

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.

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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 $0.65 \mu\text{g/L}$ 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 $5 \mu\text{g/L}$ 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 $r=0.193$, $p=0.108$).

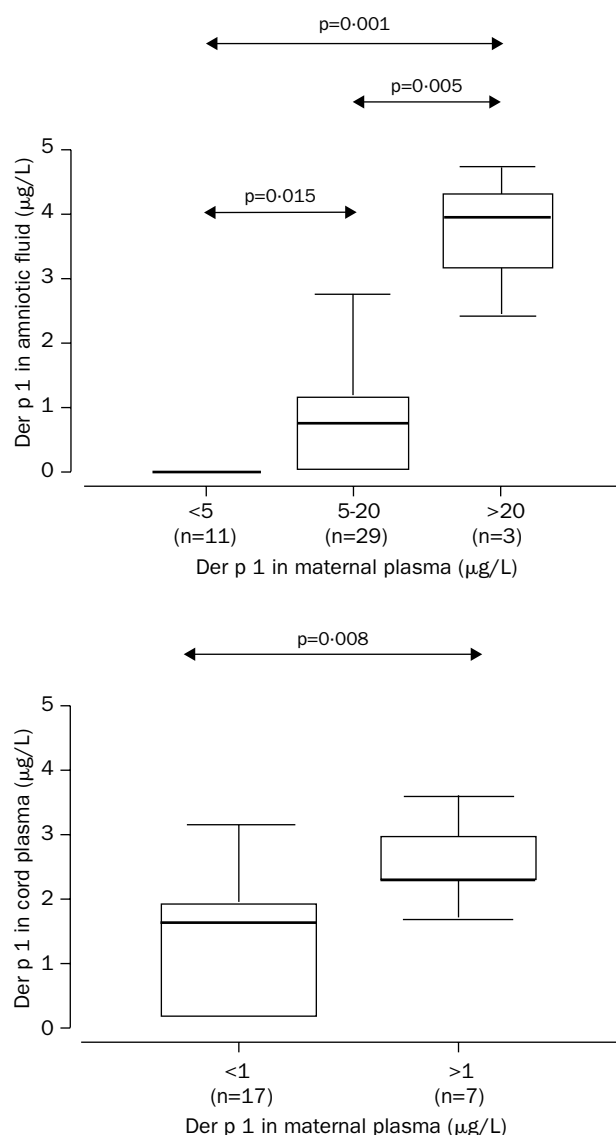


Figure 1: Relation between concentrations of Der p 1 in maternal and fetal samples (Spearman's r correlation)

Concentrations of Der p 1 in matched amniotic fluid and maternal plasma at 16–17 weeks of gestation (top), and in matched umbilical-cord plasma and maternal plasma at ≥ 37 weeks of gestation (bottom).

Matching umbilical-cord and maternal-plasma samples ($n=24$) collected from term deliveries were also tested for Der p 1. Six of 24 (25 %) maternal samples had concentrations above the detection limit, whereas 15 of 24 (63%) of their neonates had detectable concentrations. The concentrations of Der p 1 in the cord plasma were higher in samples from mothers with detectable than undetectable Der p 1 ($p=0.008$, figure 1).

There was significantly less Der p 1 in the amniotic fluid than in the matched plasma sample ($p=0.001$, $n=43$, figure 2). When detectable, the median concentration in the amniotic fluid was 9% of maternal concentrations. In contrast, Der p 1 concentrations in fetal plasma at term were higher than in the matched maternal sample ($p=0.022$, figure 2). The median concentration of Der p 1 in fetal plasma was 110% of the maternal concentrations when detectable ($n=7$).

This study shows that allergen is present in the fetal environment, being found in the amniotic fluid at 16 weeks of gestation and in the fetal circulation at term. This finding suggests that there are at least two routes of allergen transfer to the conceptus: transamniotic and transplacental. In the

