

Recovery of germinating fungal conidia from the nasal cavity after environmental exposure

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Abstract

Background The expression of fungal allergens is increased by the germination of conidia. We assessed the state of germination of fungal conidia recovered by nasal lavage after environmental exposure.

Methods Nasal lavage was performed on twenty adults at three stages: the start of the experiment, after 1 h indoors, and after 1 h outdoors. One half of the lavage liquid was immediately treated to prevent in-vitro germination and stained with periodic acid Schiff (PAS) to enable identification of germinated and ungerminated conidia. The untreated half of the lavage liquid was cultured on nutrient agar plates to enumerate and identify viable fungi.

Results PAS staining showed that both ungerminated and germinated conidia, and hyphal fragments, were present in the nasal cavity. The most prevalent fungi recovered were *Aspergillus*, *Alternaria*, *Cladosporium*, *Epicoccum*, *Penicillium*, and Yeast species. The number of viable

fungi recovered after 1 h indoors was significantly less than after 1 h outdoors ($P < 0.01$).

Conclusions Viable fungi and germinating conidia, in addition to ungerminated conidia and hyphal fragments, were present in the nasal cavity after both indoor and outdoor exposure. This provides novel insight into the pathogenicity of exposure to fungal aeroallergens.

Keywords Allergens · Fungi · Rhinitis · Rhinosinusitis

Abbreviations

PAS Periodic acid Schiff

V8 20% Vegetable juice nutrient agar

RB Rose-Bengal chloramphenicol nutrient agar

1 Introduction

Airborne fungal conidia, hyphae and associated fragments are abundant in most indoor and outdoor environments (Burge, Pierson, Groves, Strawn, & Mishra, 2000). Concentrations of these vary widely, depending on the location, season, time of day, and prevailing weather conditions (Chew, Rogers, Burge, Muilenberg, & Gold, 2003). Although inhalation of fungi has been recognized to exacerbate allergic rhinitis (Andersson, Downs,

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Mitakakis, Leuppi, & Marks, 2003) and asthma (Downs et al., 2001), the importance of exposure to fungal conidia and other fungal components in the progression of these diseases remains unclear.

Fungi can be cultured from the nasal airways of most people, including those with no respiratory disease and those with chronic rhinosinusitis (Ponikau et al. 1999). The nasal cavity is a major site for the deposition of inhaled fungi, because of the efficiency of the nose at removing particles larger than 10 μm from inhaled air (Knutsen, Bellone, & Kauffman, 2002). Although the presence of fungi in the airways does not necessarily indicate a pathological state, it may lead to symptoms of allergic or invasive disease in susceptible individuals (Braun et al., 2003). The hypothesis that colonization by fungi plays a major role in the development of chronic fungal sinusitis has recently drawn attention to their potential role in the pathology of this common condition (Braun et al., 2003).

Experiments have shown that release of allergens from fungal conidia increases substantially as a consequence of germination (Mitakakis, Barnes, & Tovey, 2001; Sporik, Arruda, Woodfolk, Chapman, & Platts-Mills, 1993). In contrast with other allergens, which are carried on non-viable particles, fungal allergens are synthesized and actively secreted at the sites of conidial deposition in the respiratory tract. Germinating conidia may also release proteolytic enzymes that induce epithelial damage (Robinson, Venaille, Mendis, & McAleer, 1990), inflammation (Reed & Kita, 2004) and lead to enhanced antigen presentation to the immune system (Kauffman, 2003). Hence, the state of germination of fungal conidia either before or shortly after deposition on the nasal mucosa may be important in determining their pathogenic potential.

Although conidia have been recovered from the nasal cavity (Karpovich-Tate et al., 2000), and hyphae and conidia have been isolated from mucin originating from inflamed nasal sinuses (Ponikau et al., 1999), no studies have investigated the state of germination of inhaled fungal conidia after normal environmental exposure. By use of an anterior nasal lavage technique (Greiff, Pipkorn, Alkner, & Persson, 1990), we have determined the relative numbers of ungerminated and germinated

conidia of a range of fungal genera recovered from the human nasal cavity after exposure to indoor and outdoor environments.

