

Research letters

An outbreak of asthma in a modern detergent factory

P Cullinan, J M Harris, A J Newman Taylor, A M Hole, M Jones, F Barnes, G Jolliffe

The striking decrease in the occurrence of protease-induced occupational asthma in the detergent industry has been attributed to enzyme encapsulation. We report an outbreak of asthma, at least equal in size to those reported in the 1960s, in a modern European factory which has exclusively used encapsulated enzymes. A survey revealed that enzyme sensitisation and work-related respiratory symptoms were positively correlated with airborne enzyme exposure. We suggest that encapsulation alone is insufficient to prevent enzyme-induced allergy and asthma.

Biological washing powders contain one or more enzymes to improve their cleaning properties. The introduction of the first type—a powdered protease—into detergent manufacture in the 1960s led to high rates of asthma among exposed workforces;¹ IgE-associated allergy also occurred among domestic users.² Consequently, enzymes used in detergent manufacture were encapsulated. Together with tighter engineering controls, this measure led to reductions in airborne enzyme concentrations and in the occurrence of asthma;³ there were no further reports of allergic reactions in consumers. Over the past decade, other encapsulated enzymes—amylases, cellulases, and lipases—have been added. Between 1994 and 1997, we saw seven employees of a modern detergent factory, six of whom were diagnosed with enzyme-induced asthma. We subsequently surveyed the entire workforce to determine the causes of this unusual outbreak.

The factory had manufactured detergents incorporating proteases since 1985; an amylase and cellulase were introduced in 1990 and 1996, respectively. All enzymes were encapsulated. Before the survey, we categorised each job by estimated airborne enzyme exposure. The highest exposures were judged to occur in certain packing tasks (refill packing), followed by other packing and production jobs, and then by distribution work. Employees in factory laboratories had lower exposures, and office staff the lowest. Factory engineers had high but intermittent exposures. In May, 1998, we collected occupational and health information, by questionnaire, from 350 employees (81% of the total workforce). We identified lower-respiratory disease by symptoms consistent with asthma, and upper-respiratory disease by symptoms of rhinitis or

ocular itching or watering. Symptoms were regarded as work-related if they started after first employment at the factory, improved during absences from work, or were provoked by being at work. 342 employees had skin-prick tests. We defined atopy as an immediate reaction (diameter ≥ 3 mm) to at least one common aeroallergen; those with one or more positive tests to 1 mg/mL detergent enzyme solutions were regarded as sensitised to enzyme. Results of skin-prick tests were in close agreement (95% for protease and cellulase and 98% for amylase) with those of specific IgE measurements in serum by radioallergosorbent assay. We calculated odds ratios for enzyme-sensitisation alone and in combination with work-related symptoms by current job title, using the risk among office staff as a point of reference. Results were adjusted for atopic status.

The average age was 34 years (range 18–60), 93 (27%) were women, 97 (28%) were atopic, and 149 (44%) were smokers. 90 (26%) employees had a positive skin-prick test to at least one detergent enzyme. Positive tests to amylase were more common than to protease or cellulase, but only 22 employees were sensitised to a single enzyme, 14 of whom were sensitised to amylase. There was a clear gradient in sensitisation across job titles (table). Work-related upper-respiratory symptoms, accompanied by enzyme sensitisation, were present in 68 (19%) employees; 54 (16%) had work-related lower-respiratory symptoms with enzyme sensitisation. Both again showed clear associations with job title. Age, sex, and smoking were not associated with any outcomes, and the odds ratio associated with atopy was only 1.59 (95% CI 0.95–2.66). 42 employees requested further investigation; 32 had, at survey, reported work-related chest symptoms and had a positive skin-prick test to at least one enzyme. We confirmed a diagnosis of occupational asthma in 24 (75%), and in a further four (40%) who had not reported such symptoms.

The survey identified a very high rate of enzyme-related sensitisation and asthma in this factory. Assuming the survey responders were representative, we estimate a total of 50 cases of occupational asthma in the current workforce, in addition to the six index cases. This rate is probably as high, or higher, than within the industry before encapsulation.¹ The patterns of

