

Letters to the Editor

Fatal posture in anaphylactic shock

To the Editor:

Since 1992, I have studied details of 214 deaths associated with anaphylaxis. For 196 of these deaths there is sufficient information from the descriptions of events during the reactions and autopsy findings to determine that 88 were from shock (in 38 of these, shock was combined with difficulty breathing), 96 were from asphyxia (lower airway, 49; upper airway, 22; upper and lower airways or airway unspecified, 25), 7 were from complications of disseminated intravascular coagulation, and 5 were from the effects of epinephrine overdose.¹

An important pattern has become clear from the 38 anaphylactic shock deaths that occurred outside hospital. In 10 of these cases, information was available to determine the postural history after onset of anaphylaxis. Four deaths occurred within seconds of a change to a more upright posture: in each of 2 cases of nut anaphylaxis, the individual stood up after sitting slumped or lying down; in 1 case of antibiotic anaphylaxis, the individual was made to sit in a chair after lying in bed; and in 1 case a driver stepped out of his vehicle during a reaction to a sting. In another 5 fatal anaphylactic reactions to stings and in another fatal reaction to an antibiotic, each individual had been supported sitting up after shock caused loss of consciousness. The postural history of the remaining 28 cases is unknown: nevertheless, the pattern being reported seems significant. It is unusual for patients to be supported upright while shocked; most shocked patients will either lie down or collapse. Patients with anaphylactic shock after being stung might recover consciousness while lying down, only to lose it again when they try to sit up: they therefore tend to remain supine while shocked.

A possible mechanism suggests itself to explain this striking pattern of sudden deaths after a change to a more upright posture. During anaphylactic shock, the capacity of the veins and capillaries expands greatly. While a shocked person is lying down, sufficient blood might return to the vena cava to maintain a reduced circulation, but on the person's sitting up or standing, this venous return stops; the vena cava will then become empty within seconds. There is then no flow through the right side of the heart, and within a few seconds more, no blood will return to the left side of the heart from the lungs. Pulseless electric activity continues, but in the absence of left ventricular filling there can be no contractions; this prevents coronary arterial flow and leads to myocardial ischemia. In less extreme cases, too, the coronary circulation, which is dependent on the diastolic pressure, is likely to become inadequate, because the blood pressure is the product of the cardiac output and the systemic vascular resistance, both of which are low in cases of anaphylactic shock.

If this hypothesis is correct, once the vena cava is empty, epinephrine—no matter where or how it is given—could not circulate and so could not reverse the

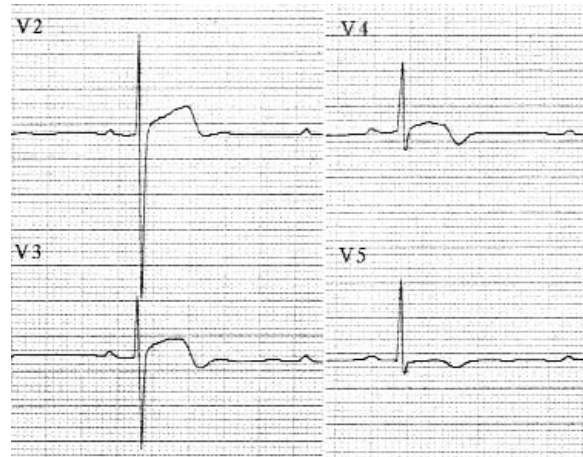


FIG 1. Electrocardiograph taken 6 hours after a hypotensive wasp stinging reaction in a 52-year-old man.

shock; nor could external cardiac massage reestablish the circulation if there is no blood in the vena cava. Anti-shock trousers or volume replacement by intravenous infusion would help refill the vena cava; raising the legs is a simple and effective alternative. A recent study of shocked patients demonstrated that the greater the deficit in the intravascular circulating volume, the greater the improvement in blood pressure consequent on raising the legs²; one would thus expect this maneuver to be highly effective in severe anaphylactic shock.

Some specific observations are consistent with this hypothesis. In a case of fatal wasp anaphylaxis, an electrocardiogram performed by a paramedic shortly after consciousness had been lost while the patient was supported sitting up showed ST elevation and T wave inversion: this electric activity continued after loss of pulse. The deceased was a fit active man with no history of heart problems: autopsy revealed that the ischemia was unlikely to have been due to coronary artery disease. Fig 1 shows an electrocardiogram taken after arrival in hospital of an athletically fit man with no history of heart disease or hypertension who had sat up to use his EpiPen and remained sitting, semiconscious, during a hypotensive wasp sting reaction. He had no angina, and his subsequent exercise electrocardiogram was normal.

