

Case Report

Triticale Allergy in a Farmer

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We present the case of a 29-year-old farmer with hay fever and atopic dermatitis since adolescence who had developed work-related asthma about 5 years earlier. He was sensitized to grass pollen, wheat and rye flour, dust from the floors of the animal facilities (cows and pigs) and grain barn, and a battery of animal feed from his farm. Work-relatedness of his asthma was demonstrated by serial measurements of spirometry and fractional exhaled nitric oxide at work and during a holiday. Immunoblot analyses revealed dominant IgE-binding to grass pollen and triticale (a hybrid of rye and wheat). IgE inhibition experiments demonstrated that sensitization to triticale was not due to cross-reactivity to grass pollen. Testing of specific IgE-antibodies to recombinant wheat allergens showed sensitizations to profilin, peroxidase, and nonspecific lipid transfer proteins type I subfamily 9.1 and 9.7. We conclude that triticale allergy may occur as a distinct allergy in farmers. Am. J. Ind. Med. 59:501–505, 2016. © 2016 Wiley Periodicals, Inc.

KEY WORDS: allergy; farmer; grain; occupational; triticale

INTRODUCTION

Respiratory disease due to grain dust may be considered a classical occupational disease [Ramazzini 1700]. Grain asthma has been reported a considerable health problem in Canadian grain elevator workers [Chan-Yeung et al., 1985]. It has been concluded from a number of studies that the airway response to grain dust represents an acute inflammatory response to inhaled endotoxins, but allergic mechanisms are of minor importance in grain workers [Clapp et al., 1994; Baur et al., 2003]. Information about immediate-type allergy in grain elevator workers is sparse. Whereas flour allergy is dominant in bakers and millers, grain allergy in farmers seems

less frequent. This view is challenged by a Polish case-control study that described 17 (41.5%) farmers with occupational respiratory diseases but only 4% of the controls with a positive skin prick test (SPT) to grain. The authors concluded that cereal farming and sensitization to cereals plays a major role in occupational asthma among farmers [Walusiak et al., 2004]. Epidemiological data from Danish farmers showed a sensitization rate to grain of about 5% [Iversen and Pedersen, 1990].

It is not known how farmers get sensitized. It has been shown that dairy farmers are exposed perennially to high grass pollen concentrations [Sudre et al., 2009], thus grain sensitization might reflect cross-sensitization to common wild grass species like *Phleum pratense* and *Lolium perenne*. Furthermore, cultured cereals like wheat, rye, and barley produce low numbers of large pollen with little dispersion which might explain selective sensitization in subjects with close contacts to grain fields, i.e., farmers [Damialis and Konstantinou, 2011]. A third possibility how farmers might get sensitized is the occurrence of cereal flours in animal feed. Asthma and airway obstruction have been described in subjects with flour sensitization after animal feeding [Valdivieso et al., 1988].

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Recently, we reported about component resolved diagnosis of baker's allergy based on specific IgE to recombinant wheat flour proteins and identified a number of allergens specific for baker's allergy without cross-reactivity to grass pollen [Sander et al., 2015]. Such studies are unavailable for farmers. It is not known whether sensitization patterns of farmers differ from those of bakers or subjects with hay fever. This has consequences for prevention, diagnosis and disease management.

METHODS

Personal data and both work and medical history were obtained by a questionnaire and a physician interview. Lung function was recorded by bodyplethysmography [Criée et al., 2011] and spirometry [Quanjer et al., 1993; American Thoracic Society, 1995] with a MasterLab (CareFusion, Würzburg, Germany). Methacholine testing was performed with a reservoir method as described recently [Polychronakis et al., 2013]. The patient performed spirometry (Spiro Pro, CareFusion) and fractional exhaled nitric oxide (eNO) measurements (NioxMino, Aerocrine, Solna, Sweden) once daily during a week of work and a two week holiday (serial measurements). Measurements were done in the evenings, i. e., after work in the working period. All data were of excellent quality and recorded electronically. Spirometry was obtained from four maneuvers by choosing the highest FEV₁ for final analysis. Medication with fluticasone and formoterol was constant during the whole period.

