

ORIGINAL PAPER

Sensitization to Empynase[®] (pronase B) in exposed hospital personnel and identification of the Empynase[®] allergen

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Clinical and Experimental Allergy

Summary

Background Empynase[®] is a proteolytic enzyme that is widely used as an anti-inflammatory drug in Korea. We evaluated the prevalence of sensitization to Empynase[®] in association with respiratory allergy symptoms in exposed hospital personnel, and identified the IgE-binding components in the Empynase[®] extract, using sera with high levels of specific IgE antibodies. **Methods** A total of 154 hospital personnel (135 nurses and 19 pharmacists) who worked in a university hospital and 123 unexposed healthy control subjects were enrolled. A questionnaire was administered that addressed demographics, job category, history of atopic diseases, diverse symptoms including nasal and lower respiratory symptoms, and the association of symptoms with work. Skin prick tests (SPTs) to common aeroallergens and Empynase[®] extract were performed. Empynase[®]-specific IgE antibody was detected by ELISA, and ELISA inhibition tests were conducted. IgE-binding components were identified by SDS-PAGE and IgE immunoblotting.

Results Forty-two subjects (27.3%) complained of work-related respiratory symptoms (WRRS). Five nurses (3.7%) and one pharmacist (5.3%) had work-related asthma symptoms, and 34 nurses (25.2%) and six pharmacists (31.6%) had work-related rhinitis symptoms. The prevalence of sensitization to Empynase[®] on SPTs was 20.1%, and tended to be higher in pharmacists (31.6%) than in nurses (18.5%). It was estimated that 3.9–8.4% of hospital personnel had WRRS attributable to Empynase[®]. The duration of exposure was longer in positive SPT responders than in negative responders (51.9 ± 27.5 vs. 39.2 ± 27.3 months, respectively; $P < 0.05$), and the prevalence of Empynase[®]-positive SPTs was significantly higher in subjects with asthma than in those without asthma (57.1% vs. 18.4%, respectively; $P < 0.05$). The levels of Empynase[®]-specific IgE antibodies were significantly higher in pharmacists (76.1 ± 83.4 OD units) and nurses (56.3 ± 103.0 OD units) than in normal controls (39.8 ± 12.7 OD units; $P < 0.05$). Seven subjects (two pharmacists and five nurses) had high serum levels of Empynase[®]-specific IgE antibodies; six of these subjects had WRRS. ELISA inhibition tests were performed with the sera of these six subjects, revealing significant inhibition only with the addition of Empynase[®]. Four strongly staining protein bands (sizes: 36, 33, 16, and 10 kDa) from Empynase[®] extract were observed to bind to the IgE antibodies of sensitized subjects.

Conclusion Exposure to Empynase[®] powder may cause rhinitis and asthma in hospital personnel, and the pathogenic mechanism appears to be IgE mediated.

Keywords Empynase[®], enzyme allergy, hospital personnel, occupational asthma, prevalence
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Introduction

Enzymes are widely used in the chemical, pharmaceutical, and food industries, and are commonly described as

occupational allergens [1]. Enzyme allergy has been recognized since the late 1960s [2, 3]. It may cause asthma, rhinoconjunctivitis, allergic contact dermatitis, and contact urticaria [4]. A community-based study

recently reported that enzymes could be a specific occupational risk [5].

Enzymes are also frequently used as therapeutic agents including digestive enzymes and anti-inflammatory drugs. There have been some reports of occupational asthma induced by these enzymes inhaled during the course of work, predominantly among pharmaceutical industry workers [6–9]. An IgE-mediated mechanism has been implicated in most of these cases. However, reports of enzyme allergy in exposed hospital personnel have been sporadic. The prevalence of occupational allergy to enzymes among hospital personnel is unknown.

Empynase[®], a formulation of pronase B, is a proteolytic enzyme that is commonly prescribed as an anti-inflammatory drug in Korea and Japan. Hospital personnel may be exposed to enzymes when mixing or otherwise handling the powdered form of Empynase[®]. A case of occupational asthma induced by pronase E has been reported in a medical laboratory technician [10].

To test the hypothesis that exposure to Empynase[®] powder may cause asthma and/or rhinitis in hospital personnel, we evaluated the prevalence of sensitization to Empynase[®] in association with work-related respiratory symptoms (WRRS), and investigated its pathogenic mechanism.

