

(wheal 7, flare 30) and negative to normal saline, bacitracin, MMR-II, and soy. Specific immunoglobulin E was positive to porcine gelatin (1.81 kU/L) and negative to bovine gelatin (ARUP Laboratories, Salt Lake City, Utah). Given her cardiac condition, intradermal testing for MMR II was performed and was positive at 1:10 dilution. (wheal 9, flare 15, with a nonreactive intradermal normal saline). This concentration was found to be nonirritant in 20 healthy volunteers.² The patient received uneventfully MMR-II vaccine using a stepwise protocol involving 6 incremental doses given subcutaneously with a 20-minute interval between each dose (0.05 mL 1:100 dilution, 0.05 mL 1:10 dilution, 0.05 mL full-strength, 0.1 mL full-strength, 0.15 mL full strength, 0.2 mL full-strength). This protocol is similar to the algorithm recommended by Wood et al³ for patients with immediate-hypersensitivity reactions to vaccines or their components. Although our patient may have been desensitized MMR-II, it is also possible that she has tolerated the small amount of hydrolyzed gelatin present in the vaccine. She has since tolerated midazolam, fentanyl, sevoflurane, ketamine, cefazolin, and lactated Ringer's.

Topical bovine thrombin has been used therapeutically since the 1940s. It may be applied alone, but it is often used in conjunction with other hemostatic aids, including absorbable agents (such as Gelfoam, collagen, and cellulose).⁴ Although the extent of the utilization of topical bovine thrombin with or without gelatin sponge is not known precisely, an estimated 1 million patients per year receive these hemostatic agents in the United States.⁵ Anaphylaxis to these products is rare overall. Clark et al⁶ performed a 21-year review of topical bovine thrombin spontaneous case safety reports submitted to FDA's adverse event reporting system, from 1986 to 2006. Of 41 cases spontaneously reported, 19 were classified as anaphylactic or anaphylactoid, although no further information was provided. Anaphylaxis to thrombin-containing preparations was otherwise reported in more details in only a few patients. Rothenberg and Moy⁷ described a patient with an anaphylactic reaction within minutes of the application of surgical gelatin sponges soaked with topical bovine thrombin. Four weeks later, this patient showed a positive skin prick test to topical bovine thrombin. Three other patients were reported in Japan in 1991 with a positive radioallergosorbent test to bovine thrombin, although the radioallergosorbent inhibition test suggested that the antigenic substance was mainly found in the contaminant rather than in the bovine thrombin itself.⁸

Gelatin, a derivative of animal skin and bones, may be a component of multiple products, including food, medications, cosmetics, and vaccines. Intraoperative anaphylaxis to gelatin-containing products is rare and has been reported in a few

papers. In a French study, 7 of 15 cases were reported to have immunoglobulin E-mediated anaphylaxis to gelatin colloids, and the overall frequency of anaphylaxis to these products was estimated to be 0.345%.⁹

We report the first case of anaphylaxis from thrombin-soaked Gelfoam application, which was secondary to porcine gelatin hypersensitivity. In addition, it is also the first report describing anaphylaxis to aspic. We recommend inquiring about gelatin allergy not only in drugs but also in food as part of the preoperative assessment when the use of gelatin-containing products is anticipated. If suspected, an allergy service consultation would be warranted before surgery.

Evelyn Khoriaty, MD*
Craig D. McClain, MD, MPH†
Perdita Permaul, MD‡
Edward R. Smith, MD§
Rima Rachid, MD*

*Department of Medicine, Division of Allergy and Immunology, Children's Hospital Boston, Harvard Medical School

†Department of Anesthesiology, Children's Hospital Boston, Harvard Medical School

‡Department of Pediatrics, Division of Pediatric Pulmonary Allergy/Immunology, Massachusetts General Hospital

§Department of Neurosurgery, Children's Hospital Boston Harvard Medical School, Boston, Massachusetts
E-mail: Rima.rachid@childrens.harvard.edu

