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Environmental exposure to *Aspergillus fumigatus* allergen (*Asp f* I)

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Summary

Asp f I is a major allergen produced by the mycelia of *Aspergillus fumigatus*. It is not present in spores and can be used as a specific marker for the detection of germination of this fungus. We investigated the domestic and outdoor concentration of *Asp f* I in Poole, U.K. and Charlottesville, VA, U.S.A. *Asp f* I was undetectable in 95% (281/296) of house dust extracts and present at low levels (<0·17 μg/g of sieved dust, mean 0·038 μg/g) in the remainder. In contrast, *Asp f* I could be detected in 65% (15/23) of cultures of house dust, suggesting the presence of viable, but ungerminated, *A. fumigatus* in the majority of homes. *Asp f* I was detectable in 80% (28/35) of extracts of leaves and compost, but present in these outdoor samples at low levels (<0·11 μg/g, mean 0·27 μg/g). Air sampling for *Asp f* I was undertaken before and after vigorous disturbances at indoor ($n=5$) and outdoor ($n=6$) sites. Airborne *Asp f* I was not detected in domestic samples or in undisturbed outdoor samples. Following disturbance it could be measured in outdoor samples (range 7·6–29 ng/m^3). The results suggest that while exposure to *A. fumigatus* is common, exposure to *Asp f* I and germinating *A. fumigatus* is uncommon. It is probable that those individuals who develop antibody responses to *Asp f* I have been exposed to *A. fumigatus* which has germinated in their respiratory tract.

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Introduction

The measurement of exposure to aero-allergens can be undertaken either by identifying and counting the particles on which they are transported, e.g. pollen grains, or by direct immunoassay of the allergen, e.g. *Fel d* I for cat or *Der p* for house dust mite. However, the measurement of exposure to fungal allergens has proved rather more difficult. This is because: (i) it is difficult to distinguish some spores (e.g. *Penicillium* and *Aspergillus* spores are morphologically identical); (ii) only a few fungal allergens have been characterized [1,2]; and (iii) some fungi of medical importance are capable of germinating on mucosal surfaces and acting as an 'intrinsic' or internal source of allergen [3–5]. In addition, while specific allergens are present on spores from some fungi, e.g. *Alternaria* [7,8] and *Basidiomycetes* [9–12], they are not found on spores in many others, being expressed only following germination, and their expression can vary depending on culture conditions [1,13]; indeed, all currently available fungal skin test reagents are derived from cultured sources. This is in marked contrast to pollen, mite or cat allergens which are always present on the particles on which they are transported and these have no ability to germinate in man or to produce more allergen.

Aspergillus species are widely distributed, usually associated with decaying vegetable matter, and are human pathogens, especially in individuals with pre-existing respiratory disease or who are immuno-compromised. Spores (conidia) have been detected in house dust, compost, indoor and outdoor air samples, with an increased frequency during the winter and spring [14–16]. Increased exposure to *A. fumigatus* spores has been associated with clinical disease in animals exposed to extreme levels and immunocompromised individuals; however, increased exposure has not been associated with disease development in normal individuals [14,17–20].

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Asp f I is a major fungal allergen produced by the mycelia of *A. fumigatus*. It is an 18 kD protein, belonging to the mitogillin family of cytotoxins and has been fully sequenced [21,22]. *Asp f* I is not present on spores, but is detectable within 12 hr of spore germination. As it is not produced by other *Aspergillus* species its presence can be used as a specific marker of *A. fumigatus* germination [23]. The clinical significance of *Asp f* I is underscored by the recent report that the majority of children with cystic fibrosis develop IgG antibodies to *Asp f* I by the age of 3 years [24]. In addition, it has been shown that the level of *A. fumigatus*-specific IgG antibodies correlated with disease activity in children and adults with cystic fibrosis [25]. We report here investigations of the relative importance of extrinsic sources of *Asp f* I, using an ELISA to measure exposure to this fungal allergen.