Methods

Subjects

Three groups were enrolled in the study: 135 nurses (group II) and 19 pharmacists (group III) who worked in a university hospital and 123 unexposed healthy control subjects (group I). All the nurses working in wards dealing with the Empynase[®], and pharmacists working in a dispensary, except three nurses who refused to, were enrolled in our study. The subjects in group I were normal healthy volunteers. They were recruited from those who had never worked in hospitals or in the pharmaceutical industry. They were asked to complete a questionnaire regarding their work history; history of atopic diseases such as asthma, allergic rhinitis, and atopic dermatitis; smoking habits; and work-related symptoms including respiratory symptoms. The questions regarding respiratory symptoms addressed whether subjects had rhinitis symptoms, such as runny nose, sneezing, or congestion, as well as asthmatic symptoms, such as shortness of breath or wheezing. The subjects who indicated that their symptoms were aggravated during work but that they experienced relief after work or during holiday periods were defined as having WRRS. Their atopy status was assessed by skin prick tests (SPTs) with nine common environmental allergens: two kinds of house dust mites (*Dermatophagoides farinae*, *D. pteronyssinus*), ragweed, mugwort, grass pollen mixtures, tree pollen mixtures, *Alternaria*,

cat, and dog (Allergopharma). The results were evaluated after 15 min. The sizes of the weals produced by each antigen and by histamine were expressed as the mean of the maximum diameter and the vertical length at the mid-portion of the maximal length. Skin reactivity was expressed as the ratio of the weal size for an allergen to that for histamine (*A/H*). For *A/H* values greater than 1, the reaction was defined as positive. A person with one or more positive SPT reactions to environmental allergens was defined as atopic. All healthy control subjects were non-atopic and showed negative responses to Empynase[®] on SPT. Blood was collected from all participants for the evaluation of specific IgE antibodies to Empynase[®]. All subjects gave their informed consent to participate in the study, which was regulated by the institutional review board of Ajou University Hospital in Suwon, Korea.

Skin prick tests to Empynase[®] and enzyme-linked immunosorbent assay for Empynase[®]-specific immunoglobulin E antibodies

To assess the sensitization of subjects to Empynase[®], SPTs were performed with Empynase[®] extract. For *A/H* values between 0.1 and 1 and erythema diameters less than 21 mm, the sensitization was rated as 1+. For *A/H* values between 0.1 and 1 and erythema diameters greater than 21 mm, the sensitization was rated as 2+. For *A/H* values between 1 and 2, the sensitization was rated as 3+. For *A/H* values between 2 and 3, the sensitization was rated as 4+. For *A/H* values greater than 3, the sensitization was rated as 5+. Subjects who demonstrated responses greater than 2+ on SPTs were defined as positive responders.

The presence of IgE antibodies specific for Empynase[®] was determined by ELISA. Briefly, microtitre plates (Corning Co., NY, USA) were coated with 100 µL of each antigen at a concentration of 10 µg/mL in a carbonate-bicarbonate buffer (pH 9.6; Sigma Co., St Louis, MO, USA) and incubated overnight at 4 °C. Each well was washed three times with 0.05% phosphate-buffered saline (PBS)–Tween (PBST), and the remaining binding sites were blocked by incubation with 350 µL of 10% fetal bovine serum (FBS)–PBS for 1 h at room temperature. The wells were then incubated for 1 h at room temperature with 50 µL of a 1 : 2 dilution of the subject's serum. The samples were washed three times with PBST, 100 µL of 1 : 1000 (vol:vol) biotin-labelled goat anti-human IgE antibody (Vector Laboratories, Burlingame, CA, USA) was added to the wells, and the plates were incubated for 1 h at room temperature. The wells were washed three times with PBST and incubated for 30 min with 1 : 1000 (vol:vol) streptavidin–peroxidase (Sigma Co.). After another washing step, the plates were incubated for 15 min at room temperature with 100 µL of 3,3',5,5'-tetraethylbenzidine (TMB; one tablet in a phosphate-citrate buffer). The reaction was stopped with the addition of 2 N H₂SO₄, and the

absorbance was read at 450 nm using an automated microplate reader. All assays were performed in triplicate. The positive cut-off value for specific IgE binding was equivalent to the mean plus 3 SD of the 123 unexposed controls.