2 Methods

2.1 Subjects

The study population consisted of twenty adults (thirteen male) including eleven atopic and nine non-atopic subjects, ranging in age from 20 to 53 years, with a mean of 30.5 years. No subject reported any recognized nasal disease. The experimental procedure was approved by the University of Sydney Human Research Ethics Committee and all participants gave their informed consent in writing.

2.2 Environmental exposure

The anterior nasal lavage procedure was performed at three consecutive intervals:

- (1) at the start of the experiment, to quantify and remove the bulk of fungi inhaled before controlled exposure;
- (2) after 1 h in an indoor environment; and
- (3) after 1 h outdoors adjacent to a grassed sports field.

There were no delays between exposure and lavage. IOM personal aerosol samplers (SKC, Eighty Four, PA, USA), worn by the subjects, were run concurrently during indoor and outdoor exposure to monitor the subjects' exposure to airborne fungi. The study was performed on consecutive days, at approximately midday, during Spring (September) in Sydney, Australia. The study coincided with mean daily maximum and overnight minimum temperatures of 21°C and 6°C, respectively. No testing was performed within 2 days of any rainfall.

2.3 Nasal lavage procedure

The nasal pool device (Fig. 1) (Greiff et al., 1990) was used to instill 10 ml warmed (approx 32°C) sterile normal saline into one nostril at a time and was administered by the subject themselves with



Fig. 1 The nasal pool device used for nasal lavage

the assistance of the researcher. The liquid was held in each nostril for 1 min, with the head tilted 30 degrees forward, and then re-drawn into the nasal pool device. This was immediately repeated in the same nostril using the same liquid for another 1 min. The liquid was then expelled from the nasal pool device into a sterile tube. The other nostril was lavaged in an identical manner, using a separate 10 mL of sterile saline. Nasal lavages from both left and right nostrils were combined and the volume of liquid recovered was recorded. In this procedure, on average, 14.4 ml of the 20 ml instilled into the nose was recovered. The nasal lavage liquid was immediately divided into two equal portions, A and B. In preliminary experiments, in which five identical nasal lavage procedures were performed consecutively, the first lavage was found to remove an average of 93% of the total culturable fungi recovered (data not shown).

2.4 PAS staining

Portion A was treated immediately with Kathon CG (Rohm and Haas, UK; 0.5% v/v) to prevent the germination of conidia subsequent to their recovery from the nasal cavity. Trial experiments confirmed that Kathon CG treatment immediately prevented conidial germination (data not shown). These samples were then treated with Sputolysin (Calbiochem, CA, USA) sufficient to

achieve a final concentration of 0.528 mg ml^{-1} dithiothreitol and incubated for 30 min at room temperature to dissolve nasal mucous. Aliquots were impacted by Cytospin centrifugation on to silanised microscope slides, then fixed in 100% ethanol and air-dried. Periodic acid Schiff (PAS) staining was performed as described elsewhere (McManus, 1946), and preparations examined by light microscopy at 200 $\times$. PAS stains fungi, mucous, and glycogen crimson, enabling enumeration and identification of conidia, hyphae, and fragments.

2.5 Nasal lavage culture

Portion B was treated with Sputolysin, as described above, to dissolve nasal mucous. An aliquot of 7 ml was then concentrated by centrifugation at 1250g for 10 min, resuspended in sterile dH₂O and plated on to 20% vegetable juice nutrient agar (V8) and rose-Bengal chloramphenicol (RB) (Oxoid, UK) nutrient agar for culture-based identification of viable fungi. Our preliminary experiments indicated that treatment with Sputolysin did not affect the viability of fungal conidia (data not shown), which confirms previous findings (Ponikau et al. 1999). Culture plates were incubated at 25°C and inspected daily for 20 days until sufficient sporulation had occurred. Fungal colonies were taxonomically identified to genus level by macroscopic and microscopic morphological features, by two observers.

2.6 IOM personal aerosol sampler counts

Fungal conidia were collected concurrently during each of the indoor and outdoor environmental exposures by IOM personal aerosol samplers, which were fitted with 1.0 μm PVDF membranes (Millipore, Bedford, MA, USA). The collected fungal propagules were identified morphologically and counted at a magnification of 200 $\times$ using light microscopy.