References

- [1] Lieberman P, Nicklas RA, Oppenheimer J, et al. The diagnosis and management of anaphylaxis practice parameter: 2010 update. *J Allergy Clin Immunol*. 2010; 126:477–80 e1–42.
- [2] Wood RA, Setse R, Halsey N. Irritant skin test reactions to common vaccines. *J Allergy Clin Immunol*. 2007;120:478–481.
- [3] Wood RA, Berger M, Dreskin SC, Setse R, Engler RJ, Decker CL, et al. An algorithm for treatment of patients with hypersensitivity reactions after vaccines. *Pediatrics*. 2008;122:e771–e777.
- [4] Lew WK, Weaver FA. Clinical use of topical thrombin as a surgical hemostat. *Biologics*. 2008;2:593–599.
- [5] Lawson JH. The clinical use and immunologic impact of thrombin in surgery. *SeminThromb Hemost*. 2006;32(Suppl 1):98–110.
- [6] Clark JA, Humphries JE, Crean S, Reynolds MW. Topical bovine thrombin: a 21-year review of topical bovine thrombin spontaneous case safety reports submitted to FDA's Adverse Event Reporting System. *Pharmacoevid Drug Saf*. 2010;19:107–114.
- [7] Rothenberg DM, Moy JN. Anaphylactic reaction to topical bovine thrombin. *Anesthesiology*. 1993;78:779–782.
- [8] Tadokoro K, Ohtoshi T, Takafuji S, et al. Topical thrombin-induced IgE-mediated anaphylaxis: RAST analysis and skin test studies. *J Allergy Clin Immunol*. 1991; 88:620–629.
- [9] Laxenaire MC, Charpentier C, Feldman L. [Anaphylactoid reactions to colloid plasma substitutes: incidence, risk factors, mechanisms. A French multicenter prospective study]. *Ann Fr Anesth Reanim*. 1994;13:301–310.

Ranitidine-induced anaphylaxis with detection of serum specific IgE to ranitidine and human serum albumin conjugates

Histamine₂ (H₂) receptor antagonists are commonly used for the treatment of peptic ulcers and gastroesophageal reflux disease.¹ They are generally well tolerated, and severe hypersensitivity reactions have been rare; however, ranitidine was the most common cause of anaphylaxis among H₂-receptor antagonists.² The pathogenic mechanism by which ranitidine induces hypersensitivity re-

actions is not well understood. One published report detected serum specific IgE to conjugates of ranitidine and human serum albumin (HSA) using enzyme-linked immunosorbent assay (ELISA) in a patient with ranitidine-induced anaphylaxis.³ The aims of this study were to observe the clinical characteristics of multiple patients with ranitidine-induced anaphylaxis and to investigate its pathogenic mechanism by detecting serum specific IgE antibodies to ranitidine-HSA conjugates.

Ten patients with ranitidine-induced anaphylaxis were enrolled and treated at Ajou University Hospital or Seoul National University Bundang

Funding support: This work was supported by Korean Science and Engineering Foundation grant MEST 2009-00786746 funded by the Korean government.

Disclosures: Authors have nothing to disclose.

Table 1
Clinical characteristics and immunologic findings of the study participants

Patient No.	Sex	Age, y	Reaction	Atopy	TEC, / μ L	Total IgE, kU/L	SPT result	Serum specific IgE antibodies to ranitidine-HSA conjugate, OD $\times$ 1,000
1	M	34	GI, U, D	P	130	535	ND	352
2	M	43	D, H, AR	P	100	1058	+	97
3	F	54	GI, U, H	P	0	820	+	117
4	M	29	U, A, D	P	ND	1424	+	26
5	F	29	U, A, D, H	P	0	31	+	32
6	F	37	GI, U, A, D, H	P	200	99	+	41
7	F	43	GI, U, D	P	0	711	+	4
8	F	44	U, A, H	P	400	305	ND	20
9	F	59	U, A, D	P	ND	50	+	10
10	M	45	U, D, H	P	160	522	–	0

Abbreviations: A, angioedema; AR, arrest; D, dyspnea; GI, gastrointestinal symptoms (including nausea, vomiting, and abdominal pain); H, hypotension; ND, not done; OD, optical density; P, presence; SPT, skin prick test; TEC, total eosinophil count; U, urticaria. Symbols: +, positive result; –, negative result.

