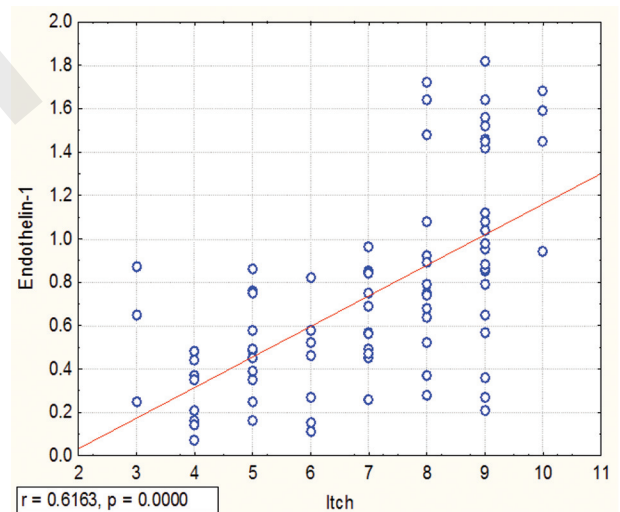


Figure 2. Correlations between plasma ET-1 levels with itch severity at the exacerbation time point in patients with AD. Endothelin-1 (fmol/mL).

els of ET-1 were positively correlated with serum IgE levels during AD exacerbation.

Pruritus is an essential feature of AD, with a great impact on the quality of life. Although the pathophysiology of AD itch is not fully understood, recent studies demonstrated that a variety of mechanisms are involved in its induction and maintenance.¹⁵ Both mast cell-mediated and mast cell-independent mechanisms underlie the AD-related pruritus.¹⁶ Clinically, histamine, the major pruritogenic mediator from mast cells, turned out to be disappointing as a target of anti-itch medications, because antihistamines exhibit an unsatisfactory effect on the AD-related itch.¹⁷ ET-1 induces

histamine-independent pruritus in mammals through activation of its G protein-coupled receptor endothelin A receptor.¹⁸ Recently, an intracellular neural peptidase-1 (ECE-1) was identified as a key regulator of ET-1-induced pruritus and neural signaling of itch.¹⁹ Analysis of our results indicated that ET-1 might have some pathophysiologic pruritogenic involvement in AD, at least during AD exacerbation.

Most of our subjects were characterized with high serum IgE, so we can claim that they were classified as having extrinsic AD. The significant positive correlation between IgE levels and objective SCORAD in our subjects was comparable with the previous study.²⁰

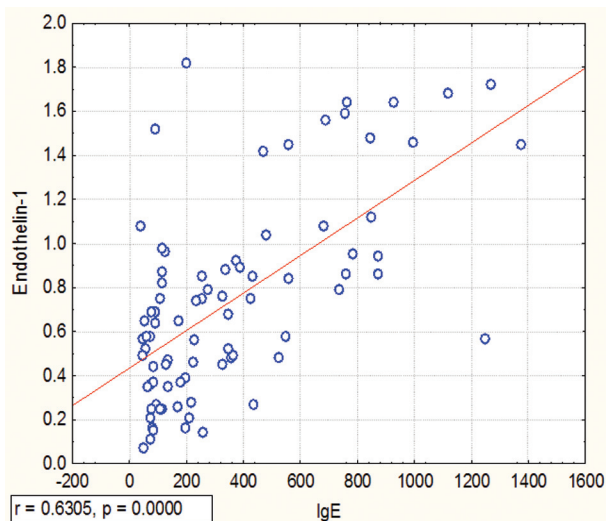


Figure 3. Correlations between plasma ET-1 levels and serum IgE levels at the exacerbation time point in patients with AD. Endothelin-1 (fmol/mL), IgE (immunoglobulin E) (kU/L).

The cellular and molecular mechanisms that regulate higher IgE production and function in AD are more complex than simply Th2 activation and B-cell class switching.²¹ There are no reports with regard to the role of ET-1 in IgE regulation in AD, and most of the previous studies investigated interactions between ET-1 and other cytokines in asthma and anaphylaxis.^{22–24} The biologic actions of IgE in allergic diseases are mainly connected with mast cell activation.²⁵ Interestingly, the monoclonal anti-IgE antibody omalizumab reduces not only serum IgE levels and the high-affinity receptor for the Fc region of immunoglobulin E (FcεRI) receptor expression on key cells in the inflammatory cascade,²⁶ but it also decreases ET-1 expression in airways of patients with persistent severe asthma.²⁷

The current understanding of the role of mast cells in the pathogenesis of AD has resemblances to the understanding of mast cells in asthma in the 1990s: several models have strongly demonstrated the lack of an increase in IgE levels and/or any significant role of IgE in causation of AD skin lesions.²⁸ Currently, little is known about the role of mast cells in modulating the clinical course of AD. Rat tissue-cultured mucosal mast cells have been shown to produce ET-1, inhibit messenger RNA expression of Th2-type cytokines (IL-4, IL-10, and IL-13), and increase IL-12 release.²⁹ Thus, ET-1 may play an important role in modulating the cytokine network by regulating Th1/Th2 cytokine production by mast cells.²⁹ The modulation of the mast cells immune response by ET-1 in AD deserves further investigation.