Previous reports have attributed ischemic changes after sting reactions to coronary artery spasm³ or shock.⁴ Either of these is possible, and the two might operate synergistically. Although coronary artery spasm has been directly observed in anaphylactic reactions during angiography,⁵ the mechanism for reactions to contrast media is not fully understood and might differ from that for reactions to stings. Histamine infusion failed to cause coronary spasm in the absence of preexisting vasospastic angina,⁶ but leukotrienes can increase coronary vascular resistance,⁷ more likely by acting on the smaller vessels

rather than acting on the main coronary arteries. Whether the ischemia is due to increased coronary resistance, decreased diastolic pressure or both, lying down with the legs raised will help restore coronary flow.

The 50 shock deaths in hospital mostly occurred in the operating room after intravenous injection; these were quicker (median time to loss of cardiac output, 5 minutes; this compared with 15 minutes outside hospital), the cases commonly involved arrhythmia rather than pulseless electric activity, and the patients were invariably rapidly treated. These facts explain why a similar pattern was not observed in this group. However, myocardial ischemia/infarction was recorded during or after the reaction in 7 of these iatrogenic anaphylactic shock deaths; autopsy confirmed infarction in 2 patients who survived 5 and 20 hours but not in 1 patient who died less than an hour after the reaction. Of the 4 patients with no autopsy information, one was a fit 26-year-old who would not have been expected to have severe coronary artery disease. In comparison, none of the 96 asphyxial anaphylactic deaths in or out of hospital were reported to involve myocardial ischemia.

Although anaphylaxis can affect many organ systems, fatal reactions have commonly been due to one dominant modality: shock, asthma, or upper airway angioedema. Most of those with severe hypotension can lie down without undue difficulty in breathing. The time to arrest in fatal anaphylactic shock outside hospital in this study is between 4 and 80 minutes; for the majority of those who died, the time was between 10 and 20 minutes. Medical assistance often takes longer than this to arrive. In comparison, asphyxial deaths outside hospital took 10 to 360 minutes (median, 35 minutes) to first respiratory arrest: most of the individuals were standing, and none is known to have been lying down. Collapse was in some cases sudden, with loss of pulse; it is possible that in these cases prolonged extreme expiratory effort contributed to the collapse by preventing cardiac refilling.

A patient with anaphylactic shock should therefore be kept lying down, with the legs supported/raised to ensure that the vena cava is, insofar as possible, the lowest part of the body. Following this recommendation during initial first aid, both lay and professional, would likely save lives. The patient should be kept in this position or fitted with antishock trousers during transport into ambulance and in hospital until the blood pressure has recovered spontaneously or responded to epinephrine, volume replacement, or other appropriate measures. There is evidence in a canine model,⁸ in a human *in vitro* model,⁹ and in clinical practice¹⁰ that epinephrine might not be able to reverse established anaphylactic shock, so care should still be taken to prevent the adverse effects of posture while a partially treated patient is being moved.

Whatever the mechanism for these deaths, there seems sufficient evidence from the observations to suggest that these postural instructions should be incorporated into both basic and advanced life support training. Patients thought to be at risk of anaphylaxis and those who might become involved in their care (teachers, babysitters,

spouses, friends, and coworkers) should be told of their need to remain lying down if they feel faint during a reaction, unless there is a greater need to sit up to overcome difficulty in breathing. The events during the nonfatal wasp sting reaction reported here suggest that those with epinephrine should be instructed to remain supine rather than sit up to use their autoinjectors if they feel faint.

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Allergy to ingested mycoprotein in a patient with mold spore inhalant allergy

To the Editor:

We would like to present a case of severe anaphylactic-type allergic reaction after the first ingestion of a Quorn product.

A range of fungus-derived meat alternative products (Quorn) has been available in several countries in Europe and is now available in the United States. The major ingredient in these products is a mycoprotein made from the large-scale cell culture of the fungus *Fusarium venenatum*. It is obtained by continuous fermentation of this fungus in a tower to which oxygen, nitrogen, sugars, minerals, and vitamins have been added. The resulting paste of fungal mycelia is mixed with egg albumin as binder, flavored and colored as desired, processed to obtain sensory meatlike characteristics, and sold in the form of pieces, mince, burgers, fillets, or nuggets.¹

Our patient is a 20-year-old woman who works in a bakery. Approximately 2 minutes after she ingested a Quorn fillet for the first time, itching in her mouth and throat occurred together with cough; her nose became completely blocked, diffuse redness appeared with heat sensation, and she also felt dyspneic. Terfenadine administered orally, a β_2 -mimetic spray, and methylprednisolone administered intravenously by her physician resulted in quick symptom relief. There was no drop in blood pressure, and the patient had no gastrointestinal symptoms.

Ever since childhood, she had had atopic dermatitis, which was still present on the hands and arms. Since the age of approximately 4 years, she had had seasonal rhinitis and mild asthma from March through August, and she is allergic to cat fur.