Methods

Quantification of Asp f *I*

Asp f I was measured by two-site enzyme-linked immunoassay using a monoclonal antibody anti-*Asp f* I for antigen capture (clone 4A6) and a rabbit anti-*Asp f* I polyclonal antibody for detection, with purified *Asp f* I as standard (0·2–270 ng/ml). Results were considered positive if three times greater than the background level (optical density ~0·15), this represented a detection limit of ~0·8 ng *Asp f* I/ml [23].

The amount of *Asp f* I produced from cultures of *A. fumigatus* spores was assessed. *Aspergillus fumigatus* was obtained from a patient with *A. fumigatus* sinusitis. Stock cultures were grown for 10 days on Sabouraud's dextrose agar at 37°C. Spores were harvested by inverting the petri dishes over paper and gently knocking the dishes. Spores (100 mg) were mixed with 2 ml phosphate-buffered saline and 0·05% Tween 20 pH 8·0 (PBS-Tween) by rotating overnight at 4°C. The hydrophobicity of the spores made complete mixing difficult. Dilutions of this stock solution were made in PBS-Tween and the concentration of spores estimated by counting in a Neubauer haemocytometer. Serial dilutions of these solutions (in volumes of 1–100 μl) were added to a sterile 100 mm petri dish containing 20 ml Sabouraud's dextrose broth (Difco Laboratories, Detroit, MI, U.S.A.) and cultured at rest for 2 days at 37°C. The culture fluid below the mycelial surface mat was then mixed by repeated pipetting and the *Asp f* I levels assayed. In addition, 500 *A. fumigatus* spores were grown in stationary cultures whose initial pH was adjusted at increments of approximately 1, between pH 2·8 and 9, by addition of 12 N HCl or 2 N NaOH. Serial spore dilutions were also added to 2 ml Sabouraud's dextrose broth and rotated for 2 days at 37°C. In addition, *A. fumigatus* spores were grown in rotary cultures to which 1·0 mm glass beads had been added. The culture fluid was then spun down and the *Asp f* I level measured in the supernatant.

Measurement of Asp f *I in domestic and outdoor samples*

House dust was collected using a hand-held vacuum cleaner from the mattress ($n=92$), bedroom floor ($n=91$) and living room ($n=113$) of houses in Charlottesville, VA, U.S.A. ($n=66$) and Poole, Dorset, U.K. ($n=230$) during the spring of 1988, autumn of 1989 and spring of 1990. The dust was sieved and stored at 4°C until assayed. Sieved dust (100 mg) was vortexed with 2 ml PBS-Tween, rotated overnight at 4°C, centrifuged and the supernatant assayed. Samples which were included from a previous study had been extracted in borate-buffered saline and 0·05% Tween 20 pH 8·0 and stored at −20°C. House dust (100 mg) was mixed in a sterile 100 mm petri dish containing 20 ml Sabouraud's dextrose broth and cultured at rest for 2 days at 37°C. The culture fluid was then mixed and the *Asp f* I levels measured. In addition, a second 100 mg of house dust, from 15 samples in which *Asp f* I had not been detected either in aqueous extracts or following culture, were added to 2 ml of Sabouraud's dextrose broth and 'spiked' with an inoculum of ~100 *A. fumigatus* spores and cultured at 37°C for 2 days with rotation or at rest ($n=10$). Leaves and decaying vegetation were collected from random sites ($n=35$) in Charlottesville, VA in the autumn of 1991. Samples (500 mg) were vortexed with 2 ml PBS-Tween, rotated overnight at 4°C, centrifuged and the supernatant assayed.

Air sampling

Indoor air samples were obtained from five houses in Charlottesville, VA. Undisturbed air was sampled at 17·5 l/min for 30 min using a glass fibre filter (Millipore Corp., Bedford, MA, U.S.A.) attached to a vacuum pump to collect total airborne particles. Air was then resampled for 30 min while the carpet was vacuumed using a Shop-Vac 6 gallon vacuum cleaner from which the filters had been removed. This caused considerable quantities of house dust to become airborne. The glass fibre filters were eluted in 1 ml PBS-Tween by rotating overnight in a 3 ml plastic syringe at 4°C and then collected by compressing the filters. During the autumn of 1991 similar outdoor air sampling for *Asp f* I was undertaken. Sites were selected in gardens and leafy glades, near compost, or leaf piles, and sampled on calm days. The air sampler was positioned 50 cm above the ground and 1 m downwind of these sites. Undisturbed air was sampled for 15 min at a flow rate of 50 l/min. The air was then re-sampled while the leaves were vigorously disturbed with a rake for 15 min. In