Competitive inhibition enzyme-linked immunosorbent assay

A competitive inhibition ELISA was performed to determine the specificity of IgE binding to Empynase[®] and cross-allergenicity with other drugs. The sera from the subjects with high levels of IgE antibody specific for Empynase[®] were pooled and pre-incubated with four concentrations (0.1, 1, 10, and 100 µg/mL) of pancreatin, α -amylase, budiastase, and *D. pteronyssinus* overnight at 4 °C. The samples were then incubated in the Empynase[®] (10 µg/mL)-coated microplate for 1 h. The ELISA was conducted as described previously. As a control, the samples were pre-incubated with equal volumes of PBS (pH 7.5) instead of inhibitors. The percentage inhibition of specific IgE binding was expressed as:

Percentage inhibition

$$= 100 - \left(\left[\frac{\text{absorbance of samples pre-incubated with allergens}}{\text{absorbance of samples pre-incubated with PBS}} \right] \times 100 \right)$$

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis and immunoglobulin E immunoblotting

Empynase[®] extract (80 µg/mL) was dissolved in sample buffer (0.5 M Tris, pH 6.8, glycerol, 10% SDS, 0.5% bromophenol blue, 2.5% β -mercaptoethanol) and heated at 100 °C for 5 min. Markers (4–250 kDa; Invitrogen, San Diego, CA, USA) and antigen solutions were loaded onto a 4–20% Tris-glycine gel (Novex, Invitrogen), and electrophoresis was performed for 2 h at a constant voltage of 125 V. The proteins were electroblotted onto a polyvinylidene difluoride membrane (Millipore Co., Bedford, MA, USA) for 2 h at 200 mA. The blotted membrane was treated with 5% skim milk-TBST for 2 h to block non-specific protein binding and then incubated at 4 °C for 15 h with the sera of the four subjects with the highest level of Empynase[®]-specific IgE antibodies and the sera of two unexposed controls. The membrane was washed with PBST and subsequently incubated with biotin-conjugated goat anti-human IgE antibody (1 : 1000, w:v; Vector Laboratories) for 1 h at room temperature. The membrane was washed again with TBST and incubated for 30 min with streptavidin-alkaline phosphatase (1 : 1000, w:v; Sigma Co.). The substrate solution, 5-bromo-4-chloro-3-indolyl phosphate/*p*-nitro blue tetrazolium chloride (Sigma Co.), was applied to the membrane until bands appeared.

Statistical analysis

The study data were analysed with SPSS 11.0 for Windows (SPSS Inc., Chicago, IL, USA). Normally distributed data were assessed using Student's *t*-test or analysis of variance (ANOVA), and data that were not normally distributed were assessed using non-parametric tests as appropriate. Data for categorical variables were assessed using the χ^2 test. Differences with *P* < 0.05 were considered to be significant.

Results

Skin prick tests and levels of Empynase[®]-specific immunoglobulin E antibody in each group

The mean ages of the 135 nurses (27.5 ± 3.1 years) and the 19 pharmacists (26.6 ± 3.6 years) were similar (*P* > 0.05). All the nurses and pharmacists were female and non-smokers. The mean age of the 123 normal controls (66 males, 57 females) was 37.3 ± 15.2 years, which was higher than the ages of the other groups (*P* < 0.05). The mean working durations of the nurses and pharmacists were 42.5 ± 26.6 and 36.0 ± 35.1 months, respectively. The prevalence among nurses and pharmacists of a

previous history of asthma (5.2% vs. 0%, respectively) or rhinitis (14.1% vs. 10.5%, respectively) did not differ. The atopy rates, 31.9% in nurses and 47.4% in pharmacists, did not differ significantly between the two groups. Thirty-one of the 154 hospital personnel showed a positive response to Empynase[®] on SPT, indicating that the prevalence of immunologic sensitization to Empynase[®] by skin test was 20.1%. The prevalence of immunologic sensitization tended to be higher in pharmacists (31.6%) than in nurses (18.5%; Table 1). When a positive response was defined as greater than 3+, the prevalence of immunologic sensitization was found to be 15.0%.

The levels of Empynase[®]-specific IgE antibody were significantly higher in pharmacists (76.1 ± 83.4 OD units) and nurses (56.3 ± 103.0 OD units) than in the normal controls (39.8 ± 12.7 OD units) and were significantly different among the three groups (*P* < 0.001; Fig. 1). Only seven subjects showed levels higher than 3 SD beyond the control mean, indicating that the prevalence of immunologic sensitization by serological test was 4.5%.

Hospital personnel with work-related respiratory symptoms

Of the 154 hospital personnel, 42 subjects (27.3%) complained of WRRS. Five nurses (3.7%) and one pharmacist

Table 1. Clinical characteristics, SPT results, and specific IgE to Empynase[®] of the study subjects

	Nurses (<i>n</i> = 135)	Pharmacists (<i>n</i> = 19)	<i>P</i> - value
Age (years, mean $\pm$ SD)	27.5 $\pm$ 3.1	26.6 $\pm$ 3.6	NS
Duration (months, mean $\pm$ SD)	42.5 $\pm$ 26.6	36.0 $\pm$ 35.1	NS
Asthma (%)	7 (5.2)	0 (0)	NS
Rhinitis (%)	19 (14.1)	2 (10.5)	NS
Atopy (%)	43 (31.9)	9 (47.4)	NS
WRRS (%)	36 (26.7)	6 (31.6)	NS
SPT_Em (%)	25 (18.5)	5 (31.6)	NS

WRRS, work-related respiratory symptoms; SPT_Em, skin prick test with Empynase extract; NS, not significant; SD, standard deviation.