White dermographism is a characteristic symptom of AD due to reduced cutaneous blood flow.³⁰ AD lesions are characterized by activation of endothelial and vas-

cular smooth muscle cells, which are ultimately involved in clinical symptoms of AD.³¹ A secondary Raynaud-like phenomenon in AD has been observed with elevated ET-1 in infants with severe AD.³² The circulating half-life of ET-1 is 2–3 minutes, and it is almost completely metabolized in the pulmonary capillaries by neutral endopeptidase.³³ Therefore, it is not clear whether an elevated level of circulating ET-1 is essential for its actions because the most biologic effects of ET-1 are detected long after the normalization of the plasma level.³⁴ Moreover, patients with AD have significantly larger wheals with ET-1 in intradermal testing and/or skin prick testing.³⁵ If the pathophysiologic role of ET-1 in AD will be confirmed in future basic and clinical studies, then ET-1 and endothelin A receptors might be potential targets for developing drugs to treat AD.³⁶

Several limitations of our study warrant consideration. First, it has to be considered that plasma levels do not accurately reflect the pathophysiologic situation at the site of skin inflammation. Second, although we observed a significant association between plasma ET-1 levels during AD exacerbation and clinical findings, at this stage, the biological mechanisms of this association are unclear, and the reasons for the apparent ET-1 elevation and ET-1 contribution to the pathophysiology of AD need further investigation. Third, for ET-1 to be a potential biomarker for AD, it should be evaluated against other known biomarkers of this disease. In previous studies, the level of thymus and activation-regulated chemokine ligand 17 (also known as thymus and activation regulated chemokine [TARC]) was shown to correlate with both the severity and disease activity of AD.^{37–39} Ideally, this marker should be used as a control in testing of ET-1 in future studies.

REFERENCES

1. Guttman-Yassky E, Nograles KE, and Krueger JG. Contrasting pathogenesis of atopic dermatitis and psoriasis–Part I: Clinical and pathologic concepts. *J Allergy Clin Immunol* 127:1110–1118, 2011.
2. Wu LC, and Zarrin AA. The production and regulation of IgE by the immune system. *Nat Rev Immunol* 14:247–259, 2014.
3. Gittler JK, Shemer A, Suárez-Fariñas M, et al. Progressive activation of T(H)2/T(H)22 cytokines and selective epidermal proteins characterizes acute and chronic atopic dermatitis. *J Allergy Clin Immunol* 130:1344–1354, 2012.
4. Yanagisawa M, Kurihara H, Kimura S, et al. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature* 332:411–415, 1988.
5. Haller H, Schaberg T, Lindschau C, et al. Endothelin increases [Ca²⁺]_i, protein phosphorylation, and O₂[•] production in human alveolar macrophages. *Am J Physiol* 261:L478–L484, 1991.
6. Uchida Y, Ninomiya H, Sakamoto T, et al. ET-1 released histamine from guinea pig pulmonary but not peritoneal mast cells. *Biochem Biophys Res Commun* 189:1196–1201, 1992.