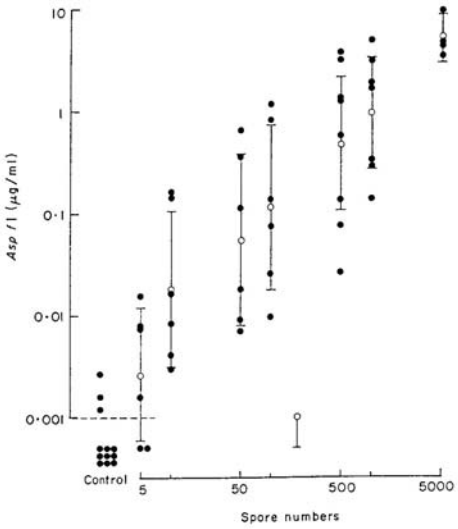

Fig. 1. Effect of the number of *Aspergillus fumigatus* spores cultured on the quantity of *Asp f* I produced in stationary culture at 37°C for 48 hr. ○, Geometric mean. Error bars represent 95% confidence interval.

addition to measuring the *Asp f* I levels in the eluate, the eluted filters were added to a petri dish containing 20 ml Sabouraud's dextrose broth and cultured at rest for 5 days at 37°C. The culture fluid was then mixed and the *Asp f* I levels assayed.

Results

The output of *Asp f* I in stationary culture was dependent on the concentration of spores incubated (Fig. 1). When more than 5000 spores were added to the petri dish, the surface colonies became confluent and the level of *Asp f* I reached a plateau. *Aspergillus fumigatus* spores formed colonies between an initial pH of 3·2 and 8 in pure culture. *Asp f* I production was highest at pH 3·2 and declined with increasing pH; at pH 8 there was a ~98% reduction of *Asp f* I levels (Table 1). The pH of stationary cultures immediately following inoculation with 100 mg of house dust ranged from 5·4 to 6·7. In the rotated samples of pure cultures the output of *Asp f* I peaked at low spore numbers (~25 ml) and then decreased with increasing concentrations (Fig. 2). The colonies formed mycelial balls; at low concentrations these were larger and more luxuriant than those at higher concentrations. If glass beads were added to the rotated samples, this completely inhibited fungal

Table 1. *Asp f* I levels from 500 *Aspergillus fumigatus* spores

Initial pH*	Final pH†	Colony description	*Asp f* I (ng/ml)
2·8	2·8	No growth	<1
3·2	3·2	Small colonies covering 5/8 plate	2812
4·2	4·6	Luxuriant covering 7/8 plate	1312
5·5	5·0	Luxuriant covering 7/8 plate	883
6·5	5·5	Luxuriant covering 7/8 plate	256
7·0	4·5	Shrivelled colonies covering 1/16 plate	22
8·0	6·1	Small colonies covering 3/4 plate	56
9·0	4·7	Shrivelled aggregates, no colonies	<1

* The pH of Sabouraud's dextrose broth was 5.6, this was adjusted with HCl or NaOH.
† After 48 hr of stationary culture at 37°C.

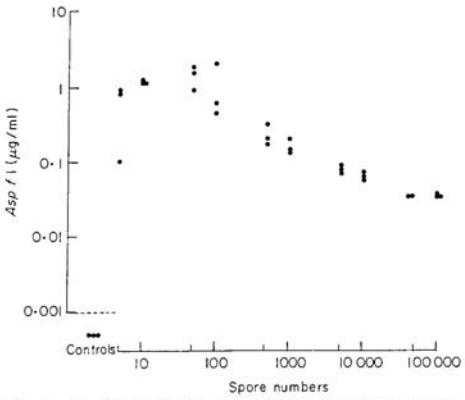

Fig. 2. *Asp f* I production by rotating cultures containing increasing numbers of *Aspergillus fumigatus* spores. Values given are the concentration found in the medium.

growth and *Asp f* I production. When house dust, from which *A. fumigatus* had not been cultured, was 'spiked' with *A. fumigatus* spores, colonies failed to grow and *Asp f* I was not detectable in stationary or rotated cultures. This suggests inhibition of *A. fumigatus* growth by house dust, either through a purely mechanical effect in rotated cultures or a direct inhibitory action.