Hospital. The patients had a certain history of recurrent anaphylaxis to ranitidine, even though the challenge tests could not be performed because of the risk. This study was approved by the ethical committee, and written informed consent was obtained from each participant.

Skin prick tests (SPTs) using 10 mg/mL of ranitidine extract were performed in 8 patients according to previously described methods.^{2–4} A positive reaction was defined as a wheal with a mean diameter of at least 3 mm with equivalent flare.

Ranitidine was conjugated to HSA using a cross-linker, as described previously,⁵ and confirmed using native polyacrylamide gel electrophoresis. To detect serum specific IgE antibodies to ranitidine, ELISA was performed using the ranitidine-HSA conjugate, according to previously described methods.⁵ A positive response was defined as a value higher than the mean absorbance values $\pm$ 3 SDs of 20 healthy controls. The binding specificity of ranitidine-HSA conjugate was confirmed by ELISA inhibition tests. Increasing amounts (1–100 μ g/mL) of free ranitidine and ranitidine-HSA conjugate were incubated with serum. Study participants were divided into 2 groups, according to the presence of ranitidine-HSA conjugate specific IgE; 3 participants showed high specificity (group 1), and 7 participants had negative results (group 2). Statistical differences between the 2 groups were assessed using Mann-Whitney and Fisher exact tests with the SPSS software package, version 12.0 (SPSS Inc, Chicago, Illinois). $P \leq .05$ was considered statistically significant.

The clinical characteristics and immunologic findings of the study participants are given in Table 1. Among 10 patients ranging in age from 29 to 59 years (mean $\pm$ SD age, 41.7 $\pm$ 9.85 years), 6 (60%) were female and all were atopic. Seven of 8 patients (88%) had positive responses to ranitidine extract by SPT. Total eosinophil counts and total IgE levels ranged from 0 to 400/ μ L and from 31 to 1424 kU/L, respectively.

The results of polyacrylamide gel electrophoresis were significantly different for ranitidine-HSA conjugate and HSA, indicating that ranitidine was successfully coupled to HSA (eFig 1A). Three patients had high serum specific IgE to ranitidine-HSA conjugate above the positive cutoff value (eFig 1B). No significant differences were found in age, sex, atopy, or total IgE levels between the 2 groups. The ELISA inhibition test demonstrated dose-dependent inhibition of conjugate binding but not free ranitidine and HSA alone (eFig 1C). This finding suggests that the IgE response is specific for a new antigenic determinant not a hapten part.

A few case reports of ranitidine-induced anaphylaxis have documented positive responses to ranitidine by SPT, suggesting that these hypersensitivity reactions were mediated by IgE.^{3,6} In our study, 7 (88%) of 8 patients showed positive responses to ranitidine by SPT, whereas 2 had high specific IgE levels. The ELISA method may be not as sensitive as SPT for detecting serum

IgE. However, when we performed SPT with 10 mg/mL of ranitidine extract in 86 healthy controls, 14% of them showed positive responses. Although SPT is a valuable tool for confirming the causative agent of a reaction,⁷ it can lead to false-positive results, and new sensitization can occur after SPT in patients with drug allergies.⁸ Insufficient knowledge about drug degradation and conjugation with body proteins is a particular problem for drugs. This observation could not demonstrate the metabolic products of ranitidine as the antigenic determinants. Further studies are needed to detect specific IgE to other antigens, using conjugates with drug metabolites or different tissue proteins.

In conclusion, we demonstrated the presence of specific IgE to ranitidine-HSA conjugate in serum samples of a small subset of patients using ELISA, suggesting that IgE is involved in the pathogenic mechanism of ranitidine-induced anaphylaxis.