7. Sanchez-Cifuentes MV, Rubio ML, Ortega M, et al. Endothelin-1 expression during early response after antigen challenge in brown-norway rats. *Pulm Pharmacol Ther* 11:215–219, 1998.
8. Shigematsu T, Miura S, Hirokawa M, et al. Endothelins promote egg albumin-induced intestinal anaphylaxis in rats. *Gastroenterology* 115:348–356, 1998.
9. Trentin PG, Fernandes MB, D'Orleans-Juste P, and Rae GA. Endothelin-1 causes pruritus in mice. *Exp Biol Med* (Maywood) 231:1146–1151, 2006.
10. Mattoli S, Mezzetti M, Riva G, et al. Specific binding of endothelin on human bronchial smooth muscle cells in culture and secretion of endothelin-like material from bronchial epithelial cells. *Am J Resp Cell Mol Biol* 3:145–151, 1990.
11. Redington AE, Springall DR, Meng QH, et al. Immunoreactive endothelin in bronchial biopsy specimens: Increased expression in asthma and modulation by corticosteroid therapy. *J Allergy Clin Immunol* 100:544–552, 1997.
12. Redington AE, Springall DR, Ghatei MA, et al. Endothelin in bronchoalveolar lavage fluid and its relation to airflow obstruction in asthma. *Am J Respir Crit Care Med* 151:1034–1049, 1995.
13. Hanifin JM, and Rajka G. Diagnostic features of atopic eczema. *Acta Derm Venereol Suppl* (Stockh) 92(suppl.):S44–S47, 1980.
14. [No authors listed]. Severity scoring of atopic dermatitis: The SCORAD index. Consensus Report of the European Task Force on Atopic Dermatitis. *Dermatology* 186:23–31, 1993.
15. Sher LG, Chang J, Patel IB, et al. Relieving the pruritus of atopic dermatitis: A meta-analysis. *Acta Derm Venereol* 92:455–461, 2012.
16. Darsow U, Pfab F, Valet M, et al. Pruritus and atopic dermatitis. *Clin Rev Allergy Immunol* 41:237–244, 2011.
17. Buddenkotte J, Maurer M, and Steinhoff M. Histamine and antihistamines in atopic dermatitis. *Adv Exp Med Biol* 709:73–80, 2010.
18. Kedziński RM, and Yanagisawa M. Endothelin system: The double-edged sword in health and disease. *Annu Rev Pharmacol Toxicol* 41:851–876, 2001.
19. Kido-Nakahara M, Buddenkotte J, Kempkes C, et al. Neural peptidase endothelin-converting enzyme 1 regulates endothelin 1-induced pruritus. *J Clin Invest* 124:2683–2695, 2014.
20. Suárez-Fariñas M, Dhingra N, Gittler J, et al. Intrinsic atopic dermatitis shows similar TH2 and higher TH17 immune activation compared with extrinsic atopic dermatitis. *J Allergy Clin Immunol* 132:361–370, 2013.
21. Poulsen LK, and Hummelshøj L. Triggers of IgE class switching and allergy development. *Ann Med* 39:440–456, 2007.
22. Xu J, and Zhong NS. The interaction of tumor necrosis factor alpha and endothelin-1 in pathogenetic models of asthma. *Clin Exp Allergy* 27:568–573, 1997.
23. Cunningham ME, Huribal M, Bala RJ, and McMillen MA. Endothelin-1 and endothelin-4 stimulate monocyte production of cytokines. *Crit Care Med* 25:958–964, 1997.
24. Zhang S, Smartt H, Holgate ST, and Roche WR. Growth factors secreted by bronchial epithelial cells control myofibroblast proliferation: An in vitro co-culture model of airway remodeling in asthma. *Lab Invest* 79:395–405, 1999.
25. Platts-Mills TA. The role of immunoglobulin E in allergy and asthma. *Am J Respir Crit Care Med* 164(8 Pt 2):S1–S5, 2001.
26. Babu KS, Polosa R, and Morjaria JB. Anti-IgE: Emerging opportunities for Omalizumab. *Expert Opin Biol Ther* 13:765–777, 2013.
27. Zietkowski Z, Skiepmo R, Tomasiak-Lozowska MM, and Bodzenta-Lukaszyk A. Anti-IgE therapy with omalizumab decreases endothelin-1 in exhaled breath condensate of patients with severe persistent allergic asthma. *Respiration* 80:534–542, 2010.
28. Kawakami T, Ando T, Kimura M, et al. Mast cells in atopic dermatitis. *Curr Opin Immunol* 21:666–678, 2009.
29. Coulombe M, Battistini B, Stankova J, et al. Endothelins regulate mediator production of rat tissue-cultured mucosal mast cells. Up-regulation of Th1 and inhibition of Th2 cytokines. *J Leukoc Biol* 71:829–836, 2002.
30. Klemp P, and Staberg B. Cutaneous blood flow during white dermographism in patients with atopic dermatitis. *J Invest Dermatol* 79:243–245, 1982.
31. Steinhoff M, Steinhoff A, Homey B, et al. Role of vasculature in atopic dermatitis. *J Allergy Clin Immunol* 118:190–197, 2006.
32. Kojima T, Taniuchi S, Aoki T, et al. Raynaud-like phenomenon in infants with atopic dermatitis: Correlation with serum endothelin-1 levels [in Japanese with English abstract]. *Arerugi* 52: 1114–1121, 2003.
33. Abassi ZA, Tate J, Golomb E, and Keiser HR. Role of neutral endopeptidase in the metabolism of endothelin. *Hypertension* 20:89–95, 1992.
34. Wagner OF, Christ G, Wojta J, et al. Polar secretion of endothelin-1 by cultured cells. *J Biol Chem* 267:16066–16068, 1992.
35. Zirbs M, Reindel U, Hermann K, et al. Reduced skin reactivity to vasoconstrictor and vasodilator substances in atopic eczema. *Eur J Dermatol* 23:812–819, 2013.
36. McQueen DS, Noble MA, and Bond SM. Endothelin-1 activates ETA receptors to cause reflex scratching in BALB/c mice. *Br J Pharmacol* 151:278–284, 2007.
37. Kakinuma T, Nakamura K, Wakugawa M, et al. Thymus and activation-regulated chemokine in atopic dermatitis: Serum thymus and activation-regulated chemokine level is closely related with disease activity. *J Allergy Clin Immunol* 107:535–541, 2001.
38. Furusyo N, Takeoka H, Toyoda K, et al. Thymus and activation regulated chemokines in children with atopic dermatitis: Kyushu University Ishigaki Atopic Dermatitis Study (KIDS). *Eur J Dermatol* 17:397–404, 2007.
39. Fujisawa T, Nagao M, Hiraguchi Y, et al. Serum measurement of thymus and activation-regulated chemokine/CCL17 in children with atopic dermatitis: Elevated normal levels in infancy and age-specific analysis in atopic dermatitis. *Pediatr Allergy Immunol* 20:633–641, 2009. □

Copyright of Allergy & Asthma Proceedings is the property of OceanSide Publications Inc. and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.