

Table 1. SPT and CAP-FEIA Inhibition Test Results After Treating Salt-Soluble Wheat Preparations With Catalytically Active Thioredoxin in Patients With Wheat-Related Allergies

Patient No. (age/sex)	Clinical features	SPT result, wheat diameter in mm			CAP-FEIA, kU/L (%)*			
		Untreated	ReTh	OxTh	Negative	Untreated	ReTh	OxTh
Present case (6 y/F)	Baker's asthma	10	4	13	24.2	1.0 (96)†	6.1 (75)	1.0 (96)
1 (7 mo/M)	Generalized urticaria	16	19	15	16.4	7.3 (55)	6.7 (59)	6.8 (59)
2 (9 y/M)	Rash and chest tightness with wheezing	24	24	24	>100.0	38.7 (>60)	41.6 (>58)	43.1 (>57)
3 (2 y/M)	Anaphylactic shock	10	9	8	2.2	<0.3 (>86)	<0.3 (>86)	<0.3 (>86)
4 (37 y/F)	Anaphylactic shock after exercise	5	4	4	3.4	0.5 (85)	0.6 (82)	0.4 (88)
5 (46 y/M)	Baker with generalized chronic dermatitis	0	1	0	66.3	19.9 (70)	16.3 (75)	18.3 (72)

Abbreviations: ReTh, reduced thioredoxin-treated salt-soluble wheat fraction; SPT, skin prick test; OxTh, oxidized thioredoxin-treated salt-soluble wheat fraction.

* The percentages in parentheses indicate the percentage of inhibition in CAP-FEIA: $(1 - \text{serum with wheat preparation} / \text{serum without wheat preparation}) \times 100$.

found to be hampered on treatment with reduced thioredoxin in the CAP-FEIA inhibition study. The results for patients with other clinical symptoms were not different in the skin reactions or in the CAP-FEIA inhibition studies (Table 1). Since thioredoxin can modulate intramolecular disulfide bonds,⁴ the structural features of proteins in the salt-soluble wheat fraction might be involved in the pathogenesis of childhood baker's asthma.

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REFERENCES

1. Baur X. Baker's asthma: causes and prevention. *Int Arch Occup Environ Health*. 1999;72:292–296.
2. Moreno-Escobosa MC, Alonso E, Ausin E, et al. Baker's asthma in a child. *Allergol Immunopathol*. 2001;29:141–143.
3. Sander I, Flaggé A, Merget R, et al. Identification of wheat flour allergens by means of 2-dimensional immunoblotting. *J Allergy Clin Immunol*. 2001;107:907–913.
4. Buchanan BB, Adamidi C, Lozano RM, et al. Thioredoxin-linked mitigation of allergic responses to wheat. *Proc Natl Acad Sci U S A*. 1997;94:5372–5377.
5. Weichel M, Glaser AG, Ballmer-Weber BK, et al. Wheat and maize thioredoxins: a novel cross-reactive cereal allergen family related to baker's asthma. *J Allergy Clin Immunol*. 2006;117:676–681.

ANAPHYLAXIS TO MARE'S MILK

To the Editor:

Allergic reactions to mare's milk (MM) are very uncommon. Mare's milk is used as an alternative in children allergic to cow's milk (CM)^{1,2} or with atopic dermatitis.³

A 53-year-old woman with rhinoconjunctivitis and mild asthma sensitized to horse epithelium (HE) developed pruritus on the palms and soles, generalized erythema, conjunctivitis, and intense dyspnea 45 minutes after her first MM intake, although she had good tolerance to goat's milk (GM) and CM before and afterward. She was evaluated 2 months after the reaction using prick-by-prick tests,⁴ and she underwent soluble and basophil surface IgE determination by means of radioallergosorbent tests (RASTs)⁵ and basophil activation tests,⁶ cross-reactivity by RAST inhibition assay,⁵ and allergenic proteins by immunoblotting⁵ using native MM, CM, and GM plus commercial HE and solid-phase disks conjugated to MM or HE. Controls consisted of 5 patients allergic to HE (group A) and 10 nonatopic persons (group B).

Good agreement was found between results of skin testing, basophil activation testing, and RAST inhibition, confirming the presence of specific IgE antibodies to MM and HE in 4 cases (the patient and controls 1, 2, and 3) and only to HE in 2 (controls 4 and 5) (Table 1). The RAST inhibition results showed that in the patient, using MM in the solid phase, the stronger inhibitor was MM, with less recognition with HE. These results were reversed with HE in the solid phase. Immunoblotting with the patient's serum showed 3 bands of MM recognized by IgE antibodies (caseins [23–36 kDa], β -lactoglobulin [18 kDa], and α -lactalbumin [15 kDa]) and only 1 band of HE (EquC1 [19 kDa] or EquC2 [18 kDa]). All 5 group A controls had the same pattern of HE recognition as

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Table 1. Allergic Study in the Index Patient (Allergic to MM) and in Group A (Allergic to HE)