2.7 Statistical analysis

Associations between the numbers of fungi quantified by each method of analysis were expressed

as Spearman correlation coefficients. Differences between the numbers of viable fungi isolated on culture plates after indoor and outdoor exposure, and differences between the numbers of fungal conidia collected by IOM personal air samplers in each exposure setting were tested using a paired *t*-test on log-normalized values. Statistical calculations were performed using Analyse-it for Microsoft Excel (Analyse-it Software, UK). The criterion for significance for all analyses was $P < 0.05$.

3 Results

PAS staining of Cytospin preparations of nasal lavage fluid enabled identification and quantification of fungal propagules in 95% of the 60 samples processed. Figure 2 shows ungerminated and germinated fungal conidia, and hyphal fragments, recovered from nasal lavages and stained using the PAS technique. Both germinated and ungerminated *Aspergillus* and *Penicillium* conidia (Fig. 2G) were by far the genera most frequently observed in the PAS-stained samples (Table 1). It was, however, necessary to analyse *Aspergillus* and *Penicillium* conidia as a combined group with regard to PAS results, because it was not possible to discriminate between these conidia morphologically in PAS-stained samples. Other species, for example *Curvularia* (Fig. 2H) were also identified, with isolated hyphal fragments, discernible by their septal junctions (Fig. 2I and 2J).

Table 1 shows the median, maximum, and minimum numbers of conidia in the nasal lavage samples from each of the 20 subjects. The most

numerous fungal conidia that were observed belonged to the *Aspergillus*/*Penicillium* group. Members of this group were also by far the most often seen to be in a state of germination.