Asp f I was rarely detected in extracts made from house dust. In 5% (15/296) of extracts *Asp f* I was detectable; the highest level measured was 0·16 μg/g from dust obtained

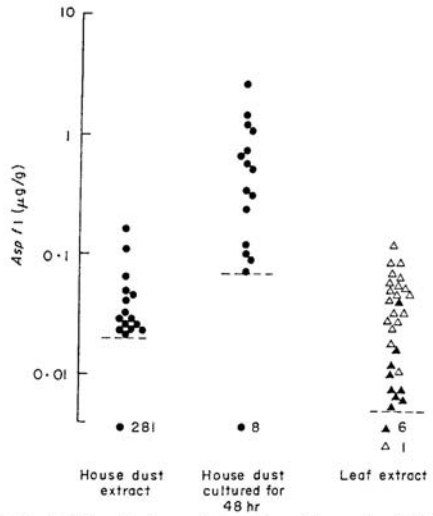

Fig. 3. *Asp f* I levels in house dust, cultures of house dust (48 hr, stationary cultures, 37°C) and outdoor samples (▲, outdoor samples collected during a dry period, △, outdoor samples collected 96 hr after heavy rain). The dashed line represents the limit of detection of the assay, which varied due to the different weights and volumes assayed.

from a mattress and the mean level was 0·038 μg/g. The collection site did not influence the *Asp f* I level. After culturing house dust, *Asp f* I was measurable in 65% (15/23) of samples demonstrating the presence of viable *A. fumigatus* (Fig. 3). *Asp f* I was detectable at low levels in extracts of most outdoor samples. The highest level measured was 0·12 μg/g and the mean of positive values was 0·27 μg/g. The specimens collected following a prolonged dry period had a lower mean value of allergen (0·009 μg/g) than those obtained 4 days after a heavy rainfall (0·044 μg/g). *Asp f* I could also be detected in puddles pigmented by the run-off from leaves at levels of 1 ng/ml.

Domestic airborne measurements failed to detect *Asp f* I, either before or after vigorous disturbance (Table 2). Outdoor airborne measurements also failed to detect *Asp f* I in undisturbed air. In contrast, following vigorous disturbances the allergen could be detected at a mean level of 14 ng/m³ (range 7·6–29 ng/m³). In addition, 5 day culture of the eluted filter resulted in heavy growth of *A. fumigatus*. The level of *Asp f* I measured in these cultures and the level measured in the eluted samples were not significantly correlated ($P = 0·06$).

Table 2. Air sampling for *Asp f* I (ng/m³)*

Site	Undisturbed	Following disturbance†
Indoor		
House A Carpeted basement	<7·2	<7·2
House B Carpeted basement	<7·2	<7·2
House C Carpeted basement	<7·2	<7·2
House D Carpeted basement	<7·2	<7·2
House E Uncarpeted first floor	<7·2	<7·2
Outdoor		
Ground surface	<7·2	—
1·5 feet above ground level	<7·2	—
4 feet above ground level	<1·6	—
Compost, lower layers	<7·2	7·6
Compost	<7·2	8
Compost	<7·2	16
Leaves	<7·2	17
Leaves	<7·2	17
Leaves	<7·2	29

* Air samples for 60 min.
† Indoor disturbance was with a vacuum cleaner with no bag or filters, outdoor disturbance was with a rake.