Participant	Skin tests, cm	RAST, % (8,000 µg/mL)				BAT, SI (20 µg/mL)				RAST inhibition, %				SDS immunoblotting			
		MM		HE		MM		HE		Solid-phase MM (8,000 µg/mL)		Solid-phase HE (8,000 µg/mL)		MM		HE	
		HE		MM		HE		MM		HE		MM		HE		MM	
		MM	HE	MM	HE	MM	HE	MM	HE	MM	HE	MM	HE	MM	HE	MM	HE
MM-allergic patient	4 × 5*	3 × 4*	4.2*	13.7*	9.0*	9.4*	9.4*	97*	79*	76*	92*	+	+	+	+	+	+
HE-allergic controls																	
1	3 × 2*	3 × 3*	1.58	6.17*	2.3*	3.0*	3.0*	ND	ND	59*	85*	+	+	+	+	+	+
2	3 × 3*	3 × 2*	1.94	21.52*	8.0*	10.0*	10.0*	ND	ND	61*	91*	+	+	+	+	+	+
3	3 × 4*	3 × 3*	1.83	11.68*	8.9*	9.6*	9.6*	ND	ND	73*	91*	+	+	+	+	+	+
4	-	3 × 4*	1.84	10.54*	1.9	20.2*	20.2*	ND	ND	43	97*	-	-	-	-	-	-
5	-	2 × 3*	1.85	19.08*	1.5	11.7*	11.7*	ND	ND	25	86*	-	-	-	-	-	-

Abbreviations: BAT, basophil activation test; CM, cow's milk; GM, goat's milk; HE, horse epithelium; MM, mare's milk; ND, not determined; RAST, radioallergosorbent test; SDS, sodium dodecyl sulfate; SI, ratio between the percentage of activated basophils with the allergen and the negative control.
 * Positive results were considered when higher than 2.5% for RAST and SI greater than 2 for BAT.

the patient. However, with MM recognition, controls 1, 2, and 3 showed the same pattern except for the 15-kDa band, and controls 4 and 5 had no recognition at all. All the test results were negative in group B. No IgE recognition to CM or GM was detected in any participant.

Although horse meat allergy has been related to hamster allergy,⁷ the anaphylaxis in this patient may be due to an association between a previous respiratory sensitization to animal epithelium and a later reaction with food allergens derived from the same animal. This may explain why the patient, previously sensitized to HE, had a reaction the first time she drank MM, although she may have had contact with the offending allergen by inadvertent contact in other foods.

The allergenic proteins in MM are α -lactalbumin and β -lactoglobulin, which show no cross-reaction with the corresponding whey proteins in CM.² Immunoblotting revealed allergen band recognition to α -lactalbumin and β -lactoglobulin and to caseins and β -lactoglobulin in 3 of the HE-allergic controls. Albumins seem to be the common protein provoking allergic cross-reactions between CM and beef.⁸ However, this patient, allergic to MM, had no IgE recognition to serum albumin. In conclusion, patients allergic to HE may also be sensitized to MM protein, although they have never had contact with that allergen. This can be due to a previous respiratory sensitization to animal epithelium. Although no shared allergens have been identified by immunoblotting, RAST inhibition assays indicate that 2 antibodies seemed to exist. Because the consumption of MM is increasing for the treatment of certain cutaneous diseases, we can expect an increase in MM allergy.

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REFERENCES

- Businco L, Giampietro PG, Lucenti P, et al. Allergenicity of mare's milk in children with cow's milk allergy. *J Allergy Clin Immunol.* 2000;105:1031-1034.
- Muraro MA, Giampietro PG, Galli E. Soy formulas and non-bovine milk. *Ann Allergy Asthma Immunol.* 2002;89:97-101.
- Gall H, Kalveram CM, Sick H, Sterry W. Allergy to the heat-labile proteins α -lactalbumin and β -lactoglobulin in mare's milk. *J Allergy Clin Immunol.* 1996;97:1304-1307.
- Mayoral M, Torres MJ, Muñoz M, Bartolomé B, Blanca M. Anaphylactic reaction following ingestion of fresh heart of palm (*Chamaerops humilis* L). *Allergy.* 2006;61:785-786.
- Mayorga C, Torres MJ, Corzo JL, et al. Immediate allergy to tetanus toxoid vaccine: determination of IgE and IgG antibodies

to allergenic proteins after the reaction and five years later. *Ann Allergy Asthma Immunol.* 2003;90:238–243.

6. Ibarrola I, Sanz ML, Gamboa PM, et al. Biological characterization of glutaraldehyde-modified *Parietaria judaica* pollen extracts. *Clin Exp Allergy.* 2004;34:303–309.
7. Cistero A, Enrique E, San Miguel MM, et al. Meat allergy and cross-reactivity with hamster epithelium. *Allergy.* 2003;58:161–162.
8. Cabanas R, Lopez-Serrano MC, Carreira J, et al. Importance of albumin in cross-reactivity among cat, dog and horse allergens. *Investig Allergol Clin Immunol.* 2000;10:71–77.