SPT were performed with a battery of 18 environmental allergens from various manufacturers. SPT with substances

from the patients' farm were performed with aqueous extracts of a mixture of maize and grass silage, maize silage, straw (all 100 mg/ml), triticale (a hybrid of wheat and rye), spring barley, winter barley (all 1000 mg/mL), dust from cow stable, pig stable, and grain barn (all 130 mg/ml), and commercial cow feed containing 38% rapeseed, 25% maize, and 13% triticale (290 mg/ml). The substances were incubated in phosphate-buffered saline (PBS) for 1 hr at room temperature, centrifuged 15 min at 3,000g, and the supernatants were sterile filtered through 0.45 µm cellulose acetate filters (Sartorius, Göttingen, Germany). Reactions were read at 20 min. Two subjects with hay fever and two non-atopic subjects were used as controls.

For specific IgE measurement and inhibition, triticale seeds from the patient's farm were crushed, homogenized and extracted at 4°C for 2 h with 3 parts v/w of buffer (10 mM potassium phosphate pH 7.0, 400 mM NaCl, 0.3% phenol) and centrifuged at 40,000g. Total IgE and specific IgE antibodies to wheat and rye flour (f4, f5), house dust mites (d1, d2), barley flour (f6) maize flour (f8), grass pollen (gx1), wheat Omega5-gliadin (Tri a 19.0101; f416), wheat nonspecific lipid transfer protein type I subfamily 9.7 (Tri a 14.0201; f433), horseradish peroxidase (HRP; o400), the carbohydrate part of bromelain (MUXF3; Ro214), the triticale supernatant, and a battery of 17 recombinant single wheat flour proteins [Sander et al., 2015] bound to streptavidin ImmunoCAPs were measured by CAP-FEIA (Thermo Fisher Scientific, Uppsala, Sweden). Expression and binding of the recombinant wheat flour proteins and binding of the triticale extract to streptavidin ImmunoCAPs were performed as described previously [Sander et al., 2005, 2011]. For IgE inhibition experiments, wheat and rye grain

TABLE I. Skin Prick Tests With Substances From the Patients' Farm

	Patient	Controls			
		Hayfever 1	Hayfever 2	Nonatopic 1	Nonatopic 2
Saline control	0	2	0	0	0
Histamine control	7	6	5	6	1
Mixture of maize and grass silage	5	2	2	0	1
Maize silage	7	3	0	2	1
Grass silage	4	2	0	1	1
Straw	3	2	0	1	1
Spring barley	5	2	0	0	1
Winter barley	6	2	0	0	1
Triticale	11	2	0	0	1
Commercial cow feed ^a	13	3	0	0	1
Dust from grain barn	11	3	0	1	1
Dust from cowhouse	7	2	2	1	1
Dust from pighouse	6	2	0	2	1

Values represent wheal diameters (mm).

^aContaining 38% rapeseed, 25% maize, and 13% triticale.