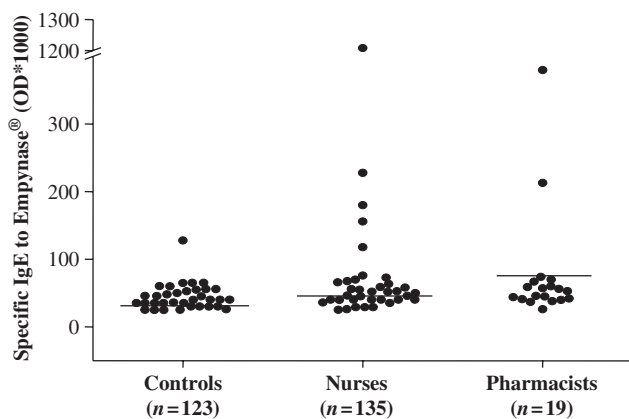


Fig. 1. Specific IgE binding to Empynase[®] detected by ELISA in the three study groups. The levels of Empynase[®]-specific IgE antibodies are different among the pharmacists, nurses, and normal control subjects. The mean values $\pm$ SD are 76.1 $\pm$ 83.4, 56.3 $\pm$ 103.0, and 39.8 $\pm$ 12.7 OD units, respectively ($P < 0.001$, between groups by the Kruskal-Wallis test).

(5.3%) experienced work-related asthma symptoms, and 34 nurses (25.2%) and six pharmacists (31.6%) experienced work-related rhinitis symptoms. These values were not significantly different between the two groups (Table 1).

Of the 42 hospital personnel with WRRS, 13 subjects (31.0%) showed a positive response to Empynase[®] on SPT, whereas only 18 (16.1%) of the 112 hospital personnel without WRRS showed a positive response. Thus, the rate of Empynase[®]-positive SPTs differed significantly depending on the presence of WRRS ($P < 0.05$). The mean level of Empynase[®]-specific IgE antibody was higher in subjects with WRRS than in those without WRRS, but the difference was not statistically significant (89.4 $\pm$ 187.0 vs. 47.3 $\pm$ 24.4 OD units, respectively; $P = 0.08$). The atopy rate did not differ with the presence or absence of WRRS (38.1% vs. 32.1%; Table. 2). By considering the results of the questionnaire, the SPTs to Empynase[®], and the

Empynase[®]-specific IgE antibody levels, it was estimated that between 3.9% (based on increased specific IgE antibody levels) and 8.4% (based on skin testing) of hospital personnel had WRRS attributable to Empynase[®].

Association between skin prick test reactivity to Empynase[®] and clinical parameters

The duration of exposure was significantly longer in positive responders than in negative responders (51.9 $\pm$ 27.5 vs. 39.2 $\pm$ 27.3 months, respectively; $P < 0.05$). The level of Empynase[®]-specific IgE antibody was higher in positive SPTs responders than in negative responders (120.0 $\pm$ 215.6 vs. 43.3 $\pm$ 11.0 OD units, respectively; $P < 0.0001$), and there was a positive correlation between the skin test reactivity and the level of Empynase[®]-specific IgE antibodies (data not shown). The rate of Empynase[®]-positive SPTs was significantly higher in subjects with asthma than in those without asthma (57.1% vs. 18.4%; $P < 0.05$), but the rate did not differ between the presence and absence of rhinitis (28.6% vs. 18.8%) or atopy (28.8% vs. 15.7%; Table. 2).

Subjects with an increased level of specific immunoglobulin E antibodies to Empynase[®]

Seven subjects, two pharmacists and five nurses, had high serum levels of IgE antibodies specific for Empynase[®] (Table 3). Although all seven subjects responded positively to Empynase[®] on SPTs, only two (28.6%) responded positively to common inhalant allergens. Six of them had WRRS; five had rhinitis symptoms, and three had asthma symptoms. Four of the five nurses (80%) complained of WRRS, and two among them had a history of asthma or rhinitis before they began working in the hospital. Both pharmacists had WRRS (100%), and neither had a previous history of asthma or rhinitis. The bronchial provocation test with Empynase[®] extract was performed in one pharmacist, according to the procedure used in previous studies [9], and an early asthmatic reaction was noted after the inhalation of a solution containing 10 mg/mL Empynase[®] extract. The latency period of WRRS in the six subjects who had both high levels of Empynase[®]-specific IgE antibodies and WRRS varied among individuals and ranged from 4 to 63 months.