Hyun Jung Jin, MD*

Jeong-Eun Kim, MD†

Young-Min Ye, MD, PhD*

Yoon-Seok Chang, MD, PhD‡

Hae-Sim Park, MD, PhD*

*Department of Allergy and Clinical Immunology
Ajou University School of Medicine
Suwon, South Korea

†Division of Pulmonology and Critical Care Medicine
Department of Internal Medicine
Samsung Changwon Hospital
Sungkyunkwan University School of Medicine
Changwon, South Korea

‡Department of Internal Medicine
Seoul National University Bundang Hospital
Seoul National University College of Medicine
Seongnam, South Korea
hspark@ajou.ac.kr

References

- Mills JG, Koch KM, Webster C, et al. The safety of ranitidine in over a decade of use. *Aliment Pharmacol Ther*. 1997;11:129–137.
- Foti C, Cassano N, Panebianco R, Calogiuri GF, Vena GA. Hypersensitivity reaction to ranitidine: description of a case and review of the literature. *Immunopharmacol Immunotoxicol*. 2009;31:414–416.
- Koh YI, Park HS, Choi IS. Ranitidine-induced anaphylaxis: detection of serum specific IgE antibody. *Allergy*. 2006;61:269–270.
- Demirkan K, Bozkurt B, Karakaya G, Kalyoncu AF. Anaphylactic reaction to drugs commonly used for gastrointestinal system diseases: 3 case reports and review of the literature. *J Invest Allergol Clin Immunol*. 2006;16:203–209.
- Suh YJ, Lee YM, Choi JH, et al. Heterogeneity of IgE response to cefteram pivoxil was noted in 2 patients with cefteram-induced occupational asthma. *J Allergy Clin Immunol*. 2003;112:209–210.
- Walker AI, Werfel S, Kick G, Przybilla B. Repeated anaphylactic responses induced by oral challenge with ranitidine. *Acta Derm Venereol*. 2010;90:189.
- Khan DA, Solensky R. Drug allergy. *J Allergy Clin Immunol*. 2010;125:126–137.

- [8] Hausermann P, Bircher AJ. Immediate and delayed hypersensitivity to ceftriaxone, and anaphylaxis due to intradermal testing with other beta-lactam antibiotics, in a previously amoxicillin-sensitized patient. *Contact Dermatitis*. 2002; 47:311–312.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.anai.2011.12.015.

Association of microbial IgE sensitizations with asthma in young children with atopic dermatitis

Atopic dermatitis (AD) is considered to be a major risk factor for the development of asthma in children. As in the general population, common food and inhalant IgE sensitizations are associated with the development of asthma in children with AD.^{1,2} A unique clinical feature of AD patients is the colonization of their skin by *Staphylococcus aureus* and fungi. Specific IgE against these microbial pathogens or their products has been associated with AD severity. However, data are limited on the role of these microbial IgE sensitizations in the development of asthma in children with AD. In the current cross-sectional study, we investigated the role of specific IgE against staphylococcal superantigens and fungi in young children with AD and persistent asthma.

The study participants consisted of 53 children ranging in age from 1 to 6 years who had mild to moderate AD and were seen in the allergy clinic. The diagnosis of AD and assessment of disease severity were based on the UK Working Party's diagnostic criteria and objective SCORing AD (SCORAD), respectively.³ The diagnosis of persistent asthma was based on the patient's current use of an asthma control medication (inhaled corticosteroid or montelukast), as prescribed by an allergist according to the current asthma guidelines, at enrollment. Total serum IgE and specific IgE for inhalant allergens (*Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, cat, dog, cockroach, western ragweed, white oak, and Bermuda grass), common food (cow's milk, egg white, peanut, wheat, and soybean), and microbial allergens (staphylococcal enterotoxins A, B, C, and D, toxic shock syndrome toxin 1, *Aspergillus fumigatus*, *Cladosporium herbarum*, *Malassezia* species, and *Candida albicans*) were measured with the ImmunoCAP system (Phadia, Uppsala, Switzerland). A specific IgE concentration greater than 0.35 kU/L was considered to be a positive result. The Pearson correlation was used to analyze the association between specific IgE level and objective SCORAD. Logistic regression models were analyzed to assess the correlation of specific IgE and persistent asthma.