Skin prick tests were performed with a standard battery of allergens (HAL-allergy, Haarlem, The Netherlands) and interpreted semiquantitatively.² The test results were positive for grass pollen, tree pollen, house dust mite, and *Alternaria alternata* (score, +++), doubtful for *Cladosporium herbarum* (score, ++), and negative for weed pollen, *Fusarium culmorum*, *Aspergillus fumigatus*, *Aspergillus niger*, *Mucor*, *Penicillium notatum*, *Rizopus*, *Helminthosporium*, wheat flour, egg, and latex. Immuno-CAP testing (Pharmacia, Sweden) gave the following results: 4.70 Ua/mL for *Alternaria tenuis*, 1.46 Ua/mL for *C herbarum*, 1.01 Ua/mL for *A fumigatus*, 0.74 Ua/mL for *Fusarium moniliforme*, and <0.35 Ua/mL for *A niger*, with a total IgE of 112 kU/L (normal range, 20-120 kU/L). CAP tests for flour, gluten, maize, soy, α -amylase, coconut, garlic, α -lactalbumin, β -lactoglobulin, and casein each had a score of <1 Ua/mL.

The result of prick-to-prick testing with the full fillet product was strongly positive in our patient but remained negative in 3 control subjects. There were no reactions with the separately tested nonfungal ingredients of the Quorn fillet—ie, garlic, mustard, cow's milk, soy, coconut, yeast, and maize.

A mycoprotein extract was prepared 1/10 (w/v) by incubating freeze-dried mycoprotein (kindly provided by the producing company, Marlow Foods Ltd, Cleveland, United Kingdom) in sodium phosphate buffer 0.02 mol/L (pH 7.5), for 3 hours at room temperature. After centrifugation, the resulting extract was filtered through a 0.45- μ m membrane and kept at -20°C until use. For skin testing, the extract was further supplemented with sodium chloride up to 0.9% and filtered through a 0.22- μ m membrane. Prick testing with undiluted extract of mycoprotein resulted in a +++ reaction; the wheal measured 7 $\times$ 6 mm and the flare 30 $\times$ 30 mm. Intradermal test results were positive in 10^{-4} v/v dilution with a score of +++, the reaction being further dose-dependent between 10^{-4} and 10^{-7} v/v. Similar reactions were elicited when tests were repeated with new extracts made on a later date. Three control individuals were tested with the highest concentration applied to the patient, but their test results remained negative.

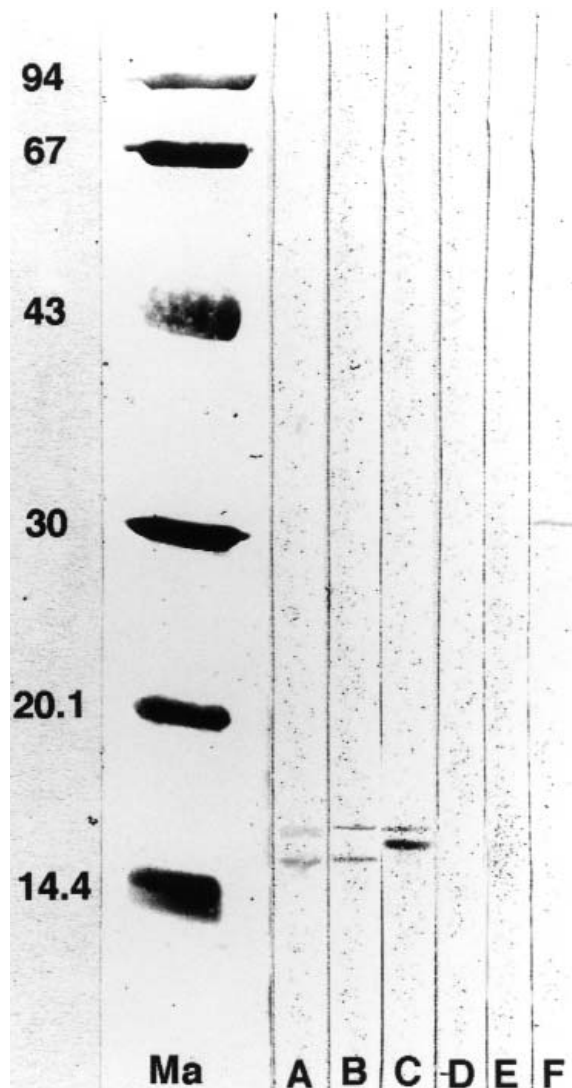


FIG 1. IgE immunoblot from SDS-PAGE gel. Patient serum was used to probe extracts of Quorn-mycoprotein (A), *A alternata* (B), *C herbarum* (C), *F culmorum* (D), *A niger* (E), and *A fumigatus* (F). Markers (Ma) are indicated (on the left) in kilodaltons.

By SDS-PAGE in reducing conditions and IgE immunoblotting with the mycoprotein extract (Fig 1), we identified IgE reactivity of the patient's serum to 2 bands at 15 and 16 kd. Two similar bands were found in home-made *A alternata* and *C herbarum* extracts. In addition, a single band at 31 kd was revealed in an *A fumigatus* extract (HAL-allergy), but no band was detected in the *F culmorum* extract (HAL-allergy). It remains unclear whether the latter observation can be explained by protein differences between the 2 *Fusarium* species (*culmorum* vs *venenatum*), by extract instability, or by changes in protein expression due to the processing required to obtain mycoprotein.