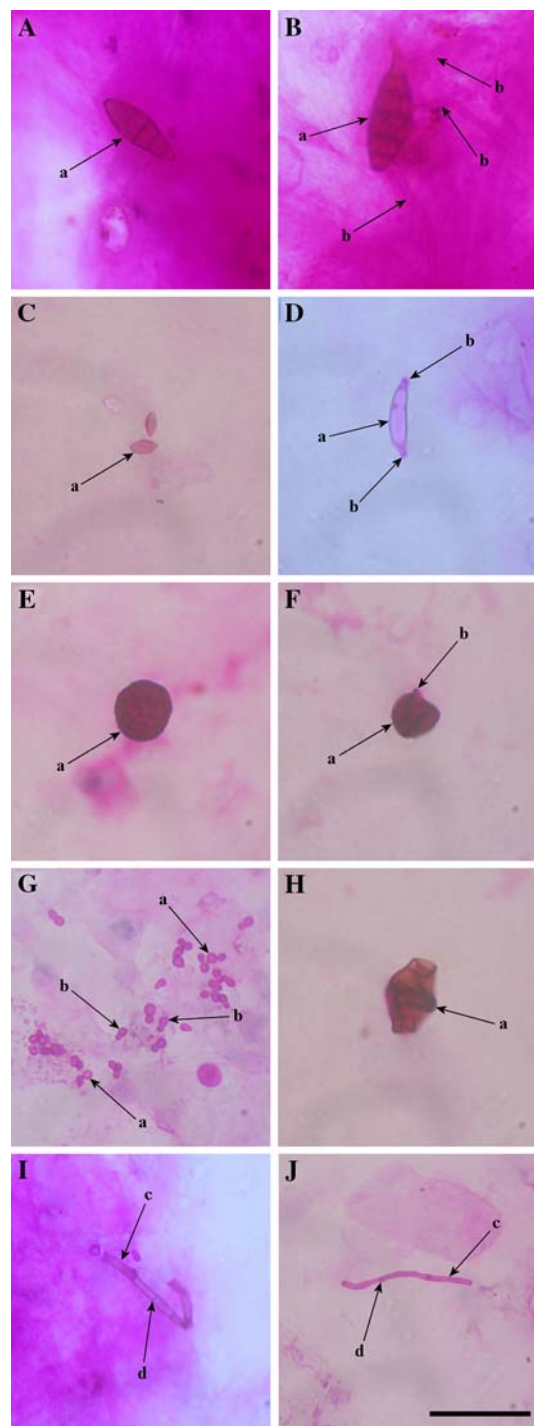


Fig. 2 Photomicrographs of ungerminated and germinated fungal conidia and hyphae recovered from nasal lavage samples and stained with PAS: (A) ungerminated *Alternaria* conidia (arrow a); (B) germinated *Alternaria* conidia (arrow a) and hyphae (arrow b); (C) ungerminated *Cladosporium* conidia (arrow a); (D) germinated *Cladosporium* conidia (arrow a) and hyphae (arrow b); (E) ungerminated *Epicoccum* conidia (arrow a); (F) germinated *Epicoccum* conidia (arrow a) and hyphae (arrow b); (G) ungerminated (arrow a) and germinated (arrow b) *Aspergillus*-*Penicillium* conidia; (H) ungerminated *Curvularia* conidia (arrow a). (I, J) fungal hyphae (arrow c) with septal junctions (arrow d). Scale bar = 30 μ m

Table 1 Recovery of different fungi from nasal lavage samples. Four recovery methods are described: V8 media culture, RBA media culture, PAS showing ungerminated conidia, and PAS showing germinated conidia. For each recovery method results for each of the environmental exposure conditions (Start, Indoor and Outdoor) are given. All counts are given as Median (Min–Max) for $n = 20$ subjects

Fungal species	Recovery method											
	V8 media culture						PAS ungerminated conidia					
	Start	Indoor	Outdoor	Start	Indoor	Outdoor	Start	Indoor	Outdoor	Start	Indoor	Outdoor
<i>Aspergillus</i>	0 (0–9)	0 (0–9)	0 (0–43)	0 (0–41)	0 (0–8)	0 (0–39)	–	–	–	–	–	–
	0 (0–94)	0 (0–15)	0 (0–9)	0 (0–69)	0 (0–9)	0 (0–8)	–	–	–	–	–	–
<i>Penicillium</i>	–	–	–	–	–	–	543 (0–5976)	208 (0–3981)	157 (11–1382)	172 (0–24336)	44 (0–8240)	61 (0–3224)
<i>Asp-Pen</i>	–	–	–	–	–	–	–	–	–	–	–	–
combined	–	–	–	–	–	–	–	–	–	–	–	–
<i>Alternaria</i>	0 (0–26)	0 (0–15)	0 (0–18)	0 (0–17)	0 (0–0)	0 (0–15)	0 (0–10)	0 (0–0)	0 (0–25)	0 (0–0)	0 (0–0)	0 (0–25)
<i>Bipolaris</i>	0 (0–0)	0 (0–0)	0 (0–9)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–620)	0 (0–13)	0 (0–0)	0 (0–12)
<i>Cladosporium</i>	0 (0–174)	0 (0–9)	8 (0–4003)	0 (0–273)	0 (0–8)	9 (0–4606)	0 (0–157)	0 (0–24)	0 (0–1538)	0 (0–0)	0 (0–0)	0 (0–471)
<i>Curvularia</i>	0 (0–8)	0 (0–0)	0 (0–97)	0 (0–0)	0 (0–0)	0 (0–71)	0 (0–36)	0 (0–0)	0 (0–12)	0 (0–0)	0 (0–0)	0 (0–0)
<i>Epicoecum</i>	0 (0–9)	0 (0–17)	0 (0–673)	0 (0–9)	0 (0–9)	0 (0–416)	0 (0–0)	0 (0–0)	0 (0–13)	0 (0–0)	0 (0–0)	0 (0–12)
<i>Exserohilum</i>	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–25)	0 (0–0)	0 (0–0)	0 (0–0)
<i>Mucor</i>	0 (0–0)	0 (0–0)	0 (0–9)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)
<i>Mycelia</i>	0 (0–33)	0 (0–0)	0 (0–248)	0 (0–41)	0 (0–17)	0 (0–35)	–	–	–	–	–	–
Sterilia	–	–	–	–	–	–	–	–	–	–	–	–
Hyphae	–	–	–	–	–	–	–0 (0–0)	0 (0–26)	0 (0–12)	0 (0–0)	0 (0–0)	0 (0–51)
Pollen	–	–	–	–	–	–	–	–	–	–	–	–