Competitive inhibition ELISA

Competitive inhibition ELISA was performed using the pooled sera from the six subjects who had both high levels of Empynase[®]-specific IgE antibodies and WRRS. Figure 2 shows the ELISA results with serial additions of Empynase[®], pancreatin, α -amylase, biodiastase, and *D. pteronyssinus* antigens. A significant dose-dependent inhibition was noted with the addition of Empynase[®],

Table 2. Comparison of clinical parameters according to the presence of work-related respiratory symptoms and skin prick test results

	WRRS			SPT_Em		
	Presence (n = 42)	Absence (n = 112)	P-value	Positive (n = 31)	Negative (n = 123)	P-value
Age (years, mean $\pm$ SD)	28.0 $\pm$ 3.0	27.0 $\pm$ 3.2	NS	27.7 $\pm$ 2.5	27.2 $\pm$ 3.3	NS
Duration (months, mean $\pm$ SD)	51.1 $\pm$ 28.0	38.3 $\pm$ 28.0	0.006	51.9 $\pm$ 27.5	39.2 $\pm$ 27.3	0.02
SPT_Em (%)	13 (31.0)	18 (16.1)	0.04			
s-IgE to Empynase [®] (OD, mean $\pm$ SD)	89.4 $\pm$ 187.0	47.3 $\pm$ 24.4	0.08	120.0 $\pm$ 215.6	43.3 $\pm$ 11.0	< 0.0001
Atopy (%)	16 (38.1)	36 (32.1)	NS	13 (28.8)	18 (15.7)	NS
Asthma (%)				16 (57.1)	36 (18.4)	0.03
Rhinitis (%)				16 (28.6)	36 (18.8)	NS

WRRS, work-related respiratory symptoms; SPT_Em, skin prick test with Empynase extract; NS, not significant; SD, standard deviation.

Table 3. Seven subjects with high levels of specific IgE to Empynase

	Group	Age (years)	BA Hx	AR Hx	Atopy	SPT (Em)	IgE (Em)	Nasal Sx	Asthmatic Sx
1	Nurse	29	—	+	Negative	Positive	1208	+	—
2	Nurse	28	+	—	Negative	Positive	228	—	+
3	Nurse	28	—	—	Negative	Positive	180	+	—
4	Nurse	32	—	—	Negative	Positive	156	+	+
5	Nurse	29	—	—	Negative	Positive	118	—	—
6	Pharmacist	29	—	—	Positive	Positive	380	+	+
7	Pharmacist	26	—	—	Positive	Positive	213	+	—

BA Hx., history of asthma; AR Hx., history of rhinitis; SPT (Em), skin test reactivity to Empynase[®]; IgE (Em), level of specific IgE to Empynase[®] (optical density [OD] unit); Nasal sx., work-related nasal symptoms; Asthmatic sx., work-related asthmatic symptoms; +, presence; —, absence.

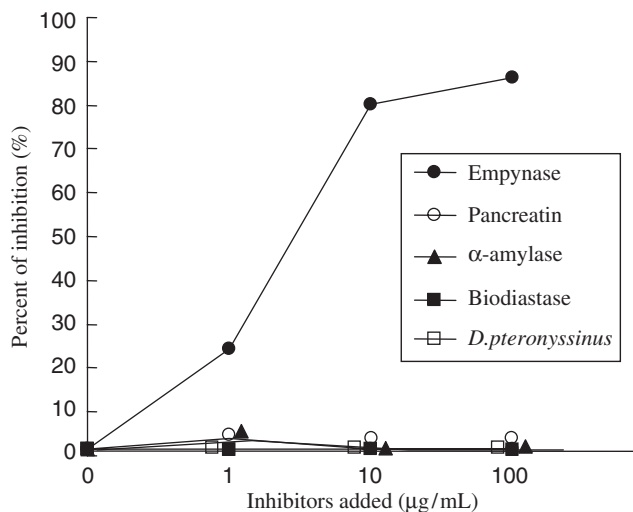


Fig. 2. IgE ELISA inhibition in Empynase[®]-coated wells with serial additions of pancreatin, α -amylase, Empynase[®], biodiastase, and *Der-matophagoides pteronyssinus*. ELISA inhibition tests with pooled sera from subjects with high levels of Empynase[®]-specific IgE antibodies show significant dose-dependent inhibition with the addition of Empynase[®] but minimal inhibition with the addition of pancreatin, amylase, biodiastase, and *Dermatophagoides pteronyssinus* antigens.

whereas minimal inhibition was observed with the addition of pancreatin, amylase, biodiastase, or *D. pteronyssinus* antigens.