Inhibition experiments showed that the staining of the 2 mycoprotein bands at 15 and 16 kd could be inhibited by preincubation with *A alternata* and *C herbarum* extracts

but not by preincubation with *A fumigatus* and *F culmorum* extracts. Similarly, mycoprotein extract could inhibit the reactions to *A alternata* and *C herbarum* but not the reactions to *A fumigatus*. This provides evidence of shared allergenic determinants at 15–16 kD between the mycoprotein, *A alternata*, and *C herbarum*; in contrast, the 31-kD band of *A fumigatus* seems to be unrelated to any mycoprotein band. It has to be emphasized that no allergen has yet been described with such molecular mass in *Cladosporium* extracts.³ In *Alternaria* extracts, the reduced form of Alt a 1 is known to be detected at such molecular mass,³ but inhibition experiments with recombinant Alt a 1 (Biomay, Vienna, Austria), in reduced and nonreduced form, demonstrated no link between the major allergen of *Alternaria* and the proteins recognized by our patient, which thus represent newly described allergens.

Until now, according to information obtained from the company itself, there have been 5 proven positive skin prick test results in individuals who have apparently reacted to mycoprotein in a manner suggestive of IgE involvement. In the literature, the immunologic status of 12 workers involved in mycoprotein production and 10 individuals who presented with vomiting or diarrhea after its ingestion has been investigated.⁴ Sensitization to the product has been demonstrated in 2 workers, but they had no symptoms. Data from the complaining consumers led the authors of that study⁴ to the conclusion that the mechanisms involved were probably not IgE-mediated. In the same study, RAST-inhibition experiments with mold-sensitized sera demonstrated immunologic cross-reactivity between mycoprotein and *A alternata*, *C herbarum*, and *A fumigatus*, which therefore indicated that patients allergic to molds might react to mycoprotein.

If food-pollen cross-reactivity and food-latex cross-reactivity are well-known syndromes,⁵ food-mold spores cross-reactivity is in comparison very poorly documented. A single case report has recently been published that describes cross-reactivity between mushroom and mold spores.⁶ This case and ours suggest the existence of mold spore-food cross-reactivity as a new inhalant-associated food allergy syndrome. In such syndromes, it is generally considered that inhalant allergy precedes and induces food allergy.⁵ The fact that our patient, to the best of her knowledge, had never previously eaten a Quorn product indicates a primary sensitization by inhaled mold spores. If this is the case, patients sensitized to mold spores (at least *Cladosporium* and *Alternaria*) could be at risk when eating such a product, even for the first time.

Although this new type of inhalant-associated food allergy seems to be rare and the kind of cross-reactivity presented here rather exceptional, special attention should be given to its occurrence and to the potential for a rise in its incidence as the consumption of products containing edible fungi or food ingredients derived from fungal fermentations continues to increase.

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Association of thromboxane A2 receptor (TBXA2R) with atopy and asthma

To the Editor:

Human thromboxane A2 (TBXA2) is a potent constrictor of the airway smooth muscle and an inducer of acetylcholine release and of airway microvascular leakage.¹ It may play a part in the thickening and remodeling of the airway wall that may in turn contribute to airway hyperresponsiveness, a typical feature of asthma. The receptor for TBXA2, thromboxane A2 receptor (TBXA2R, MIM# 188070), is a member of the family of G protein-coupled receptors and performs an essential role in hemostasis by interacting with thromboxane. TBXA2R is located on chromosome 19p13.3¹ and contains 4 exons with total length of 15 kb, composed of 343 amino acids.² TBXA2 and its receptor (TBXA2R) are involved in the constriction of vascular and respiratory smooth muscles. Significantly, 1 polymorphism in the TBXA2R gene (*TBXA2R*+924T > C) was recently found to be associated with asthma and atopic asthma.^{3,4}

To assess whether genetic variants of the TXA2 receptor gene relate to the asthma phenotypes, we conducted an association study of genetic polymorphisms of TXA2 receptor (*TBXA2R*+795T > C and *TBXA2R*+924T > C) in a Korean asthma cohort (n = 721). In our article, we present a positive association of genetic polymorphisms with asthma and atopy development in the Korean population.