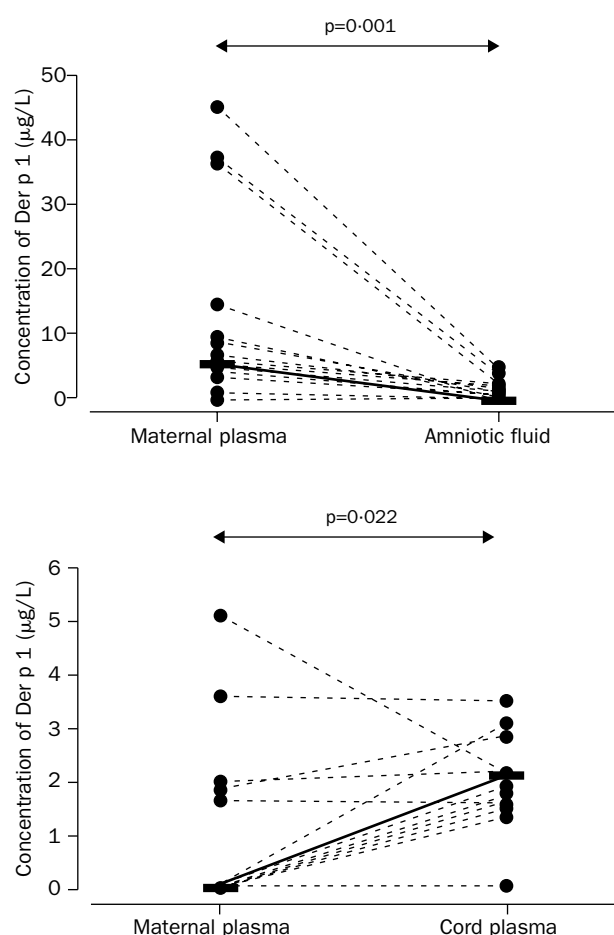


Figure 2: Relation between concentrations of Der p 1 in maternal and fetal samples (Wilcoxon's signed-rank test)

Concentrations of Der p 1 in matched pairs of amniotic fluid and maternal plasma at 16–17 weeks of gestation (top), and in umbilical-cord plasma and maternal plasma at ≥ 37 weeks of gestation (bottom).

amniotic fluid, allergen will gain access to the gut-associated lymphoid tissue, where Peyer's patches are structurally mature by 19 weeks of gestation, with the M cells that sample the luminal contents already in place.³ Allergen in the fetal circulation will also encounter immunocompetent antigen-presenting cells. Other molecules such as inulin, interleukin 6, tumour necrosis factor α , and interleukin 1 α are known to pass to the fetus via the transamniotic route. Ovalbumin (which has a molecular weight of 45 kDa) can be detected in the amniotic fluid of rats after maternal ingestion.² Therefore, Der p 1—a protein of 25 kDa—could be expected to cross the fetal membranes from the maternal vasculature of the decidua into the amniotic fluid.

The presence of Der p 1 in the umbilical-cord plasma infers that Der p 1 can also cross the placenta. Der p 1 concentrations were higher in the fetal circulation than the maternal circulation. There is evidence that other molecules such as cotinine and inulin can accumulate in fetal compartments—including the fetal circulation—at higher concentrations than the mother, but these are smaller molecules than Der p 1. Also, it has been shown that both Bet v1 and β -lactoglobulin can traverse the human placenta in an in-vitro perfusion model, which is increased by human immunoglobulin.⁴ Since IgG is actively transported to the fetus across the placenta from the maternal vasculature, Der p 1 might be carried in complexes with IgG. The acquisition via the transamniotic route may also contribute to the concentration effect, since by term the fetus is swallowing

700 mL of amniotic fluid per day and is able to absorb 10% of the macromolecules within the fluid.

The presence of allergen and the initiation of antigen-specific responses via the gut in the second trimester could be modified in the third trimester by circulating allergen IgG complexes. The possibility that maternal antigen-specific IgG protects against the development of IgE to the same allergen has been shown in human beings.⁵ Further modification of the antigen-specific immune response could also occur postnatally with the acquisition of and response to the commensal gut flora. Thus these two routes of allergen exposure in utero are critical to the establishment of an immune response and could influence the development of allergic diseases such as asthma. A greater understanding of the initiation and modification of allergen-specific responses before and after birth is needed before trials of any intervention strategies can be considered.