Discussion

The results show that a major *A. fumigatus* allergen is rarely found in extracts of house dust obtained from Virginia and Southern England, although viable *A. fumigatus* is present. This suggests that *A. fumigatus* generally does not germinate in house dust. This observation is in keeping with the numerous reports demonstrating the growth of *Aspergillus* species from house dust and Van Bronswijk's observation that 'as far as humidity conditions are concerned, house dust must be considered as a borderline habitat, even for the most dry resistant fungi' [26]. The relative humidity found on floors ranges from 30 to 90% with an average value of ~65%. The highest values are found in damp basements, although when ventilation is poor local pockets with even higher relative humidities can exist in other areas of the house. While *A. fumigatus* can germinate at a relative humidity of ~80%, optimum growth occurs at a relative humidity of 98% [27]. Other factors which limit the growth of *A. fumigatus* spores in the domestic environment are the relatively cool temperatures (optimal growth occurs at 37°C, although this fungus can grow at a wide range of temperatures, 10–55°C) and their vulnerability to ultraviolet damage, since they lack heavy pigmentation. *Aspergillus fumigatus* could, however, be cultured from house dust and its presence and specificity identified by

measuring *Asp f* I using ELISA techniques. Caution must be used in extrapolating the results of *Asp f* I production from pure cultures to that from cultures of house dust in estimating the number of viable spores. Culture conditions were critical and it also appeared that growth was inhibited by house dust. In addition, no attempt was made to quantify the percentage germination of spores in pure culture, which may vary greatly depending on their source and the conditions of storage. These factors limit the usefulness of this method to that of a semi-quantitative assay. The poor growth in rotating cultures may be partly explained by the fact that fungi are aerobic saprophytes which depend on the absorption of enzymatically degraded food sources. The reason for the sharp decrease in *Asp f* I production per spore observed in rotating cultures is not clear. However, there was a parallel decrease in mycelial growth, and further experiments are being carried out to understand this phenomenon. Culture in closed containers and disturbances of this local milieu, either by motion, mechanical disruption or alterations of pH may also impair growth.

Domestic aero-allergens of known importance such as house dust mite, cat and cockroach can be detected in dust at concentrations up to 200 μg/g, approximately 1000 times higher than the highest level of *Asp f* I detected. Indeed, the levels of *Asp f* I measured were at the limit of detection of this monoclonal-based assay. Compared with the levels of other domestic allergens, *A. fumigatus* cannot be regarded as an important indoor allergen in the majority of homes. However, if the level of humidity is excessive, *A. fumigatus*, which is present, could germinate. Indeed, in tight damp houses with visible mould growth, airborne *A. fumigatus* allergen has been detected using RAST inhibition (Dr Mark Swanson, personal communication). By contrast, *Asp f* I could be detected more frequently in outdoor samples, provided there was adequate moisture to ensure germination, albeit in low concentrations. The outdoor presence of *Asp f* I was confirmed by air sampling, although vigorous disturbance was necessary to make the allergen airborne and the filters contained considerable particular matter. Not only did *Asp f* I become airborne, but so did viable *A. fumigatus*. The levels were then comparable with indoor airborne levels of mite allergen obtained following disturbance, and an order of magnitude lower then those recorded for cat allergen. The sampling period during November has been shown to be a period, both in the U.K. and the U.S.; when airborne spore counts are elevated, so these results may represent the highest level of *Asp f* I to be found during the year.

These results suggest that most people are exposed to very low levels of *A. fumigatus* allergen. In general, population studies of the prevalence of IgE antibodies, as judged by skin prick testing to *A. fumigatus*, give results of 0–1% increasing to 20% in allergic subsets. In marked contrast, among patients with cystic fibrosis, the prevalence of IgE antibodies has been reported to be between 35% and 56% [28–30]. Specific IgG antibodies to *A. fumigatus*, which have been shown to be a better correlate of disease progression [25], have been reported in 1% of controls and 35% of children with cystic fibrosis when measured by precipitation techniques [31]. If more sensitive techniques are used specific IgG antibodies to *Asp f* I have been demonstrated in 6% of control children and 98% of children with cystic fibrosis [24]. These results could be explained by differences in exposure; however this appears unlikely. An alternative explanation is that any allergen that is inhaled is retained for a longer period due to the impaired muco-ciliary clearance, and is therefore more likely to induce an immune response. This is not supported by the studies of the prevalence of sensitization to other aero-allergens which was lower among children with cystic fibrosis than that found in either a population selected from an allergy clinic [29] or a normal population [30]. A more plausible explanation is that ungerminated spores enter the lung, or sinuses, have sufficient time to germinate in an environment which provides ideal conditions of temperature and humidity, and act as an 'intrinsic' or internal source of allergen [31]. It is known that *A. fumigatus* is not only a source of a cytotoxin (*Asp f* I), but also of gliotoxin which inhibits phagocytosis and induces apoptosis in macrophages [32] and inhibits T-cell proliferation [33]. The presence of such an organism in the lungs may itself be detrimental to respiratory function [34] and calls for a re-evaluation of the contention that colonization by *A. fumigatus* can be harmless. Indeed, given the efficacy of topical [35] and oral anti-fungal agents, these may have a therapeutic role in slowing the deterioration of respiratory function in children with cystic fibrosis.