Subjects were recruited from the Asthma Genome Research Center, which consists of 4 tertiary hospitals in Korea (Soonchunhyang University Hospital, Ajou University Hospital, Choong-Ang University Hospital, and Ulsan University Hospital). Ethical approvals were obtained from the institutional review board of each hospital. All patients had clinical symptoms and a physical examination compatible with asthma. Each patient

showed airway reversibility as documented by an inhalant bronchodilator-induced improvement of more than 15% of FEV₁ or an airway hyperreactivity of less than 10 mg/mL of methacholine. Normal subjects were recruited from spouses of the patients and the general population who answered negatively to a screening questionnaire for respiratory symptoms and had an FEV₁ greater than 75% predicted, PC₂₀ methacholine greater than 10 mg/mL, and normal findings on a simple chest radiogram. Total IgE and specific IgE to *Dermatophagoides farinae* and *D pteronyssinus* were measured using the CAP system (Pharmacia Diagnostics, Sweden). Atopy was defined by the presence of an immediate skin reaction (histamine of > 3 mm in diameter) to 1 or more of 24 common aeroallergens (dust mites [*D farinae* and *D pteronyssinus*], cat fur, dog fur, cockroach, grass, tree pollens, and ragweed), or by the positive response (score ≥ 1) of specific IgE to *D pteronyssinus* or *D farinae*. (The clinical parameters are available at http://www.snp-genetics.com/user/news_content.asp?board_idx=141). Sequences of amplifying and extension primers for *TBXA2R*+795T > C and *TBXA2R*+924T > C were forward-5'-gagtgaccctggatctca-3', reverse 5'-gcgctctgtccacttct-3', extension-5'-cgcagcactgtctgggc-3' (*TBXA2R*+795T > C), and extension-5'-tcctggaccctgggtga-3' (*TBXA2R*+924T > C). A single base extension method was used for genotyping of single nucleotide polymorphisms (SNPs) in *TBXA2R* as described previously⁵ (available at http://www.snp-genetics.com/user/news_content.asp?board_idx=140). The genotype distribution of *TBXA2R* SNPs and haplotypes between (1) patients with bronchial asthma and normal subjects, and (2) atopic and nonatopic subjects were analyzed by logistic regression models controlling for age (continuous value), sex (male = 0, female = 1), and smoking status (categorized as nonsmoker = 0, exsmoker = 1, and smoker = 2) as covariables. Three SNPs have been reported previously in *TBXA2R* (*TBXA2R*+179(R60L) G > T, *TBXA2R*+795T > C, and *TBXA2R*+924T > C). But the nonsynonymous SNP in exon 1 (*TBXA2R*+179(R60L) G > T) was excluded in genotyping because of extremely low frequency in the Japanese population (freq = 0.003, n = 165).³ Two SNPs in *TBXA2R* (*TBXA2R*+795T > C and *TBXA2R*+924T > C) showed complete linkage disequilibrium (ID'1 = 1 and $d^2 \neq 1$), so only three haplotypes were constructed. *TBXA2R*-ht1[C-C] and *TBXA2R*-ht3[T-T] were equivalent with *TBXA2R*+795T > C and *TBXA2R*+924T > C, respectively. The allele frequencies of *TBXA2R*+795T > C, *TBXA2R*+924T > C, and *TBXA2R*-ht2[C-T] were 0.38, 0.20, and 0.17 in the Korean population (n = 721), respectively.

Genetic associations of two known variants in *TBXA2R* with asthma and atopy were analyzed. The statistical analyses are summarized in Tables I and II. In a comparison of genotype distribution in patients with asthma and normal controls, no significant differences were detected by single SNPs. And the frequency of *TBXA2R*-ht2[C-T] containing genotype in patients with asthma was lower than that in normal controls (28.8% vs 35.6%, $P = .004$). In subgroup analyses with atopic sub-

jects only, the T allele of *TBXA2R*+795T > C in patients with asthma was more frequent in them than in normal controls (42.5% vs 27.8%, $P = .004$). Also, a higher frequency of *TBXA2R*-ht2[C-T] containing genotype in patients with asthma was detected (69.2% vs 59.0%, $P = .007$). Although it was not statistically significant, *TBXA2R*-ht2[C-T] containing genotype in patients with asthma was also higher than in normal controls without atopy (75.9% vs 69.4%, $P = .31$). In analyses of association with atopy (Table II), the other SNP (*TBXA2R*+924T > C), which showed no signal for development of asthma, revealed a significant association with atopy in all subjects and asthma patients ($P = .006-.008$). *TBXA2R*-ht2[C-T] also seemed to have weak signals with the same direction for atopy ($P = .05-.17$) as in asthma development.