Our data showed significant positive correlations between objective SCORAD and specific IgE levels for the fungi *A. fumigatus*, *Malassezia* species, *C. herbarum*, and *C. albicans* ($r = 0.41–0.61$; $P < .03$; Pearson correlation). No significant correlations were found between other specific IgE levels and objective SCORAD except for cat ($r = 0.48$; $P = .01$) and staphylococcal enterotoxin A ($r = 0.47$; $P = .06$).

Logistic regression analyses showed that persistent asthma was significantly associated with IgE sensitization to *C. albicans* (odds ratio [OR] = 3.5; $P = .03$) and *C. herbarum* (OR = 4.3; $P = .01$) (Table), whereas other specific IgE sensitizations were not associated with persistent asthma. Because persistent asthma may be difficult to diagnose in young infants, we further analyzed an older subgroup of the study participants (≥ 3 years) ($n = 38$). Persistent asthma was again significantly associated with IgE sensitization to *C. albicans* (OR = 4.7; $P = .03$) and *C. herbarum* (OR = 6.1; $P = .01$). In addition, persistent asthma was signifi-

Table

Association of specific IgE sensitizations with persistent asthma in young children with atopic dermatitis

	Odds ratio (95% confidence interval)		P value
	No control medication (n = 31)	Control medication (n = 22)	
<i>Candida albicans</i> IgE	1	3.5 (1.1–11.2)	.03
<i>Cladosporium herbarum</i> IgE	1	4.3 (1.3–13.7)	.01
<i>Malassezia</i> IgE	1	2.7 (0.9–9.3)	.10
<i>Aspergillus fumigatus</i> IgE	1	2.5 (0.8–7.8)	.10
Cat IgE	1	2.8 (0.9–8.6)	.10
Egg white IgE	1	1.9 (0.6–6.2)	.30

cantly associated with allergic sensitization to *Malassezia* species (OR = 4.2; $P = .05$), cat (OR = 4.7; $P = .03$), and egg white (OR = 4.2; $P = .05$) in the older subgroup.

There is increasing evidence that skin barrier defects play a primary role in the pathogenesis of AD. Multiple studies worldwide have confirmed that mutations in filaggrin (*FLG*), a skin barrier protein, is associated with AD.⁴ These studies also suggested that *FLG* in AD may facilitate IgE sensitization through the skin. The significant correlation between specific IgE levels of fungi with AD severity shown in the current study is consistent with these observations and the role of fungi in the cutaneous inflammation of AD. Of note, a recent study has confirmed the presence of fungi, including *C. albicans*, *Malassezia*, *Cladosporium*, and *Aspergillus* species on the skin of AD patients.⁵

Our data further showed that persistent asthma was associated with fungal IgE sensitizations, particularly to *C. albicans* and *C. herbarum*, in young children with AD. The mechanism(s) of how specific IgE sensitizations may lead to the development of asthma in young children with AD is an area of active research. Potential mechanisms include atopic march.⁶ Alternatively, increasing evidence has emerged that epithelial defects may play a primary role in the pathogenesis of asthma.⁷ This is of particular interest because fungal allergic sensitizations have been implicated directly in the pathogenesis of asthma.⁸ Further longitudinal or cross-sectional studies that include a control group of asthmatic patients without AD would be helpful in elucidating the potential mechanisms.

