
Extrinsic allergic alveolitis from a proteolytic enzyme

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Background: Subtilisins are proteolytic enzymes of bacterial origin found in detergents. They are high-molecular-weight antigens and have been implicated in allergic rhinitis and asthma.

Objective: This report describes a case of extrinsic allergic alveolitis due to subtilisins in a liquid cleaner.

Methods: Clinical, radiologic, and serologic information were used to make the diagnosis.

Case Report: A 53-year-old woman developed respiratory symptoms while working with a cleaner containing subtilisins. Her symptoms intensified in the work environment and improved away from work. A computed tomography scan demonstrated alveolar and interstitial infiltrates with subsequent scarring. A pulmonary function study revealed a restrictive pattern with diminished diffusion capacity. Bronchoalveolar lavage showed lymphocytosis and all cultures were negative. Precipitating antibodies to the enzyme were found in the patient's serum. Her symptoms improved once she changed her occupation.

Conclusions: The combination of the patient's clinical history, physical, laboratory, and radiologic findings support the diagnosis of extrinsic allergic alveolitis from the enzyme contained in the cleaner.

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INTRODUCTION

Extrinsic allergic alveolitis (EAA) or hypersensitivity pneumonitis is an immunologic syndrome caused by many inhaled antigens, including thermophilic actinomycetes, fungi, animal proteins, chemicals, and drugs.¹ Although the immunopathogenesis is not completely understood, it is believed to involve both humoral and cellular responses.^{1,2} Lymphocytes and macrophages are the predominant cells found in lung biopsies. IgE-mediated mechanisms that underlie asthma and rhinitis are not believed to contribute significantly to the inflammatory responses observed in EAA. Precipitating antibodies of the IgG class against the causative antigen, however, are nearly

always present in patients with EAA and probably represent evidence of exposure to the antigen.

Subtilisins, proteolytic enzymes derived from *Bacillus subtilis*, have been used in the detergent industry since the 1960s.^{3,4} Previous reactions were mostly documented in detergent industry workers; however, case reports of sensitization in consumers also exist.⁵⁻⁷ To date, only IgE-mediated respiratory diseases such as asthma and allergic rhinitis have been attributed to subtilisin exposure.^{3,8,9} We present a case of a patient who developed EAA from a detergent containing subtilisins. To our knowledge, this is a first report of a non-IgE-mediated respiratory disease from subtilisins.

CASE REPORT

A 53-year-old woman with a history of allergic rhinitis had been working with a liquid detergent (Klenzyme; Steris Corporation, St Louis, MO) for 2 years. She had no history of asthma or any respiratory diseases. The patient noted progressively worsening dyspnea on exertion, a dry cough, fatigue, and malaise for 1½ years before presentation. She required multiple office

visits to her primary physician and one emergency room visit. She denied fevers, chills, rash, arthritis, or gastrointestinal symptoms. The patient associated worsening symptoms at work as well as nocturnal worsening of the cough and dyspnea. Her symptoms improved significantly during a 2-week vacation from work. Her work consisted of washing surgical instruments as well as cleaning operating room surfaces with Klenzyme. According to the Material Safety Data Sheets, Klenzyme contains subtilisins and sodium tetraborate. She wore latex gloves and a mask for protection during work. Skin testing for possible IgE antibody sensitization to latex was negative. No additional environmental exposures were identified in her work place.

Her past medical history was significant for hypothyroidism and hypertension for which she was taking levothyroxine and nifedipine, respectively. There was no history of any travel and she did not have any pets. Smoking history was significant for 10 pack years and she had quit 10 years previously. Family history was noncontributory.