One of the genetic variants in the TXA2 receptor (*TBXA2R*+795T > C) was reported previously to have an association with serum IgE concentrations in atopic dermatitis,⁷ increasing susceptibility to asthma³ and atopic asthma⁴ developing. The results might be controversial, because the effects of *TBXA2R*+795T > C were statistically marginal in the Chinese population ($P = .04$) and in the Japanese population ($P = .01-.03$).^{3,4} In this study, we could reproduce the association of genetic variants in *TBXA2R* with developing asthma (*TBXA2R*-ht2 and *TBXA2R*+795T > C) and with developing atopy (*TBXA2R*+924T > C) in the Korean population. Similarly, with study of the Chinese population,⁴ we could not find a significant relationship of *TBXA2R*+795T > C with serum total IgE (data not shown) reported in the Japanese population in patients with atopic dermatitis.⁷ Although asthma is a multifactorial condition, the strongest risk factor in the etiology of asthma is atopy. An atopic individual has a significantly greater probability of asthma developing, and persons with a family history of atopic disease are at greatest risk of asthma. It is accepted that an immunologic response to various allergenic stimuli, including pet dander, dust mites, cockroaches, fungi, and foods, is a major triggering factor in asthma symptom development. The allergic response to dust mites involves an immediate hypersensitivity reaction, including increases in specific IgE antibodies and T cells of the T_H2 phenotype. The associations of *TBXA2R* polymorphisms with asthma and atopy simultaneously in this study provide further evidence for a common genetic etiology of these two highly correlated phenotypes. The roles of *TBXA2R* in asthma and association results including this study suggest that *TBXA2R* polymorphisms might play a critical role in the development of asthma and atopy. In addition, positive signals from *TBXA2R* haplotype suggest that the effects of the *TBXA2R* variant could be the result of a combined genotype (haplotype) rather than single polymorphisms. A clinical study showed that responders and nonresponders to TBXA2 receptor antagonist exist in patients with asthma.⁶ This raises the possibility that *TBXA2R* polymorphism can influence the responsiveness to TBXA2 receptor antagonist in patients with asthma or that patients with asthma have a different pharmacoge-

TABLE I. Genotype distribution of *TBXA2R* SNPs in bronchial asthmatic and normal subjects in a Korean population

					P value*		
Locus	Group	Genotype			Co-dominant	Dominant	Recessive
All							
TBXA2R+795T>C	BA	T 217 (40.7%)	CT 240 (45%)	C 76 (14.3%)	.11	.13	.29
	NC	58 (35.4%)	79 (48.2%)	27 (16.5%)			
TBXA2R+924T>C	BA	T 338 (63.7%)	CT 169 (31.8%)	C 24 (4.5%)	.39	.44	.55
	NC	105 (64%)	52 (31.7%)	7 (4.3%)			
TBXA2R-ht2	BA	—/— 378 (71.2%)	—/ht2 135 (25.4%)	ht2/ht2 18 (3.4%)	.003	.004	.10
	NC	105 (64.4%)	49 (30.1%)	9 (5.5%)			
Atopy							
TBXA2R+795T>C	BA	T 157 (42.5%)	CT 156 (42.3%)	C 56 (15.2%)	.04	.004	.88
	NC	22 (27.8%)	49 (62%)	8 (10.1%)			
TBXA2R+924T>C	BA	T 248 (67.6%)	CT 103 (28.1%)	C 16 (4.4%)	.86	.57	.39
	NC	50 (64.1%)	27 (34.6%)	1 (1.3%)			
TBXA2R-ht2	BA	—/— 254 (69.2%)	—/ht2 97 (26.4%)	ht2/ht2 16 (4.4%)	.02	.007	.87
	NC	46 (59%)	29 (37.2%)	3 (3.8%)			
Nonatopy							
TBXA2R+795T>C	BA	T 60 (37%)	CT 82 (50.6%)	C 20 (12.3%)	.88	.28	.09
	NC	36 (42.4%)	30 (35.3%)	19 (22.4%)			
TBXA2R+924T>C	BA	T 89 (54.9%)	CT 65 (40.1%)	C 8 (4.9%)	.25	.09	.56
	NC	55 (64%)	25 (29.1%)	6 (7%)			
TBXA2R-ht2	BA	—/— 123 (75.9%)	—/ht2 37 (22.8%)	ht2/ht2 2 (1.2%)	.12	.31	.06
	NC	59 (69.4%)	20 (23.5%)	6 (7.1%)			

BA, Bronchial asthmatic; NC, normal.

*P values for logistic analyses of 3 alternative models (codominant, dominant, and recessive) are shown, controlling age and sex as covariables. *TBXA2R*-ht1 and -ht3 were not analyzed because of equivalent models with *TBXA2R*+795T>C and *TBXA2R*+924T>C.

nomic pattern according to *TBXA2R* polymorphism. In summary, polymorphisms (SNPs and haplotypes) in *TBXA2R* showed a significant relationship with asthma and atopy, although either the 795T > C or 924T > C variants do not confer an amino acid change. The frequency of the *TBXA2R*-ht2[C-T] containing genotype in patients with asthma was lower than that in normal controls (28.8% vs 35.6%, $P = .004$). In addition, *TBXA2R*+924T > C revealed a significant association with atopy in all subjects and asthma patients ($P = .006$ -.008). The results of this study could be helpful in understanding the important function of *TBXA2R* in asthma and atopy development, and also could be a useful target for drug development for asthma and atopy.