Job	Number with positive skin-prick tests				Odds ratio (95% CI) [†]	Number with work-related upper respiratory symptoms and positivity to at least one enzyme	Odds ratio (95% CI) [†]	Number with work-related chest symptoms	Odds ratio (95% CI) and positivity to at least one enzyme
	Amylase	Protease	Cellulase	At least one enzyme					
Packing (refill line) (n=22)	17 (77%)	17 (77%)	10 (45%)	17 (77%)	144.0 (15.6–1332.5)	14 (64%)	73.2 (8.4–640.1)	12 (55%)	50.5 (5.8–437.6)
Production (n=131)	38 (29%)	30 (23%)	28 (21%)	43 (33%)	21.0 (2.8–158.2)	33 (25%)	14.0 (1.8–106.1)	25 (19%)	10.1 (1.3–77.3)
Packing (other) (n=51)	11 (22%)	8 (16%)	7 (14%)	15 (29%)	17.7 (2.2–140.9)	10 (20%)	10.2 (1.3–83.9)	4 (8%)	3.6 (0.4–33.4)
Distribution (n=21)	4 (19%)	3 (14%)	1 (5%)	4 (19%)	10.3 (1.1–99.5)	3 (14%)	7.2 (0.7–74.4)	3 (14%)	7.3 (0.7–75.7)
Engineering (n=23)	6 (26%)	4 (17%)	3 (13%)	7 (30%)	19.4 (2.2–171.7)	6 (26%)	15.5 (1.7–139)	6 (25%)	15.8 (1.7–142)
Laboratory work (n=26)	2 (8%)	2 (8%)	2 (8%)	2 (9%)	3.6 (0.3–42.5)	0	..	2 (7%)	3.7 (0.3–42.8)
Other (n=26)	1 (4%)	1 (4%)	0	1 (4%)	1.8 (0.1–30.5)	1 (4%)	1.8 (0.1–30)	1 (4%)	1.8 (0.1–30.8)
Office work* (n=42)	1 (2%)	1 (2%)	0	1 (2%)	1.0	1 (2%)	1.0	1 (2%)	1.0
Total (n=342)	80 (23%)	66 (19%)	51 (15%)	90 (26%)	..	68 (19%)	..	54 (16%)	..

*Reference category. [†]Positivity for at least one enzyme adjusted for atopy.

Enzyme sensitisation alone and in combination with work-related symptoms by current job

risk by job title indicate that sensitisation and disease were determined mainly by level of exposure to airborne enzyme. Atopic status did not materially affect the relation between job title and any of the clinical outcomes.

Without reliable measurements over the entire period of relevance, we do not know the concentrations of airborne enzyme at which these diseases occurred. Reliable measurements of airborne protease concentrations ($n=1767$) were available for 1997. The geometric mean was 4.25 ng/m^3 (95% CI $4.12\text{--}4.38$), 5% were above industry guidelines of 15 ng/m^3 , and the highest value was 57 ng/m^3 . Of 74 employees who started work in or after 1997, seven had a positive response to one or more enzymes: five to protease, five to amylase, and four to cellulase. Two of these workers, both refill packers, reported work-related chest symptoms; five reported work-related upper-respiratory symptoms.

Given the frequency of multiple sensitisation, we cannot easily attribute particular risk to one enzyme type, although sensitisation to amylase was most common. Purchase records between 1992 and 1998 showed a progressive increase in the use of protease and amylase—the former at about twice the volume of the latter, suggesting that airborne amylase concentrations would consistently be half those of protease. Subsequent measurements tend to confirm this: in 1998, geometric mean enzyme concentrations were 2.4 ng/m^3 ($2.3\text{--}2.5$) and 1.5 ng/m^3 ($1.4\text{--}1.6$) for protease and amylase, respectively. Our findings support animal data which suggest that bacillary amylase is more allergenic than proteases, either innately or through potentiation.⁴

Enzyme encapsulation is commonly believed to have effectively eliminated occupational asthma in the detergent industry. These results, from a factory that exclusively used encapsulates, indicate that this assumption is not necessarily correct; during manufacture, encapsulates were probably crushed into a respirable size, a mechanism postulated as being responsible for a small outbreak of protease-sensitisation in France.⁵ Our findings have several important implications for this expanding industry: engineering improvements that occurred in parallel with the introduction of encapsulates have been critical in the reported reductions in protease-induced asthma; the introduction of new enzymes of uncertain allergenic potential should be accompanied by meticulous health surveillance; and the rate of occupational sensitisation described here, which is at least as high as that reported in the 1960s, suggests that the issue of sensitisation outside detergent manufacture and factory environments should be re-examined.

We thank Janet Helm, Jennifer Welch, Warwick Atkinson, Isobel Barnes, and Maria Barnes.