Physical examinations performed at various office visits were unremarkable except for crackles in the left lung base. She was initially treated for new onset asthma with albuterol, corticosteroid inhalers, and several courses of prednisone without improvement. A chest roentgenogram performed 4 months after symptoms started showed a new right perihilar infiltrate compared with a chest x-ray performed 1 year previously. Despite antibiotics, the infiltrate persisted on a repeat chest x-ray. A high resolution chest computed tomography (CT) scan performed 10 months after symptoms developed demonstrated an interstitial infiltrate in the left upper lobe and a mild interstitial and alveolar infiltrate in the right lower lobe (Figs 1 and 2). Complete blood count with differential, serum electrolytes including liver function

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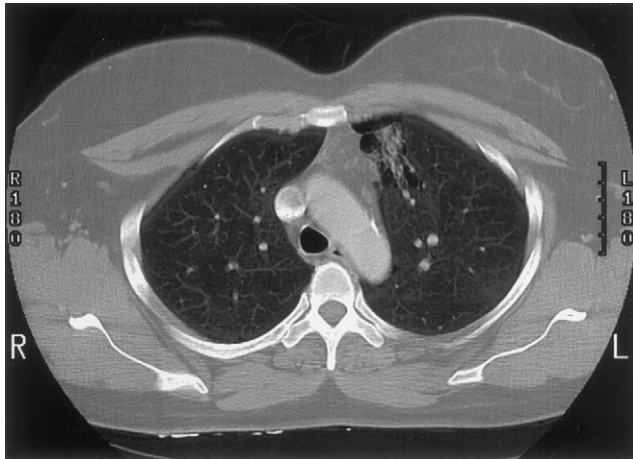


Figure 1. Chest CT demonstrating an interstitial infiltrate in the left upper lobe.

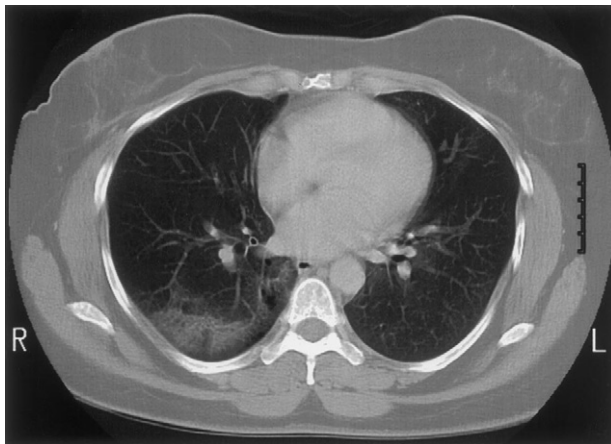


Figure 2. Chest CT demonstrating an interstitial and alveolar infiltrate in the right lower lobe.

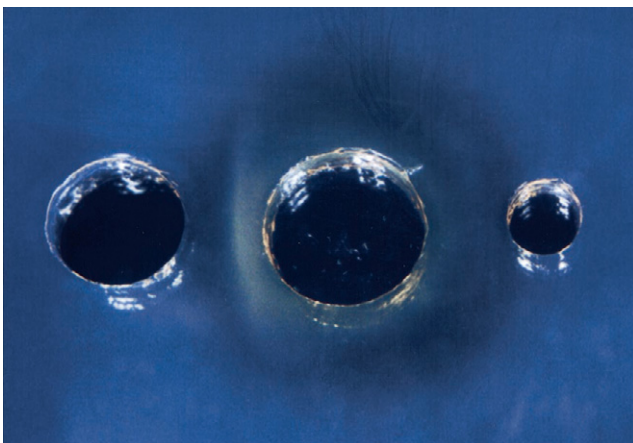


Figure 3. Photograph of the precipitin reaction between the patient's serum (left) and Klenzyme (center). A precipitin reaction was absent for the nonatopic, nonexposed control serum (right).

tests, urinalysis, erythrocyte sedimentation rate, antinuclear antibody, and rheumatoid factor were normal. Blood and sputum cultures were negative. Bronchoalveolar lavage (BAL) fluid cell-differentiation showed 60% lymphocytes, 17% macrophages, 12% neutrophils, and 1% eosinophils. Lymphocyte cell marker studies were not performed on the BAL fluid. Stains and cultures for bacteria, fungi, and mycobacteria as well as cytology were negative.