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TABLE II. Genotype distribution of *TBXA2R* SNPs in atopic and nonatopic subjects in a Korean population

					P value		
Locus	Group	Genotype			Co-dominant	Dominant	Recessive
All							
<i>TBXA2R</i> +795T>C	No atopy	T 109 (36.7%)	CT 139 (46.8%)	C 49 (16.5%)	.81	.86	.82
	Atopy	218 (38.6%)	261 (46.2%)	86 (15.2%)			
<i>TBXA2R</i> +924T>C	No atopy	T 171 (57.2%)	CT 112 (37.5%)	C 16 (5.4%)	.01	.006	.49
	Atopy	376 (66.9%)	162 (28.8%)	24 (4.3%)			
<i>TBXA2R</i> - <i>ht2</i>	No atopy	—/— 212 (71.4%)	—/ <i>ht2</i> 76 (25.6%)	<i>ht2</i> / <i>ht2</i> 9 (3%)	.03	.05	.16
	Atopy	371 (66.1%)	164 (29.2%)	26 (4.6%)			
Asthmatic							
<i>TBXA2R</i> +795T>C	No atopy	T 60 (37%)	CT 82 (50.6%)	C 20 (12.3%)	.79	.39	.50
	Atopy	157 (42.5%)	156 (42.3%)	56 (15.2%)			
<i>TBXA2R</i> +924T>C	No atopy	T 89 (54.9%)	CT 65 (40.1%)	C 8 (4.9%)	.02	.008	.71
	Atopy	248 (67.6%)	103 (28.1%)	16 (4.4%)			
<i>TBXA2R</i> - <i>ht2</i>	No atopy	—/— 123 (75.9%)	—/ <i>ht2</i> 37 (22.8%)	<i>ht2</i> / <i>ht2</i> 2 (1.2%)	.04	.07	.11
	Atopy	254 (69.2%)	97 (26.4%)	16 (4.4%)			
Normal control							
<i>TBXA2R</i> +795T>C	No atopy	T 36 (42.4%)	CT 30 (35.3%)	C 19 (22.4%)	.83	.05	.04
	Atopy	22 (27.8%)	49 (62%)	8 (10.1%)			
<i>TBXA2R</i> +924T>C	No atopy	T 55 (64%)	CT 25 (29.1%)	C 6 (7%)	.51	.98	.11
	Atopy	50 (64.1%)	27 (34.6%)	1 (1.3%)			
<i>TBXA2R</i> - <i>ht2</i>	No atopy	—/— 59 (69.4%)	—/ <i>ht2</i> 20 (23.5%)	<i>ht2</i> / <i>ht2</i> 6 (7.1%)	.44	.17	.38
	Atopy	46 (59%)	29 (37.2%)	3 (3.8%)			

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Evidence for human mast cell nonreleaser phenotype

To the Editor:

Mast cells and basophils express high amounts of FcεRI. In basophils the concentration of anti-IgE or antigen required for maximal histamine release through FcεRI varies among donors, a concept termed "releasability." Some donors fail to release histamine in response to any concentration of IgE-mediated stimulus

(nonreleaser)¹⁻⁴ and were subsequently found to lack the protein tyrosine kinase Syk.⁵ Although the nonreleaser phenotype is common (ie, present in 10% to 20% of all donors), it is not known whether mast cells exhibit a similar IgE-mediated nonresponsiveness. We report here a lung mast cell preparation that did not respond to IgE-mediated stimuli.

Mast cells were dispersed from human lung tissue and enriched as described previously.⁶ This donor was undergoing a thoracotomy for lung cancer and was not receiving steroid therapy. These studies were approved by the Human Studies Internal Review Board at Virginia Commonwealth University. Mast cells were positively selected with mouse anti-c-kit receptor antibodies (YB5B8, a gift from Leonie Ashman) and bead-conjugated anti-mouse antibodies, as per the manufacturer's instructions (Miltenyi, Auburn, Calif). A total of 3.2×10^7 cells were isolated from 30 g of tissue. The purity of mast cells was 76%, as assessed with toluidine blue staining. The mast cells were cultured at 1×10^6 cells/mL in RPMI medium supplemented with 10% CPSR-1 (Sigma, St Louis, Mo), 10 μmol/L HEPES, 50 μmol/L 2-mercaptoethanol, 4 mmol/L L-glutamine, 1× MEM amino acids, 1× vitamin solution, 100 U/mL penicillin, 100 μg/mL streptomycin (Gibco, Gaithersburg Md), and 100 ng/mL recombinant human stem cell factor (Biosource, Camarillo, Calif).