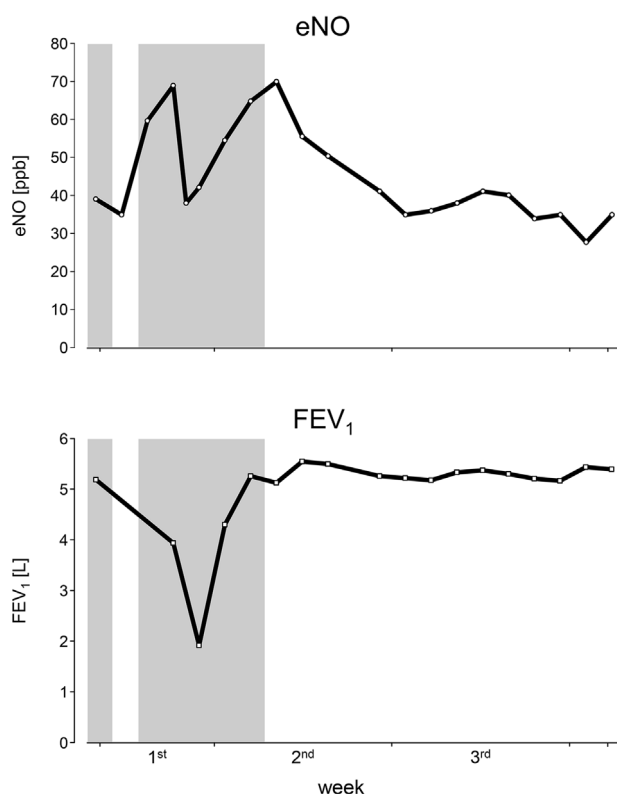


FIGURE 1. Serial daily measurements of fractional exhaled nitric oxide (eNO) and FEV₁ at work (shaded area) and during 2 weeks holiday.

extracts produced as previously described [Sander et al. 2005], the triticale extract and a grass pollen mixture including *L. perenne*, *Poa pratensis*, *Dactylis glomerata*, *Festuca pratensis*, and *P. pratense* (all from Allergon, Angelholm, Sweden) were used in protein concentrations of 10 mg/ml. 50 μ l of the patients' serum were mixed with 10 μ l of inhibition solution or PBS before specific IgE concentrations were measured in CAP-FEIA.

SDS-polyacrylamide electrophoresis, silver stain, and immunoblots of the substances from the patient's farm and the same wheat grain and grass pollen mixture as used for inhibition were performed as described previously [Sander et al. 2009] using the patient's serum and a hay fever patient's serum with specific IgE to grass pollen (4.37 kU_A/L) as control. This case study was part of a study about serial measurements of eNO and FEV₁ at work and during a holiday. It was approved by the ethics committee of the Ruhr-University Bochum and the patient gave written informed consent. Control subjects for SPT with substances from the patient's workplace gave informed consent for the tests.

CASE REPORT

The 29-year-old farmer with hay fever and atopic dermatitis since adolescence developed work-related asthma

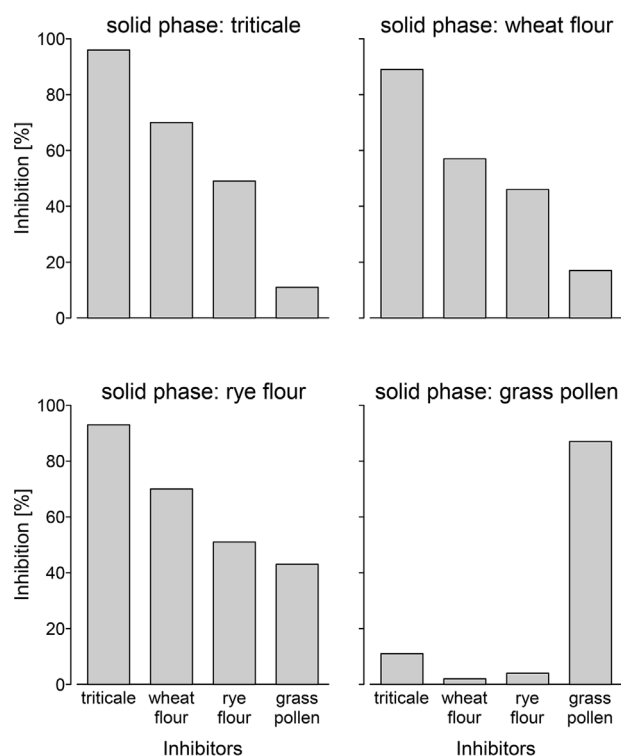


FIGURE 2. ImmunoCAP inhibition experiments with serum from the patient. Triticale, wheat and rye flour as well as grass pollen were bound to the solid phase and used as inhibitors.

about 5 years earlier. Additionally he reported itching of the throat after eating apples, skin rashes after eating nuts, and nausea after banana and sea food consumption. He grew up on his parents' farm with about 100 cows and 300 pigs. He cultivated barley, wheat, maize, triticale, and rapeseed. The cereals were milled on the farm, thus contact to cereal dust occurred during grinding, transport, and animal feeding. He reported that asthma occurred mainly after contact to grain and straw.