Measurement of *Asp f* I has provided a novel marker for detection of *A. fumigatus*. It has also enabled the measurement of exposure to a fungal allergen to be undertaken. The results confirm that while it is essentially an outdoor organism, *A. fumigatus* can also be detected in house dust, although conditions in most houses are too dry for its germination. Overall, the results suggest that significant exposure to this fungal antigen is most likely to occur from growth of *A. fumigatus* on mucosal surfaces.

Acknowledgments

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References

1 Bush RK. Aerobiology of pollen and fungal allergens. J Allergy Clin Immunol 1989; 84:1120–3.

2 Marsh DG, Goodfriend L, King TP, Lowenstein H, Platts-Mills TAE. Allergen nomenclature [published erratum J Allergy Clin Immunol 1988; 81:918]. J Allergy Clin Immunol 1988; 90:639–45.

3 Gourley DS, Whisman BA, Jorgensen NL, Martin ME, Reid MJ. Allergic *Bipolaris* sinusitis: clinical and immunopathologic characteristics. J Allergy Clin Immunol 1990; 85:583–91.

4 Ward GW Jr, Karlsson G, Rose G, Platts-Mills TAE. Trichophyton asthma: sensitization of bronchi and upper airways to Dermatophyte antigen. Lancet 1989; 1:859–62.

5 Bardana EJ Jr. The clinical spectrum of aspergillosis. Part 2: Classification and description of saprophytic, allergic and invasive variants of human disease. CRC Crit Rev Clin Lab Sci 1981; 13:85–159.

6 Slavin RG, Bedrossian CW, Hutcheson PS, Pittman S, Salinas-Madrigal L, Tsai CC, Gleich GJ. A pathologic study of allergic bronchopulmonary aspergillosis. J Allergy Clin Immunol 1988; 81:718–25.

7 Agarwal MK, Swanson MC, Reed CE, Yunginger JW. Immunochemical quantification of airborne short ragweed, *Alternaria*, antigen E, and *Alt*-I allergens: a two year propsective study. J Allergy Clin Immunol 1983; 72:40–5.

8 O'Hallaren MT, Yunginger JW, Offord KP, Somers MJ, O'Connell EJ, Ballard DJ, Sachs MI. Exposure to an aeroallergen as a possible precipitating factor in respiratory arrest in young patients with asthma. New Engl J Med 1991; 324:359–63.

9 De Zubiria A, Horner WE, Lehrer SB. Evidence for cross-reactive allergens among basidiomycetes: Immunoprint-inhibition studies. J Allergy Clin Immunol 1990; 86:26–33.

10 Lopez M, Voigtlander JR, Lehrer SB, Salvaggio JE. Bronchoprovocation studies in basidiospore-sensitive allergic subjects with asthma. J Allergy Clin Immunol 1989; 84:242–6.

11 Herxheimer H, Hyde HA, Williams DA. Allergic asthma caused by fungal spores. Lancet 1966; i:572–3.

12 Nakazawa T, Tochigi T. Hypersensitivity pneumonitis due to mushroom spores (*Pholiota nameko*). Chest 1989; 95:1149–51.

13 Kauffman HF, de Vries K. Antibodies against *Aspergillus fumigatus*. I. Standardization of the antigenic composition. Int Arch Allergy Appl Immunol 1980; 62:252–64.