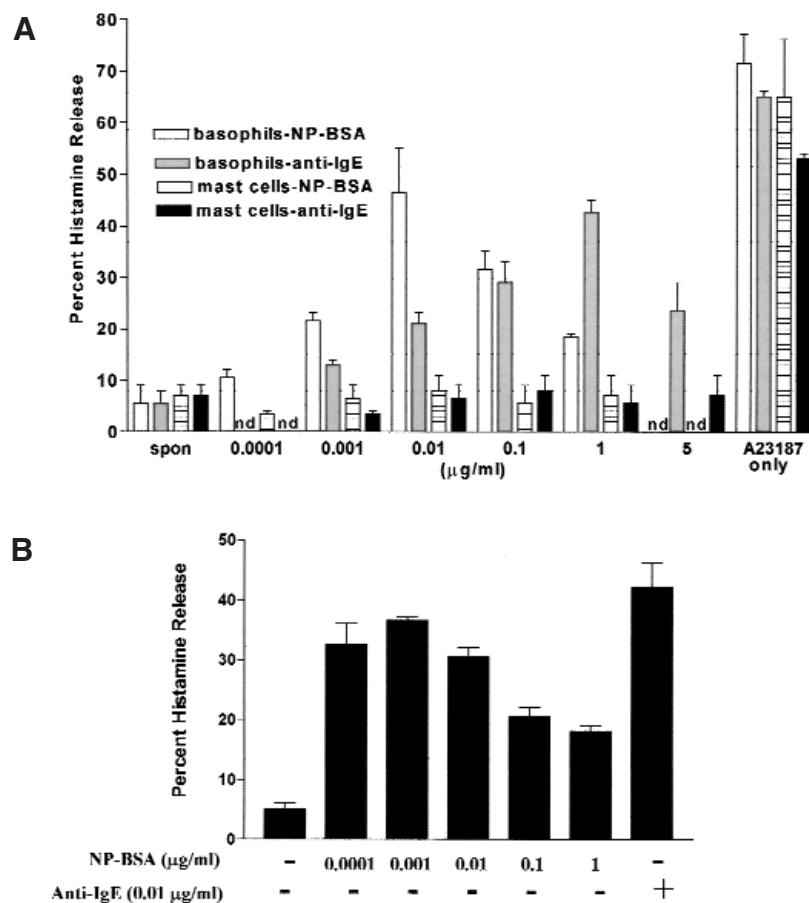


FIG 1. A, Specific impairment of FcεRI-dependent degranulation in lung mast cells. Data are from 1 experiment and are representative of 2 other experiments, each done in duplicate and performed with mast cells (mean ± SEM, n = 3). *ND*, Not done. **B,** Isolation procedure for lung mast cells does not induce FcεRI non-responsiveness.

Nitrophenacetyl-specific IgE (NP-IgE; 5 μg/mL) was added on day 2 of culture. After 3 days, we performed FACS analysis by using the mouse anti-human FcεRI α chain (22E7, gift from Dr J. Kochan) and c-kit receptor antibodies. These mast cells expressed high amounts of FcεRI and the c-kit receptor (data not shown). No obvious morphologic differences were observed by means of light microscopy when comparing toluidine-stained mast cells from cells responsive to IgE-mediated stimuli (not shown).

Initial degranulatory experiments (routinely performed in our laboratory)⁵⁻⁸ with IgE-sensitized cells cultured for 3 days showed this preparation did not respond to IgE stimulus (not shown). We hypothesized that these mast cells were defective in their ability to degranulate in response to IgE-mediated stimulus. To test this hypothesis, we used peripheral blood basophils from a separate known releaser donor in parallel with the lung mast cells to ensure all reagents were working properly. IgE-sensitized lung mast cells or freshly isolated basophils (sensitized with NP-IgE for 4 hours)⁷ were washed and challenged with different concentrations of anti-IgE or NP-BSA, A23187 (500 ng/mL), or no stimulus (spontaneous)

for 30 minutes. The supernatants were measured for histamine release by using a kit from Beckman-Coulter (Marseille, France), and the percentage of histamine release was calculated as described previously.⁸ As expected, basophils released histamine in response to antigen or anti-IgE antibodies. However, by using identical reagents and experimental conditions, the lung mast cells failed to release histamine in response to any concentration of antigen or anti-IgE (Fig 1, A). In addition, no β-hexosaminidase release was detected in the supernatants of the mast cells (data not shown). Although FcεRI-induced secretion was absent, the mast cells secreted normally when challenged with Ca²⁺ ionophore, which induces secretion in part by direct effects on cytoplasmic Ca²⁺ levels. The methods for mast cell purification from the tissue did not cause IgE nonresponsiveness because a previous mast cell preparation (84% purity; isolated, sensitized, and activated as above) released histamine when challenged with varying concentrations of antigen (Fig 1, B). Therefore these mast cells had a specific defect in their IgE-mediated signal transduction pathway because their ability to degranulate remained intact.

We have not detected other mast cell preparations that did not degranulate in response to FcεRI-mediated stimulus (n = 4). We conclude that the mast cells in these experiments were defective in their IgE-mediated signaling and might reflect a heretofore unknown mast cell nonreleaser phenotype. More studies are needed to confirm these preliminary findings.

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