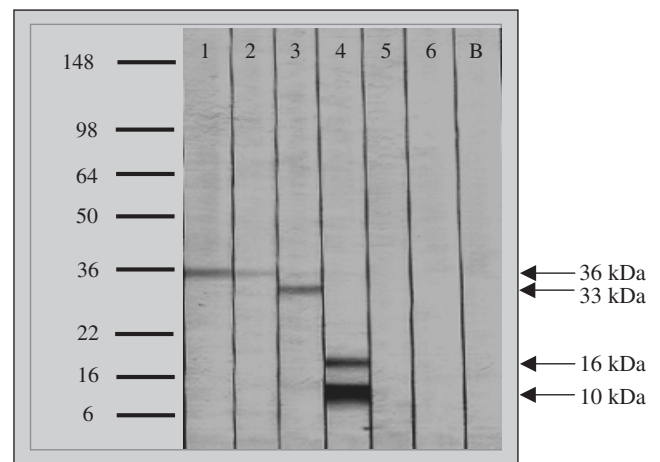


Fig. 3. IgE immunoblot analysis of Empynase[®] extract in the sera from sensitized patients (lanes 1–4), non-atopic controls (lanes 5 and 6), and buffer control (B). Four strongly staining protein bands (36, 33, 16, 10 kDa) are seen, representing Empynase[®] antigen bound to IgE antibody in the sera from the four sensitized subjects. No bands are seen in the sera from the two control subjects.

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis and immunoglobulinE immunoblotting

Figure 3 shows the proteins from the Empynase[®] extract that bound the specific IgE in the sera of four sensitized subjects (1–4) and two control subjects (5 and 6) after blotting onto a nitrocellulose membrane. Four IgE-

binding components (36, 33, 16, and 10 kDa) were observed in the Empynase[®] extract using the sera of sensitized subjects, whereas no IgE-binding bands were noted using the control sera.

Discussion

The present study demonstrates that exposure to Empynase[®], a proteolytic enzyme, may cause asthma and rhinitis in hospital personnel, including nurses and pharmacists. Many of the subjects in our study had WRRS including symptoms of asthma and rhinitis (26.7% in nurses and 31.6% in pharmacists), and many of those with WRRS were sensitized to Empynase[®]. Approximately 3.9–8.4% of exposed hospital personnel had WRRS attributable to Empynase[®].

The prevalence of immunological sensitization to Empynase[®] was very high in exposed subjects: 20.1% as defined by SPTs and 4.5% as defined by serological tests. This suggests that Empynase[®] is a major occupational sensitizer in exposed hospital workers. Most studies reporting occupational asthma in hospitals have been case reports [11, 12]. There have been few studies evaluating the prevalence of sensitization to or work-related symptoms caused by an occupational allergen other than latex [13, 14]. One study examined sensitization to psyllium among health personnel working in chronic care hospitals [15], and one reported sensitization to glutaraldehyde among endoscopy nursing staff [16]. Our study demonstrated that biological enzymes such as Empynase[®] may be an occupational allergen in hospital personnel. To the best of our knowledge, this is the first report of the prevalence of sensitization and occupational allergy to enzymes in hospital personnel.

The extent of exposure is considered an important risk factor for sensitization to Empynase[®]. In our study, the period of exposure was significantly longer in sensitized subjects than in non-sensitized subjects. There was a significant difference in the level of Empynase[®]-specific IgE antibodies among the pharmacists, nurses, and normal control subjects. The prevalence of positive SPTs was slightly higher in pharmacists than in nurses, although the difference was not statistically significant. Empynase[®] is usually administered in tablet form; however, it is commonly prepared and administered in powdered-form for tube-feeding patients. Considering that pharmacists grind and mix the medication in confined spaces and that nurses deliver the medication to patients in open wards, pharmacists are likely to be exposed to Empynase[®] powder more frequently. Our findings suggest that sensitization to Empynase[®] may be associated with the extent of exposure. Epidemiological studies of occupational allergies to other enzymes in bakers and enzyme-detergent workers have shown an exposure-response relationship for IgE sensitization [17–19].

