

## Occupational allergic respiratory disease (rinoconjunctivitis and asthma) in a cheese factory worker

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### Clinical Implications

- This is the first report of occupational IgE-mediated allergic respiratory disease caused by chymosin, an enzyme present in rennet used for the coagulation of milk in the production of cheese. The disease was severe and could be controlled only with omalizumab (decrease in symptoms, improvement in respiratory auscultation and spirometry), in addition to reducing exposure to the agent (use of mask) and pharmacological treatment (salmeterol/fluticasone, 500 µg/12 h and montelukast 10 mg/d).

### TO THE EDITOR:

The list of allergens involved in rinoconjunctivitis and, especially, in occupational allergic asthma continues to grow because of technological advances and because of a better knowledge of occupational allergic respiratory diseases (ARDs).<sup>1</sup> The dust from enzymes is known to be a causative agent in ARDs, usually of occupational origin and IgE-mediated.<sup>2</sup>

For a long time, calf rennet was commonly used in cheese industries for the curdling of milk. Currently, because calf rennet is difficult to obtain, various animal, plant, and microbial sources have been exploited as possible alternatives to calf rennet.<sup>3</sup> Rennet is a mixture of proteolytic enzymes (pepsin and chymosin) that are high-molecular-weight agents. Exposure to enzyme dusts has long been known to cause occupational immediate hypersensitivities.

A 37-year-old male patient with a history of rinoconjunctivitis and seasonal bronchial asthma due to allergy to grass, olive, and Chenopodiaceae pollen had been working in a cheese factory for the last 17 years. His job was to heat and then add the rennet to the milk to induce the coagulation of its proteins, a necessary procedure for cheese making. The patient presented with perennial asthma for the last 7 years. He complained of dry cough and dyspnea with wheezing, naso-ocular itching, sneezing, and rhinorrhea 3 to 4 minutes after handling rennet, which contained 50% of porcine pepsin and 50% of bovine chymosin. Asthma subsided with the inhalation of 200 µg of salbutamol, but it took 4 to 5 hours for the patient to become asymptomatic. The patient improved on weekends and was asymptomatic in vacations. The physical examination of the patient revealed some rhonchi and disseminated wheezing.

Baseline spirometry demonstrated moderate obstruction (Table 1) with a positive response to bronchodilator. The fractional exhaled nitric oxide was 183 ppb.

The allergy study showed positive skin prick test results to *Dermatophagoides pteronyssinus* (3.4 mm), grass pollen (10.1 mm), Olive tree (10.1 mm), Russian thistle pollen (9.1 mm), rennet powder 1/10 (w/v) (18.6 mm), and liquid rennet (8.0 mm) with a positive histamine control of 5.5 mm (mean wheal diameter).

Blood analysis revealed eosinophilia (580 eosinophils/mL), a total IgE level of 1611 kU/L, and specific IgE (kU/L) level of more than 100 (class 6) to grass pollen, 26 (class 3) to Russian thistle pollen, 24 (class 3) to Olive, 0.5 (class 1) to *D pteronyssinus*, and 11.6 (class 3) to chymosin. Specific IgE was negative to Alternaria, pepsin, and dog and cat epithelium.

To calculate the rennet concentration with which to start the bronchial provocation test, we performed intradermal skin tests with liquid rennet at 1/1000 (w/v), but after 5 minutes, the patient developed naso-ocular symptoms and bronchospasm with audible wheezing. These symptoms subsided in 1 hour with intramuscular epinephrine 0.5 mL, intravenous methylprednisolone 125 mg, and Polaramine 5 mg. We did not perform a specific bronchial challenge test with rennet because of the severity of asthma and because we considered diagnosis confirmed with the intradermal tests that induced a systemic reaction.

SDS-PAGE immunoblot analysis with calf chymosin extract and porcine pepsin was performed according to the method of Laemmli.<sup>4</sup> Reactive bands were detected with the ability to bind to IgE in the calf chymosin alone, not in pepsin. The molecular weight of these bands was between 25 and 45 kD, especially between 35 and 43 kD (Figure 1).

In addition to allergy to pollen, the patient was diagnosed with rinoconjunctivitis and persistent bronchial asthma due to allergy to chymosin contained in the rennet used to produce cheeses. We advised the patient to request a change in the tasks performed at his job within the cheese factory to avoid exposure to the rennet or, alternatively, to use liquid rennet instead of the lyophilized one. We also advised a mask and prescribed pharmacological treatment (inhaled salmeterol + fluticasone 500 µg every 12 hours and oral montelukast 10 mg every 24 hours). For economic reasons, the patient did not request a change in the tasks performed at his job within the cheese factory.