Because of persistent symptoms, the patient took a leave of absence from her work for 1 month and had considerable improvement in her symptoms. On return to work, her symptoms recurred within 1 week. One and one-half years after symptoms developed, the patient was evaluated in the allergy-immunology outpatient facility. Percutaneous tests to common aeroallergens were positive to tree pollen and dust mites. Serology for allergic bronchopulmonary aspergillosis was negative. Percutaneous tests with varying concentrations of Klenzyme (5% wt/vol) were negative. The skin tests with Klenzyme were negative in two nonexposed control participants. Strongly positive precipitins to Klenzyme were found in the patient's serum by using the Ouchterlony gel diffusion technique (Fig 3).¹⁰ Pulmonary function tests revealed a mild restrictive ventilatory defect with diminished diffusion capacity (Table 1). Based on these findings, the patient was diagnosed as having EAA with Klenzyme as the probable causative antigen.

Subsequently, the patient quit her work at the sterile processing division in the surgical department of the hospital and opened a day care center at home. A chest CT scan performed 1 year after she changed her work demonstrated scarring in the left upper and right lower lobes, but no progression of the previous infiltrates. A mild restrictive defect persists in her spirometry. She still has occasional cough and dyspnea with upper respiratory infections but, otherwise, is asymptomatic.

Table 1. Pulmonary Function Studies 1 Year After Onset of Symptoms

Function	Quantity	Percent predicted
FEV ₁	2.03 L	74
FVC	2.58 L	72
Thin layer chromatography	4.38 L	80
Right ventricle	1.57 L	80
Carbon monoxide diffusing capacity of the lungs	17.3 mL/min/mm Hg	69
FEF _{25%-75%}	1.41 L	47

DISCUSSION

Pepys et al⁴ and Flindt¹¹ were the first to report IgE-mediated allergic respiratory diseases in detergent industry workers exposed to *B. subtilis* enzymes.^{4,11} Measures aimed at reducing exposure to the proteolytic enzymes have significantly decreased the rates of IgE sensitization and respiratory diseases.¹² A recent case report implicating subtilisin as a cause of occupational asthma, however, indicates that sensitization still occurs.⁵ The patient presented in this report was originally evaluated for asthma given that exposure to high-molecular-weight agents such as the subtilisins are known to cause this condition. However, her clinical history, laboratory analysis, pulmonary function tests, and radiologic studies favor a diagnosis of EAA.

The temporal relationship between the patient's symptoms and her work environment suggested that the offending antigen was present at her work place. Improvement during absence from work with exacerbation on return confirmed this. An open-lung biopsy was initially planned but was not pursued due to progressive improvement. Infectious etiologies and other respiratory diseases such as sarcoidosis were thought to be unlikely due to lack of supporting laboratory data or other clinical findings. Negative skin tests, pulmonary infiltrates, and restrictive lung volumes with low diffusion capacity, as observed in our patient, are not compatible with an IgE-mediated obstructive airway disease. Besides the detergent, the patient was not exposed to any other potential antigen or organic dust. The detergent does contain another ingredient, sodium tetraborate,

which can act as a respiratory irritant. Borates can cause symptoms of dry mouth, nasal irritation, and shortness of breath. The respiratory effects of borates, unlike those observed in our patient, are reversible and exposed individuals feature normal lung volumes.^{13,14} Therefore, sodium tetraborate was unlikely to have contributed to the symptoms observed in our patient.

Our patient's disease course characterized by exacerbations and remissions coinciding with work exposure, the BAL fluid with lymphocytosis, restrictive lung volumes with reduced diffusion capacity, and precipitins to Klenzyme provides sufficient criteria to be considered EAA secondary to subtilisins.¹⁵⁻¹⁷ This report should alert physicians to the diagnosis of EAA in subtilisin-exposed individuals. This is important because timely diagnosis of EAA and avoidance of the causative antigen may prevent progressive impairment.

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