He showed generalized atopic dermatitis, otherwise physical examination, blood chemistry, X-rays of the chest, and electrocardiogram were normal, but differential blood count showed 5% eosinophils. Spirometry was normal with a postbronchodilator-FEV₁ of 117% predicted [Quanjer et al. 1993] but asthma was corroborated by the demonstration of bronchial hyperresponsiveness (PD₂₀FEV₁ 199 μ g; the cut-off in our laboratory is 300 μ g). Spiroergometry showed a small increase of the endexpiratory lung volume during exercise, but was otherwise normal with a maximal oxygen consumption of 2.8 L/min.

Specific IgE antibodies were detected to triticale (7.76 kU_A/L), wheat, rye, barley, and maize flour (f4: 6.35 kU_A/L, f5: 3.6 kU_A/L, f6: 5.44 kU_A/L, f8: 1.39 kU_A/L), grass pollen (gx1: 7.41 kU_A/L) and house dust mite (d1: 0.53 kU_A/L). Furthermore, the following wheat allergen

components bound specific IgE: profilin (Tri a 12.0102: 1.13 kU_A/L, nonspecific lipid transfer proteins type I subfamily 9.1 and 9.7 (Tri a 14.0101: 1.13 kU_A/L, Tri a 14.02: 9.16 kU_A/L), wheat peroxidase (0.8 kU_A/L), and MUXF3 (1.29 kU_A/L).

SPT were positive with grass pollen (10 mm wheal), rye pollen (10 mm), barley pollen (15 mm), and small SPT reactions of 3 mm wheal diameter were also obtained with mixtures of tree and herbs pollen. There was no sensitization to cow dander. SPT with a battery of animal feed including triticale and dust from the floors of the animal facilities (cows and pigs) and grain barn of his farm yielded positive reactions. These SPT reactions were not observed in two hayfever patients and two nonatopic controls who showed only minor skin reactions to the noncommercial extracts (Table I).

Work-relatedness of his asthma was demonstrated by serial measurements of spirometry (decrease of FEV₁ during work) and eNO (increase during work) (Fig. 1). CAP inhibition experiments demonstrated that sensitization to triticale was not due to cross-reactivity to grass pollen (Fig. 2). Whereas the highest inhibition rates of IgE-binding to all cereals coupled to the solid phase were observed with triticale as inhibitor, grass pollen as inhibitor gave the lowest rates. IgE-binding to the grass pollen solid phase could not be inhibited with cereals. Immunoblot analyses revealed dominant IgE-binding to grass pollen and triticale, the latter was absent in a control subject with hay fever (Fig. 3).

A diagnosis of hay fever and allergic occupational asthma due to cereals (especially triticale) was made.

DISCUSSION

This case study demonstrates primary sensitization to cereals and especially triticale, a hybrid cereal of rye and wheat in a farmer. Sensitization was demonstrated by SPT, CAP, and IgE immunoblot analyses. In addition, it was shown by serial measurements of spirometry and eNO during a holiday and a work period that farm work exacerbated the patient's asthma. As there were no further sensitizations to occupational allergens, it is highly probable that the decrease of FEV₁ and the increase of eNO during work are due to exposure to triticale. Thus we considered an inhalation challenge in the laboratory unnecessary.

The patient was also sensitized to grass pollen and demonstrated IgE antibodies to recombinant wheat allergen components that are not exclusively reacting with bakers' IgE but also with IgE from hay fever patients [Sander et al. 2015]. This explains his symptoms of hay fever since adolescence. However, as shown by immunoblot and CAP inhibition experiments, his sensitization pattern cannot be explained by cross-sensitization to grass pollen. As a practical consequence, cereals should be considered as occupational allergens in farmers.