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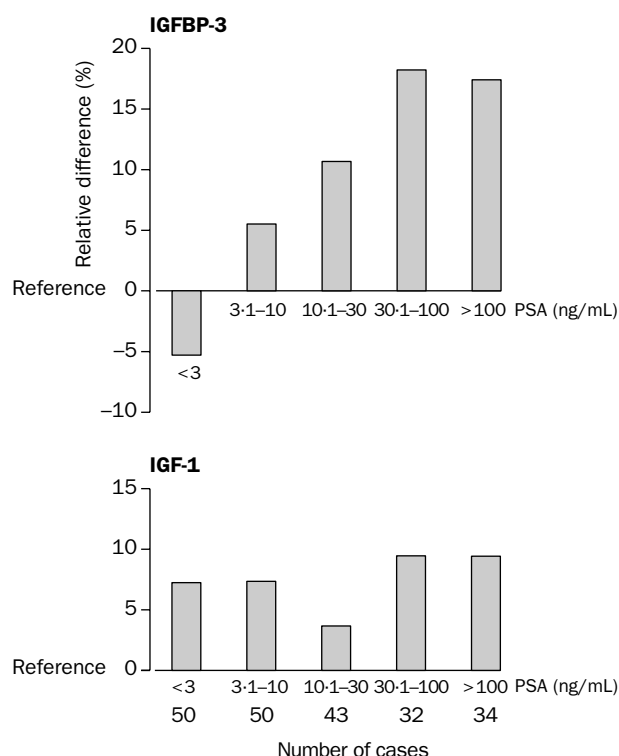
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Can measurements of IGF-1 and IGFBP-3 improve the sensitivity of prostate-cancer screening?

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Further well designed studies are urgently needed to clarify if use of prostate-specific antigen (PSA) as a diagnostic test for prostate cancer can be improved by incorporating measurements of serum insulin-like growth factor 1 (IGF-1) and insulin-like growth factor binding-protein 3 (IGFBP-3), and if these measurements might also identify men at higher risk.

Epidemiological studies indicate that higher concentrations of circulating insulin-like growth factor 1 (IGF-1) are associated with a greater risk of prostate cancer.^{1,2} Insulin-like growth factor binding-protein 3 (IGFBP-3) serum concentrations were inversely associated with prostate cancer risk in a prospective study in which serum samples were collected before onset of disease,¹ whereas in a case-control study serum samples were collected at the time of disease diagnosis and no such association was evident.² Since prostate-specific antigen (PSA) is an IGFBP-3 protease, which greatly lowers



Relative differences between mean serum concentrations of IGF-1 and IGFBP-3 in newly diagnosed patients with prostate cancer grouped according to PSA concentrations

the affinity of IGFBP-3 for IGF-1,³ we postulated that the apparent discrepancy between results from these two studies might be explained by differences in PSA concentrations.

In a population-based case-control study, we collected blood samples for analyses of IGF-1, IGFBP-3, and PSA from 209 patients with a newly diagnosed, untreated prostate cancer and from 221 healthy men matched for age.² IGF-1 and IGFBP-3 concentrations in serum samples (stored at -70°C) were analysed using immunoradiometric assays (Diagnostic Systems Laboratories, Webster, TX, USA). In unconditional logistic regression models, we used 141 controls with $\text{PSA} \leq 3 \text{ ng/mL}$ as a reference group.

Among patients with prostate cancer, mean IGF-1 concentrations were increased compared with controls (158.4 [SD 53.8] vs 147.7 [48.5] ng/mL, respectively), but did not differ appreciably at different serum concentrations of PSA ($p=0.77$; figure). Concentrations of IGFBP-3, however, increased substantially with higher PSA concentrations ($p=0.004$). Patients with prostate cancer and $\text{PSA} \leq 3 \text{ ng/mL}$ had lower mean IGFBP-3 concentrations than controls (2345 [801] vs 2475 [745] ng/mL, respectively).

Among patients with a normal PSA ($\leq 3 \text{ ng/mL}$), we recorded a 2.5-fold increased risk of prostate cancer per 100 ng/mL increase in IGF-1 concentration and an inverse association with IGFBP-3 (table). Results in this subgroup with low PSA concentrations are in agreement with results based on prospectively collected blood.¹ IGFBP-3 binds IGF-1, and thus prevents IGF-1 from binding to its receptors at the prostate cell surface. IGFBP-3 has also been reported to have an IGF-independent apoptotic function. By contrast, we recorded no association between IGF-1 and prostate cancer among patients with raised PSA ($>10 \text{ ng/mL}$). Decreased expression of type-1 IGF receptor mRNA encoding the cognate receptor for IGF-1, seen in advanced disease,⁴ might explain this finding.