Atopy was not associated with sensitization to Empynase[®]. Although the prevalence of positive SPTs

tended to be higher in atopic subjects than in non-atopic subjects, five of the seven subjects (71.4%) who were positive for Empynase[®]-specific IgE antibodies were non-atopic. Atopy has been reported as a risk factor in earlier studies [20–22], but other studies found that atopy was not a significant risk factor for sensitization to enzymes [23, 24]. In our study, a history of asthma was a risk factor for sensitization to Empynase[®]. Although smoking has been reported to be a marked risk factor for sensitization in some studies [25], we could not evaluate the role of smoking as a risk factor because all of our subjects were reportedly non-smokers. Recent prospective studies have shown that the single strongest influence on the incidence of occupational allergy caused by high-molecular-weight allergens is exposure to the responsible agent; other factors, such as atopy and smoking, were found to be less important [26–29].

The pathogenic mechanism of Empynase[®]-related asthma and/or rhinitis may be an IgE-mediated reaction. Many people who complained of WRRS showed a positive response to Empynase[®] extract in SPTs and had high levels of Empynase[®]-specific IgE antibodies. This is consistent with other reports of occupational asthma induction by biological enzymes inhaled during the course of work [7, 9, 11, 30]. The ELISA inhibition test results suggested the specificity of IgE binding to Empynase[®]. Furthermore, IgE immunoblot analysis showed IgE-binding components for Empynase[®]. To the best of our knowledge, this is the first study to demonstrate IgE-binding components for Empynase[®] in sensitized subjects. Our findings suggest that SPTs and serological tests for specific IgE may be used for the early detection of sensitized subjects among exposed personnel.

It is well known that early detection and avoidance of the causative agent are the most effective strategies in the management of occupational allergic diseases [31, 32]. Given the high prevalence of sensitization and WRRS in exposed hospital personnel, early detection methods are critical. We suggest that regular SPTs and/or serological tests of exposed workers be used for the early detection of sensitization.

The present study has some limitations. Firstly, we did not measure the exposure intensity in detail on an individual basis. Secondly, as the study was cross-sectional, bias may have affected the results regarding prevalence and the exposure–effect relationship [33]. Thirdly, we could not perform the bronchoprovocation test with Empynase[®] in all the symptomatic workers. Only one pharmacist consented to challenge testing with both methacholine and Empynase[®] and showed an early asthmatic response. Other symptomatic subjects did not agree to undergo bronchoprovocation testing. However, in this study, a high prevalence of WRRS correlated with a high Empynase[®]-positive rate on SPTs, and a significant association was found between skin test prevalence and

the presence of specific IgE in the serum. Based on these findings, Empynase[®] may be considered as a causative allergen in exposed hospital personnel.

In conclusion, exposure to Empynase[®] powder may cause rhinitis and asthma in hospital personnel, and the pathogenic mechanism is suggestive of an IgE-mediated one.