INHIBITION OF GUT BACTERIA BY DIETARY CHEMICALS AND THE HYGIENE HYPOTHESIS

To the Editor:

The high incidence of allergic and autoimmune diseases, such as asthma, allergic rhinitis, atopic dermatitis (eczema), inflammatory bowel disease (Crohn disease and ulcerative colitis), multiple sclerosis, and type 1 diabetes mellitus, has long been a big puzzle in developed countries.^{1–3} The hygiene hypothesis, which was proposed more than 2 decades ago, suggests that the increase in these allergic and autoimmune diseases is caused by reduced exposure to bacteria and other microorganisms attributable to improved hygiene.^{1,2} Interestingly, recent studies have revealed that these allergic and autoimmune diseases seem closely related to the various microbial species in the gastrointestinal tract.^{4,5} For instance, even asthma, an allergic reaction of the lung to inhaled antigens, has been shown to have an increased relationship to reduced exposure to foodborne and orofecal microbes, in addition to the amount of allergens in the air and the exposure to airborne microbes.^{6,7} Therefore, alteration in the types or concentrations of gastrointestinal microbes may play an important role in the development of these diseases.

The gut flora are established at birth. After that, a relatively stable gut flora could probably be sustained even without continuous refreshment from sources such as food and water. In this sense, the gut flora ecosystem may be more prone to be disturbed by chemicals infiltrating the gut. People have paid much attention to improved hygiene and the use of antibiotics because of the hygiene hypothesis but probably should pay more attention to dietary chemicals. For instance, saccharin has been widely used as a sweetener at tabletops and in light foods, soft beverages, baked goods, jams, chewing gums, canned fruits, candies, dessert toppings, and salad dressings.⁸ However, saccharin was initially used as an anti-septic agent and food preservative to inhibit the growth of bacteria,⁸ which is in accordance with the observation that it inhibits gut flora in animals.⁹ Actually, the pattern of its consumption fits well with the epidemiologic characteristics of inflammatory bowel disease, and saccharin might be a causative factor in inflammatory bowel disease.^{10,11}

Many kinds of dietary chemicals have been widely used as food preservatives, colors, emulsifiers, thickening agents, ex-

traction solvents, carrier solvents, flavors (synthetic), flavor enhancers, nonnutritive sweeteners, and processing aids.¹² However, for most of them, their effect on gut flora remains largely unknown. Therefore, more research in this area would be worthwhile to determine whether and to what extent they have contributed to the many diseases related to the western lifestyle.

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REFERENCES

1. Prescott SL. Allergy: the price we pay for cleaner living? *Ann Allergy Asthma Immunol.* 2003;90:64–70.
2. Bach JF. The effect of infections on susceptibility to autoimmune and allergic diseases. *N Engl J Med.* 2002;347:911–920.
3. Matricardi PM. Prevalence of atopy and asthma in eastern versus western Europe: why the difference? *Ann Allergy Asthma Immunol.* 2001;87:24–27.
4. Noverr MC, Huffnagle GB. Does the microbiota regulate immune responses outside the gut? *Trends Microbiol.* 2004;12:562–568.
5. Laiho K, Ouwehand A, Salminen S, Isolauri E. Inventing probiotic functional foods for patients with allergic disease. *Ann Allergy Asthma Immunol.* 2002;89:75–82.
6. Matricardi PM, Rosmini F, Panetta V et al. Hay fever and asthma in relation to markers of infection in the United States. *J Allergy Clin Immunol.* 2002;110:381–387.
7. Matricardi PM, Rosmini F, Riondino S et al. Exposure to foodborne and orofecal microbes versus airborne viruses in relation to atopy and allergic asthma: epidemiological study. *BMJ.* 2000;320:412–417.
8. Cranmer MF, Scherr GH. *Saccharin: A Report.* Park Forest South, IL: Pathotox Publishers; 1980.
9. Anderson RL, Kirkland JJ. The effect of sodium saccharin in the diet on caecal microflora. *Food Cosmet Toxicol.* 1980;18:353–355.
10. Qin XF. Impaired inactivation of digestive proteases by deconjugated bilirubin: the possible mechanism for inflammatory bowel disease. *Med Hypotheses.* 2002;59:159–163.
11. Qin X. Is the incidence of inflammatory bowel disease in the developed countries increasing again? is that surprising? *Inflamm Bowel Dis.* December 19, 2006. Epub ahead of print.
12. Berglund F. Food additives. *Arch Toxicol Suppl.* 1978;1:33–46.

HOW THE FIRST EFFECTIVE INHALED CORTICOSTEROID WAS ALMOST DISCARDED AS USELESS

To the Editor:

The recent supplement to the *Annals*¹ does not mention the very early history of inhaled corticosteroids, which may be of interest to readers, particularly because beclomethasone dipropionate was very nearly discarded as ineffective in 1970 by the manufacturers, Allen & Hanburys Ltd (London, England).