14 Vernon DRH, Allan F. Environmental factors in allergic bronchopulmonary aspergillosis. Clin Allergy 1980; 10:217–27.

15 Hudson HJ. Aspergilli in the air-spora at Cambridge. Trans Br Mycol Soc 1969; 52:153–9.

16 Davies RR. Viable moulds in house dust. Trans Br Mycol Soc 1960; 43:617–30.

17 Raper KB, Fennell DI. The genus Aspergillus. Baltimore: Williams & Wilkins, 1965:92–7.

18 Dewhurst AG, Cooper MJ, Khan SM, Pallett AP, Dathan JR. Invasive aspergillosis in immunosuppressed patients: potential hazard of hospital building work. Br Med J 1990; 301:802–4.

19 Seaton A. Invasive pulmonary aspergillosis in non-immunocompromised patients. Br Med J 1991; 302:906.

20 Kurup VP, Kumar A. Immunodiagnosis of aspergillosis. Clin Micro Reviews 1991; 4:439–56.

21 Arruda LK, Platts-Mills TAE, Fox JW, Chapman MD. *Aspergillus fumigatus* allergen I, a major IgE-binding protein, is a member of the mitogillin family of cytotoxins. J Exp Med 1990; 172:1529–32.

22 Lamy B, Moutaouakil M, Latge JP, Davies J. Secretion of a potential virulence factor, a fungal ribonucleotoxin, during human aspergillosis infections. Mol Microbiol 1991; 5:1811–5.

23 Arruda LK, Mann BJ, Chapman MD. Selective expression of a major allergen and cytotoxin *Asp f* I in *Aspergillus fumigatus* implications for the pathogenesis of Aspergillus related disease. J Immunol 1992; in press.

24 El-Dahr J, Fink R, Selden R, Arruda LK, Platts-Mills T, Heymann P. J Allergy Clin Immunol 1990; 85:241 (abstr).

25 Forsyth KD, Hohmann AW, Martin AJ, Bradley J. IgG antibodies to *Aspergillus fumigatus* in cystic fibrosis–a laboratory correlate of disease activity. Arch Dis Child 1988; 63:953–7.

26 Van Bronswijk JEHN. House dust biology. Zeist, The Netherlands: Distributor NIB Publishers, 1981:151–64.

27 Panasenko VT. Ecology of microfungi. Botan Rev 1967;33:189–215.

28 Allan JD, Moss AD, Wallwork JC, McFarlane H. Immediate hypersensitivity in patients with cystic fibrosis. Clin Allergy 1975; 5:255–61.

29 Warner JO, Taylor BW, Norman AP, Soothill JF. Association of cystic fibrosis with allergy. Arch Dis Child 1976; 51:507–11.

30 Silverman M, Hobbs FDR, Gordon IRS, Carswell F. Cystic fibrosis, atopy and airways liability. Arch Dis Child 1978; 53:873–7.

31 Mearns M, Longbottom J, Batten J. Precipitating antibodies to *Aspergillus fumigatus* in cystic fibrosis. Lancet 1967; 1:538–9.

32 Waring P, Eichner RD, Mullbacher A, Sjaarda J. Gliotoxin induces apoptosis in macrophages unrelated to its antiphagocytic properties. J Biol Chem 1988; 263:18493–9.

33 Mullbacher A, Eichner RD. Immunosuppression *in vitro* by a metabolite of a human pathogenic fungus. Proc Natl Acad Sci USA 1984; 81:3835–7.

34 Knutsen AP, Hutcheson PS, Mueller KR, Slavin RG. Serum IgE and IgG anti *Aspergillus fumigatus* in patients with cystic fibrosis who have allergic bronchopulmonary aspergillosis. J Lab Med 1990; 116:724–7.

35 Jeffery GM, Beard ME, Ikranm RB, Chua J, Allen JR, Heaton DC, Hart DN, Schousboe MI. Intranasal amphotericin B reduces the frequency of invasive aspergillosis in neutropenic patients. Am J Med 1991; 90:685–82.