- 1 Flindt M. Pulmonary disease due to inhalation of derivatives of *Bacillus subtilis* containing proteolytic enzyme. *Lancet* 1969; **1**: 1177–81.
- 2 Belin L, Hoborn J, Falsen E, Andre J. Enzyme sensitisation in consumers of enzyme containing washing powder. *Lancet* 1970; **2**: 1153–57.
- 3 Juniper CP, How MJ, Goodwin BFJ, 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.
- 4 Sarlo K, Ritz HL, Fletcher R, Schrotel KR, Clark ED. Proteolytic detergent enzymes enhance the allergic antibody responses of guinea pigs to nonproteolytic detergent enzymes in a mixture: implications for occupational exposure. *J Allergy Clin Immunol* 1997; **100**: 480–87.
- 5 Perdu D, Lavaud F, Cossart C, et al. Enzymes des lessives: le risque de sensibilisation professionnelle a-t-il disparu? *Rev Mal Resp* 1992; **9**: 443–48.

Department of Occupational and Environmental Medicine, Imperial College School of Medicine, London SW3 6LR, UK (P Cullinan MD, J M Harris MSc, Prof A J Newman Taylor FRCP, A M Hole, M Jones PhD, F Barnes PhD, G Jolliffe MRCP)

Correspondence to: Dr P Cullinan
(e-mail: p.cullinan@ic.ac.uk)

Detection of house-dust-mite allergen in amniotic fluid and umbilical-cord blood

Judith A Holloway, John O Warner, Gillian H S Vance, Norma D Diaper, Jill A Warner, Catherine A Jones

Mononuclear cells in umbilical-cord blood display allergen-specific reactivity, but how allergen exposure occurs in utero is unknown. We investigated the presence of a common inhalant allergen (Der p 1), to which mothers are exposed throughout pregnancy, by ELISA in matched maternal blood and amniotic fluid samples at 16–17 weeks of gestation, and in matched maternal and umbilical-cord blood at term (≥ 37 weeks of gestation). Der p 1 was detectable in 24 of 43 amniotic fluid samples where it was also present in maternal blood, and in 15 of 24 cord-plasma samples at significantly higher concentrations than in the maternal plasma ($p=0.022$). The detection of Der p 1 in the amniotic fluid and the fetal circulation provides direct evidence of transamniotic and transplacental allergen exposure.

Allergen-specific reactivity by umbilical-cord-blood mononuclear cells is evident at birth and as early as 23 weeks of gestation.¹ Thus, fetal exposure to allergens, both dietary and inhalant, must occur from at least midway through gestation. Cotinine—the main metabolite of nicotine, HIV-1 p24, and inulin have all been found in amniotic fluid, fetal blood, and fetal urine when the mother has been exposed to these substances, and dietary antigens have been shown in the fetal environment in rats.² However, the presence of allergen in the human fetal environment has not been shown. Therefore, we investigated whether an allergen to which mothers are exposed throughout pregnancy—the house-dust-mite antigen Der p 1—is detectable in the maternal circulation and matched amniotic-fluid or fetal-blood samples.

Matched samples of maternal blood and amniotic fluid were collected from women undergoing diagnostic amniocentesis (16–17 weeks' gestation). Only women who proceeded with a healthy pregnancy were included in this study. Matched samples of maternal blood and umbilical-cord blood were also collected from a different group of women after delivery at term (≥ 37 weeks). These women were selected from a larger series ($n=100$) with high concentrations of house-dust-mite antigen in their home dust. All samples were collected with the mother's consent and the approval of the Southampton and SW Hants Joint Research Ethics Committee.

Concentrations of Der p 1 were measured by ELISA with the Dermatophagoides group 1 allergen kit (Indoor Biotechnologies, Chester, UK). The detection limit was $0.65 \text{ } \mu\text{g/L}$ Der p 1. The assay was regarded as specific for Der p 1 since there was no signal with different antigens or with biotinylated secondary antibody to different antigens, and since the signal could be eliminated by heating the plasma samples at 71°C for 15 min (this treatment did not destroy the total IgG signal as measured by an in-house ELISA). Statistical analysis was done with Spearman's r correlation coefficient to calculate the relation between maternal and fetal samples, and Wilcoxon's signed-rank test for the matched data (SPSS version 10.0).

Der p 1 was detectable in 43 of 206 (21%) samples of maternal blood from women undergoing amniocentesis at 16–17 weeks' gestation. Amniotic fluid from women with detectable circulating concentrations was then tested alongside retested maternal plasma. Der p 1 was detectable in the amniotic fluid of 24 of 43 (56%) mothers, all of whom had Der p 1 concentrations of greater than $5 \text{ } \mu\text{g/L}$ in their plasma (figure 1). Der p 1 concentrations in the amniotic fluid increased with circulating concentrations, although this relation was not significant (Spearman's $r=0.193$, $p=0.108$).