Peck Y. Ong, MD

Ronald M. Ferdman, MD

Joseph A. Church, MD

Division of Clinical Immunology and Allergy

Children's Hospital Los Angeles

Department of Pediatrics

Keck School of Medicine

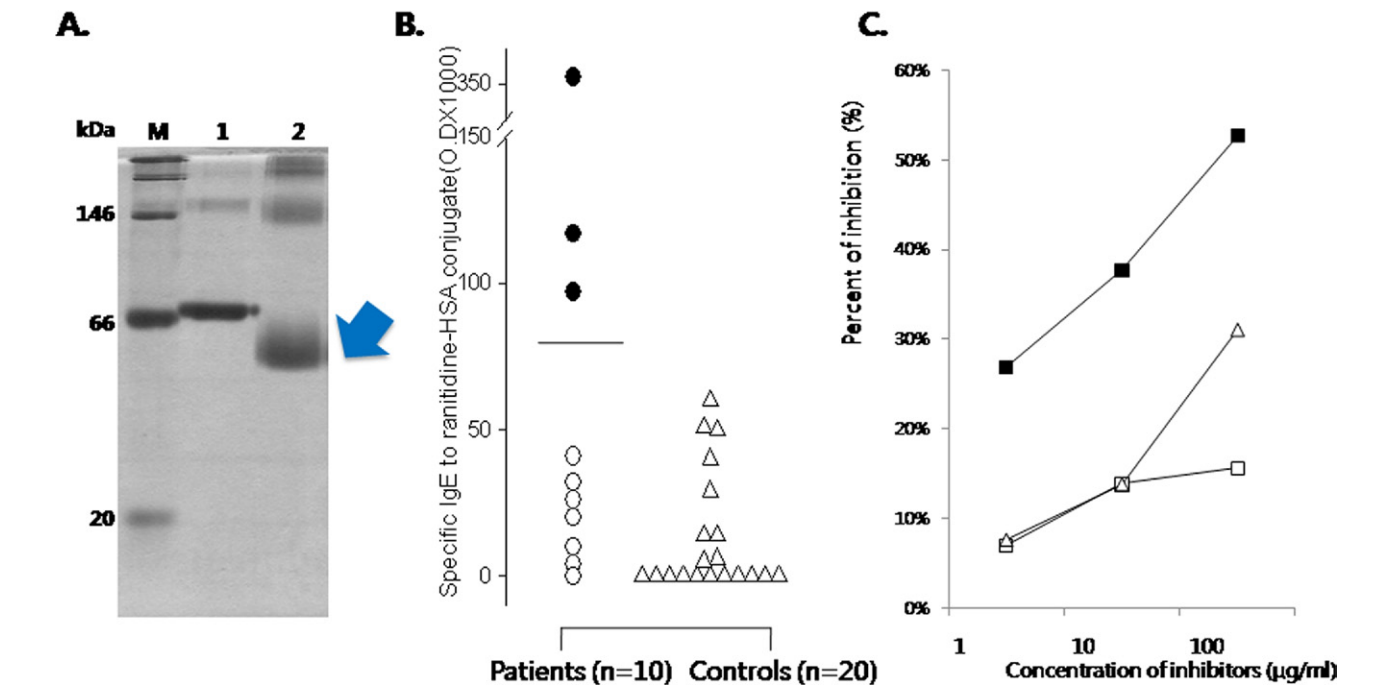
University of Southern California

Los Angeles, California

pyong@chla.usc.edu

Disclosures: Authors have nothing to disclose.

Funding Sources: This work was supported by CReFF award MO1 RR 00043 to Dr Ong from the General Clinical Research Center, National Center for Research Resources, University of Southern California.



eFig. 1. The finding of native gel (10% gel) of ranitidine–human serum albumin (HSA) conjugate (A), levels of serum specific IgE antibodies to ranitidine-HSA conjugate by enzyme-linked immunosorbent assay (ELISA) (B), and effect of ranitidine-HSA conjugate, free ranitidine, or HSA alone in an inhibition ELISA (C). Lane M, marker; lane 1, HSA; and lane 2, ranitidine-HSA conjugate. Horizontal bar indicates mean $\pm$ 3 SDs. ●, Positive responders; ○, Negative responders; △, normal controls; —■—, ranitidine-HSA; —△—, free ranitidine; —□—, HSA alone.