Because of the comparatively low numbers of other species that were found to be germinated, meaningful analysis of the percentage of conidia germinated was only possible for the *Aspergillus/Penicillium* group. The mean percentages of *Aspergillus/Penicillium* conidia observed to be germinated in nasal lavage samples were 32% for the start condition, 36% after 1 h indoors and 33% after 1 h outdoors. There were no significant differences ($P > 0.05$) between the percentage germination of *Aspergillus/Penicillium* observed after each exposure condition. The percentage germination of *Aspergillus/Penicillium* conidia observed in each individual subject, by environmental exposure condition, is shown in Table 2.

Viable fungi were recovered from the nasal lavage fluids of 95% of subjects participating in the study (Table 1). The most commonly cultured fungi from the nasal lavages were *Alternaria*, *Aspergillus*, *Cladosporium*, *Epicoccum*, *Penicillium*, and Yeast species. The diversity of fungal genera and the total number of fungi recovered varied among subjects and exposure conditions.

Table 2 Percentage germination of *Aspergillus* and *Penicillium* conidia recovered from nasal lavage samples, grouped by experimental subject and environmental exposure

Subject number	Percentage germination of <i>Aspergillus/Penicillium</i> conidia for each subject (%)		
	Start	Indoor	Outdoor
1	81.4	71.2	75.6
2	0	16.7	0
3	27.8	37.5	47.1
4	22.2	28.9	23.5
5	31.3	0	22.2
6	27.3	0	25.9
7	69.9	66.4	58
8	34.5	0	40
9	31.9	31.3	34.4
10	13.1	28.2	16.2
11	31.2	44.1	58.6
12	63.9	74.3	73.6
13	10	6.7	0
14	7.5	2.9	0
15	71.4	100	50
16	0	0	0
17	26.3	18.2	23.1
18	92.3	88.2	52
19	0	5.9	66.7
20	0	100	0

The total number of all viable filamentous fungi recovered after 1 h of outdoor exposure was significantly greater than after 1 h of indoor exposure on both V8 ($P = 0.0024$) and RB ($P = 0.0019$) nutrient media. The number of all filamentous fungi combined, isolated on V8 nutrient media, correlated significantly with that isolated on RB nutrient media ($r_s = 0.96$, $P < 0.001$).

The total number of fungal conidia collected by the IOM samplers was highly variable among individuals and there was a significant difference between the IOM counts obtained from the two environmental exposure settings ($P < 0.0001$). There was also a significant association between the numbers of fungi collected on the IOM personal air sampler and the culture colony counts on V8 nutrient media ($r_s = 0.73$, $P < 0.001$) and on RB nutrient media ($r_s = 0.58$, $P < 0.001$). Speciation of the conidia captured on the IOM samplers, and determination of their state of germination, was not possible, because of the opaque nature of the filter membrane.

4 Discussion

Fungal propagules, including germinated and ungerminated conidia and hyphal fragments were recovered from the nasal cavity of 95% of subjects after different environmental exposures in typical everyday settings. For the *Aspergillus* and *Penicillium* group, over 30% of conidia recovered from nasal lavage samples were in a state of germination. Numbers of viable conidia estimated by culture on both V8 and RB nutrient media correlated significantly ($P < 0.001$) with the corresponding IOM personal air sampler measurements of each subject's environmental fungal exposure. The results of this study demonstrate for the first time that fungal conidia inhaled as part of ordinary environmental exposure can exist in the nasal cavity in a germinated state. These findings implicate the germination of conidia as a major factor in natural exposure to fungal allergens.

We found that numbers of fungi recovered from the nasal lavages differed between subjects.