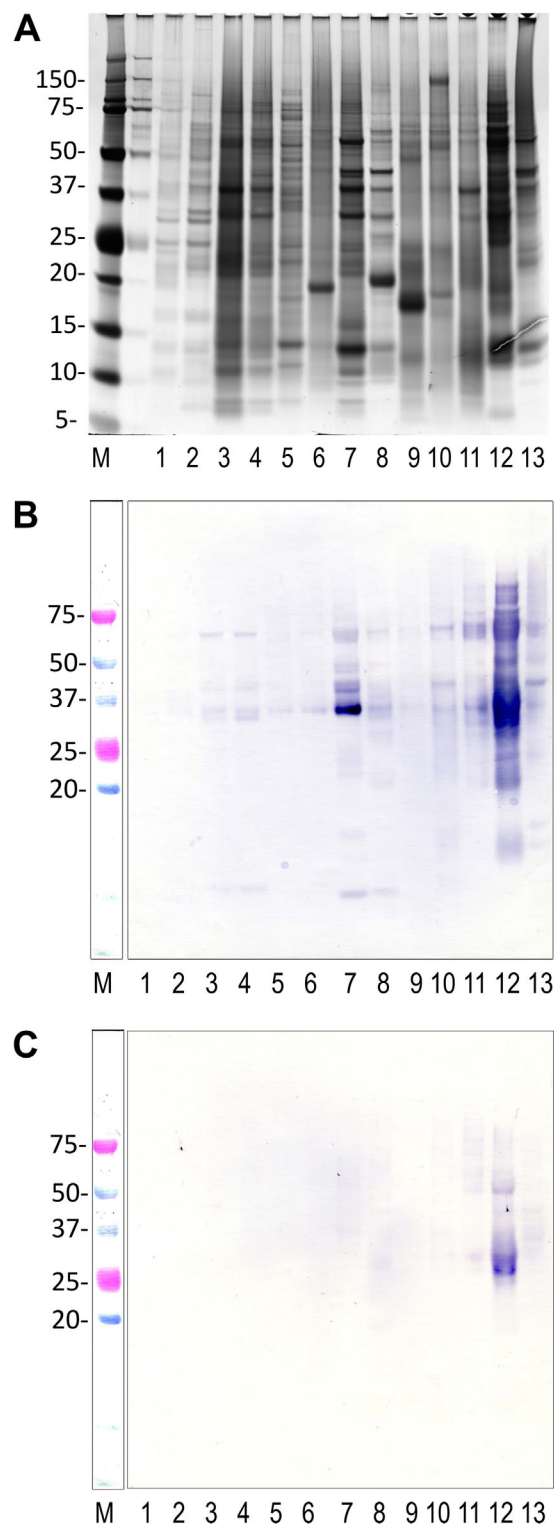


FIGURE 3. SDS-PAGE (A), IgE immunoblot with the patient's serum (B) and a control subject with hay fever (C). The lanes represent cow feed (1), maize silage (2), spring barley (3), winter barley (4), grass silage (5), straw (6), triticale (7), commercial cow feed containing 38% rapeseed, 25% maize, and 13% triticale (8), dust from cow stable (9), dust from pig stable (10), dust from grain barn (11), grass pollen (12), and wheat flour (13).

The route of sensitization is unknown. Three possibilities need discussion: the sensitization of the patient may have occurred: (1) by contact to cereals when feeding the animals; (2) by milling of cereal grains that was performed by himself; and (3) close contact to grain pollen. Close contact to grain dust in grain elevator workers only rarely induced sensitization to grain despite high-dust concentrations [Baur et al., 2003], thus we consider feeding of the animals with grain not a likely cause of his disease. Of course we cannot exclude that flour in pre-mixed animal feed formula induced sensitization during feeding. The milling of grain was done by this farmer, a task that is usually done by millers, who may be sensitized to flour [Smith et al., 2000]. Dust exposure reduction during milling operations (but also in general during harvesting, transport, and feeding of grain) can be considered as major recommendations.

After the use of a powered air-purifying respirator, the patient reported improvement of his symptoms but continued to experience asthma attacks at his workplace. Thus we recommended total exposure cessation. When we contacted the patient about a year after this examination he was still working as a farmer and his symptoms as well as medication were unchanged.

AUTHORS' CONTRIBUTION

All authors contributed as recommended by the ICJME.

DISCLOSURE BY AJIM EDITOR OF RECORD

Steven Markowitz declares that he has no competing or conflicts of interest in the review and publication decision regarding this article.

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