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References

- Zentner A, Jeep S, Wahl R, Kunkel G, Kleine-Tebbe J. Multiple IgE-mediated sensitizations to enzymes after occupational exposure: evaluation by skin prick test, RAST, and immunoblot. *Allergy* 1997; 52:928–34.
- Pepys J, Longbottom JL, Hargreave FE, Faux J. Allergic reactions of the lungs to enzymes of *Bacillus subtilis*. *Lancet* 1969; 1:1181–4.
- Flindt ML. Pulmonary disease due to inhalation of derivatives of *Bacillus subtilis* containing proteolytic enzyme. *Lancet* 1969; 1:1177–81.
- Laraqui C, Harourate K, Belamalle I, Benhaymoud N, Verger C. Occupational respiratory risks in workers exposed to enzymes in detergents. *Rev Mal Respir* 1996; 13:485–92.
- Zock JP, Cavalle N, Kromhout H *et al*. Evaluation of specific occupational asthma risks in a community-based study with special reference to single and multiple exposures. *J Expo Anal Environ Epidemiol* 2004; 14:397–403.
- Dolan TF Jr, Meyers A. Bronchial asthma and allergic rhinitis associated with inhalation of pancreatic extracts. *Am Rev Respir Dis* 1974; 110:812–3.
- Cartier A, Malo JL, Pineau L, Dolovich J. Occupational asthma due to pepsin. *J Allergy Clin Immunol* 1984; 73:574–7.
- Aiken TC, Ward R, Peel ET, Hendrick DJ. Occupational asthma due to porcine pancreatic amylase. *Occup Environ Med* 1997; 54:762–4.
- Park HS, Nahm DH. New occupational allergen in a pharmaceutical industry: serratal peptidase and lysozyme chloride. *Ann Allergy Asthma Immunol* 1997; 78:225–9.
- Kempf W, Oman H, Wuthrich B. Allergy to proteases in medical laboratory technician: a new occupational disease? *J Allergy Clin Immunol* 1999; 104:700–1.
- Park HS, Kim HY, Suh YJ *et al*. Alpha amylase is a major allergenic component in occupational asthma patients caused by porcine pancreatic extract. *J Asthma* 2002; 39:511–6.
- Di Stefano F, Siriruttanapruk S, McCoach J, Burge PS. Glutaraldehyde: an occupational hazard in the hospital setting. *Allergy* 1999; 54:1105–9.
- Vandenplas O, Delwiche JP, Evrard G *et al*. Prevalence of occupational asthma due to latex among hospital personnel. *Am J Respir Crit Care Med* 1995; 151:54–60.
- Garabrant DH, Schweitzer S. Epidemiology of latex sensitization and allergies in health care workers. *J Allergy Clin Immunol* 2002; 110:S82–95.
- Malo JL, Cartier A, L'Archeveque J *et al*. Prevalence of occupational asthma and immunologic sensitization to psyllium among health personnel in chronic care hospitals. *Am Rev Respir Dis* 1990; 142:1359–66.
- Vyas A, Pickering CA, Oldham LA *et al*. Survey of symptoms, respiratory function, and immunology and their relation to glutaraldehyde and other occupational exposures among endoscopy nursing staff. *Occup Environ Med* 2000; 57:752–9.
- Juniper CP, How MJ, Goodwin BF, Kinshott AK. *Bacillus subtilis* enzymes: a 7-year clinical, epidemiological and immunological study of an industrial allergen. *J Soc Occup Med* 1977; 27:3–12.
- Houba R, Heederik DJ, Doekes G, van Run PE. Exposure-sensitization relationship for alpha-amylase allergens in the baking industry. *Am J Respir Crit Care Med* 1996; 154:130–6.
- Houba R, Heederik D, Doekes G. Wheat sensitization and work-related symptoms in the baking industry are preventable. An epidemiologic study. *Am J Respir Crit Care Med* 1998; 158:1499–503.
- Vanhanen M, Tuomi T, Hokkanen H *et al*. Enzyme exposure and enzyme sensitisation in the baking industry. *Occup Environ Med* 1996; 53:670–6.
- Cullinan P, Lowson D, Nieuwenhuijsen MJ *et al*. Work related symptoms, sensitisation, and estimated exposure in workers not previously exposed to laboratory rats. *Occup Environ Med* 1994; 51:589–92.
- Weill H, Waddell LC, Ziskind M. A study of workers exposed to detergent enzymes. *JAMA* 1971; 217:425–33.
- Johnsen CR, Sorensen TB, Ingemann Larsen A *et al*. Allergy risk in an enzyme producing plant: a retrospective follow up study. *Occup Environ Med* 1997; 54:671–5.
- Vanhanen M, Tuomi T, Tiikkainen U, Tupasela O, Voutilainen R, Nordman H. Risk of enzyme allergy in the detergent industry. *Occup Environ Med* 2000; 57:121–5.
- Venables KM, Topping MD, Howe W, Luczynska CM, Hawkins R, Taylor AJ. Interaction of smoking and atopy in producing specific IgE antibody against a hapten protein conjugate. *Br Med J (Clin Res Ed)* 1985; 290:201–4.
- Gautrin D, Ghezzi H, Infante-Rivard C, Malo JL. Incidence and host determinants of work-related rhinoconjunctivitis in apprentice pastry-makers. *Allergy* 2002; 57:913–8.
- Gautrin D, Infante-Rivard C, Ghezzi H, Malo JL. Incidence and host determinants of probable occupational asthma in apprentices exposed to laboratory animals. *Am J Respir Crit Care Med* 2001; 163:899–904.
- Gautrin D, Ghezzi H, Infante-Rivard C, Malo JL. Incidence and determinants of IgE-mediated sensitization in apprentices: a prospective study. *Am J Respir Crit Care Med* 2000; 162:1222–8.
- Cullinan P, Cook A, Gordon S *et al*. Allergen exposure, atopy and smoking as determinants of allergy to rats in a cohort of laboratory employees. *Eur Respir J* 1999; 13:1139–43.
- Bardy JD, Malo JL, Seguin P *et al*. Occupational asthma and IgE sensitization in a pharmaceutical company processing psyllium. *Am Rev Respir Dis* 1987; 135:1033–8.
- Ameille J, Descatha A. Outcome of occupational asthma. *Curr Opin Allergy Clin Immunol* 2005; 5:125–8.
- Wild LG, Lopez M. Occupational asthma caused by high-molecular-weight substances. *Immunol Allergy Clin North Am* 2003; 23:235–50.
- Gautrin D, Newman-Taylor AJ, Nordman H, Malo. Controversies in epidemiology of occupational asthma. *Eur Respir J* 2003; 22:551–9.