This is likely to reflect their different exposure during the study period, as demonstrated by the significant correlations between nasal lavage culture counts and the IOM personal air sampler counts collected concurrently. Other sources of variation include the subject's individual breathing patterns, nasal anatomy, and nasal lavage technique. Overall, the numbers of many fungal genera recovered were low in several of the samples, which is consistent with previous studies (Braun et al., 2003) that describe the difficulties encountered in recovering fungi from nasal mucous. Our preliminary experiments demonstrated that the nasal lavage procedure recovered a mean of 93% of recoverable fungi in the first lavage alone. It is not known, however, if there is a subset of fungi that occur in the nasal cavity with greater adhesion to the mucosa than those that can be collected by lavage. Such fungi, because of the intimate and ongoing contact they would have with the mucosa and the difficulty of removal by normal mucociliary clearance, would most probably result in an even greater allergen challenge than transient, less adherent fungal species.

The results of the study show that in several samples the conidia of up to 5 of the 7 genera identified in PAS stained samples were in a state of germination. Conidial germination has been linked with a substantial increase in the expression of allergens after in vitro experiments using monoclonal antibodies (Mitakakis et al., 2001; Sporik et al., 1993) and fungus-specific human IgE from individuals sensitized to fungi (Green, Mitakakis, & Tovey, 2003). Conidial germination is also associated with protein synthesis, protein release, and enzymatic activity at the hyphal tips and sites of germination. Previous studies have shown that on germination of *Alternaria* conidia an Alt a 1 homologue was differentially expressed during hyphal growth (Cramer & Lawrence, 2003), and it was also demonstrated that a greater amount of Alt a 1 could be detected by immunostaining with Alt a 1 specific monoclonal antibodies after conidial germination (Mitakakis et al., 2001). Although conidial germination is a natural part of the life cycle of the fungus, it has also been shown to lead to expression of specific pathogenic enzymes that have been shown to

decrease the clearance of conidia from the respiratory mucosa (Robinson et al., 1990), promote adhesion to the respiratory epithelium (Yang, Jaeckisch, & Mitchell, 2000), and inhibit macrophage function (Kauffman, Tomee, van de Reit, Timmerman, & Borger, 2000; Yang et al., 2000). Fungal protease enzymes are also known to stimulate protease-activated receptors, directly amplifying IgE production, eosinophil degranulation, and inflammation (Reed & Kita, 2004). These protease-activated receptors are present on many of the cells throughout the airways which are involved in rhinitis and asthma (Cocks & Moffatt, 2001). Because of the combination of the above factors, the presence of germinating fungi on the nasal epithelium of a sensitized atopic individual is likely to increase both the probability and severity of an IgE-mediated allergic reaction. In this study we have shown that substantial numbers of germinated fungal conidia can, and do exist, in contact with the nasal epithelium after normal respiration in indoor and outdoor settings.

The objective of this study was to demonstrate that fungal conidia were present in the nasal cavity while undergoing the process of germination after short (1 h) periods of environmental exposure. The microscopic techniques used in our experiments enabled us to identify the first stages of germination corresponding to the emergence of a germ tube from the conidia. We were, however, unable to conclude that these conidia began germinating *only* subsequent to inhalation. Irrespective of whether the germinating conidia we observed began their germination before or after their inhalation, we can conclude that germinating conidia are present on the nasal mucosa in normal individuals after environmental exposure.

The knowledge that viable fungal conidia are common in the nasal cavity, and that fungi can be cultured from the nasal airways of most people (Ponikau et al., 1999) does suggest, however, that germination of fungal conidia could continue, or even begin, after inhalation. The requirements for conidial germination include specific ranges of temperature and humidity and the availability of nutrients. The site of deposition of fungal propagules in the nasal cavity depends on their

aerodynamic characteristics as part of the inhaled air stream (Proctor, Anderson, & Lundqvist, 1973). Within the nasal cavity there are a variety of temperature zones, which relate to regions of nasal anatomy (Keck, Leiacker, Riechelmann, & Rettinger, 2000) and might function as a selective condition in the determination of conidial germination, especially for thermotolerant species (Lake et al., 1991).