After 1 month of using mask at work and carrying out the treatment described above, the patient reported partial improvement in the symptoms and the spirometry at that time showed mild obstruction (Table 1). Despite these measures, the patient remained symptomatic and therefore we added omalizumab to the treatment, with a significant improvement in both the symptoms and the spirometric values over the next several months.

Rennet is a mixture of the proteolytic enzymes pepsin and chymosin, which is usually obtained from the fourth stomach of young ruminants. Although pepsin is also used in the pharmaceutical industry, both enzymes (pepsin and chymosin) are used

**TABLE I.** Spirometry at various time points in the patient's course

| Time point                                                              | FEV <sub>1</sub> | FVC        | FEV <sub>1</sub> /FVC | PEF        |
|-------------------------------------------------------------------------|------------------|------------|-----------------------|------------|
| Manipulating rennet (powder)                                            | 2.4 (52%)        | 3.2 (67%)  | 52%                   | 4.3 (48%)  |
| Postbronchodilation                                                     | 3.4 (73%)        | 3.6 (75%)  | 97%                   | 5.8 (65%)  |
| Manipulating rennet (liquid) + face mask during work and drug treatment | 2.8 (72%)        | 4.1 (87%)  | 68%                   | 7.88 (86%) |
| After 6 mo with omalizumab                                              | 3.2 (90%)        | 4.5 (102%) | 71%                   | 8.1 (88%)  |

FVC, Forced vital capacity; PEF, peak expiratory flow.

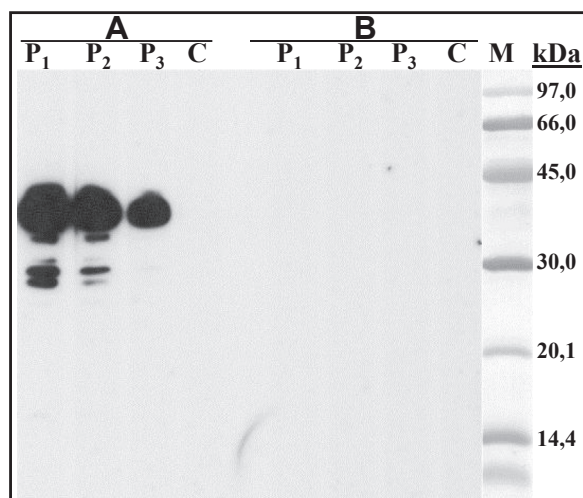


**FIGURE 1.** SDS-PAGE Immunoblotting. **A**, Renin of calf chymosin. **B**, Pork pepsin. Lane P: PS dil. 1/3 Lane P2: PS dil. 1/8 Lane P3: PS dil. 1/20 Lane C: Control serum (serum mixture of nonatopic people). Lane M: Pattern of molecular masses. PS, patient serum.

for the coagulation of milk protein in the manufacture of cheese.<sup>5</sup>

Proteases have been recognized as environmental allergens for years. Recently, a murine model of occupational asthma by a protease has been studied, characterizing the main molecular pathways involved in allergy sensitization to subtilisin that potentially contribute to the initiation of an ARD.<sup>6</sup>

In cheese workers, hypersensitivity pneumonitis due to penicillium, pepsin, and other agents has also been described.<sup>7</sup> Atopic patients, such as our patient, who are exposed to enzyme dust are more at risk of sensitization to these agents and therefore more likely to develop ARD than the general population.<sup>8</sup>

Both the history and the spirometric data (moderate-severe obstructive pattern with positive bronchodilator test result)<sup>9</sup> suggested that the patient's asthma had evolved from mild seasonal asthma due to pollen allergy to more severe perennial asthma of occupational origin due to allergy to chymosin.

As far as we know, no cases of ARD due to allergy to chymosin have been published. A case of occupational rhinitis caused by exposure to dust from another protease (pepsin) has been reported, but this patient worked in a slaughterhouse, not in a dairy; an IgE-binding band of a similar molecular weight (43 kDa) was detected in SDS-PAGE Immunoblotting (Figure 1).<sup>10</sup>

At the outset, the patient's respiratory medical examinations were monthly because his asthma was uncontrolled, but with

omalizumab, both the patient's symptoms and his respiratory function tests improved, and the reviews were spaced every 2 to 3 months.

In conclusion, we report an atopic patient who, after several years of working in an industrial dairy, developed ARD caused by chymosin allergy. The patient was diagnosed on the basis of work-related exposure to the enzyme and on the basis of skin tests. These tests have to be performed with caution because they triggered a systemic reaction in this patient. He could not completely avoid the exposure to the causative agent and had to be treated with omalizumab, with a good response. Physicians should stay alert to exposure to occupational agents in any adult with new-onset asthma or with newly uncontrolled asthma exposed to known occupational sensitizers.

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