Whether germination will occur in the nasal cavity depends largely on the opposing factors of germination time and the time required for mucociliary clearance. Germination time can vary markedly between different fungal genera and species. For example, *A. alternata* germinates, with substantial lengthening of new hyphae, after 14 h at room temperature, as shown in previous publications from our laboratory (Mitakakis et al., 2001). Our recent experiments (data not shown) indicate that *A. alternata* conidia begin germination within 2 h at 30°C and 95% RH. After 4 h the total length of hyphae emerging from each conidium is a mean of 50 µm. Other authors have reported that for some common fungal species germination in culture at 37°C can begin after 2 h for *Rhizopus microsporus* and after 5 h for *Aspergillus fumigatus* (Meletiadiis, Meis, Mouton, & Verweij, 2001). At 25°C in conditions of high water availability *Aspergillus flavus* and *Aspergillus parasiticus* spores can germinate within 2.4 h (Passone, Resnik, & Etchverry, 2005). In the most relevant studies performed to date, *Epicoccum nigrum* and *Bipolaris spicifera* have been shown to germinate within 1 h when incubated under conditions known to occur in different regions of the nasal cavity (Green et al., 2006).

When fungal conidia have been inhaled and are in contact with the lining of the nasal airway, their residence time depends on the rate of mucociliary clearance. Although our experiments were restricted to sampling fungi that had been deposited after natural environmental exposure, we can relate our findings to studies performed using radiolabelled particulate matter of a size and density approximating that of fungal spores. Radiolabelled alginate and Sephadex microspheres (1.3 and 24.6 µm in diameter, respectively) inhaled on to the human nasal mucosa had

clearance half-lives of 4 h and 2 h, respectively (Tafaghodi, Abolghasem Sajadi Tabassi, Jaafari, Zakavi, & Momen-Nejad, 2004). For particles of a size equivalent to small fungal spores, 12.5% of the particles inhaled may therefore remain on the nasal mucosa 12 h after inhalation. This time is substantially longer than is necessary for many fungal species to germinate under the conditions of temperature and humidity which occur in the nasal cavity. Given the large numbers of viable fungal conidia that are inhaled, germination within the nasal cavity is likely to occur to some extent.

Because the nasal sinuses are continuous with the nasal cavity, it can be expected that the sinuses to some extent reflect the population of microorganisms present in the nasal cavity. It is known that the sinus cavities are not sterile, even in disease-free states (Su, Liu, Hung, & Tsai, 1983), and that infants within four months of birth have populations of fungi in nasal mucous samples similar to those of adults (Lackner et al., 2004). Interaction of viable fungi with the mucosa of the nasal sinuses, inducing specific inflammatory responses, has been proposed as the root cause of the hyper-immune disorder chronic fungal sinusitis (CFS) (Schubert, Hutcheson, Graff, Santiago, & Slavin, 2004). Clinical intervention intended to reduce the amount of viable fungi in the nasal cavities of CFS patients and in indoor air have been tested, on the premise that these treatments will also have some effect on the sinuses. Such treatments have been identified as being effective in some studies (Dennis, 2003; Ponikau, Sherris, Kita, & Kern, 2002) and not in others (Weschta et al., 2004). Our results confirm that viable fungi are common in the nasal cavity, and suggest that any anti-fungal treatment applied nasally would have to cope with the constant influx of newly inhaled fungi.

Our findings demonstrate that not only are fungal conidia present in the nasal cavities of normal individuals, reflecting recent environmental exposure, but also that these conidia are in a state of germination. These findings are in agreement with those of Braun et al. (2003) who identified viable fungi in the nasal mucous of 91.3% of normal controls. These authors suggested that positive nasal fungal cultures should be considered a normal finding, but that chronic

rhinosinusitis (a more broad definition of CFS) can be identified by fungal elements and clumps of eosinophils present in the mucus.

In this study we used two types of nutrient agar to enable recovery of viable fungi from the nasal lavage samples. Our choice of vegetable juice and rose Bengal chloramphenicol nutrient media was based on the findings of other studies, which reported that at least two types of medium are necessary to recover representatives from the full spectrum of environmental fungi, while protecting against overgrowth, which could result in errors in counting. The choice of one selective and one non-selective nutrient media is often recommended for environmental fungal air sampling (Burge, Solomon, & Boise, 1977). Our observation in the current study, that the numbers of fungal colonies isolated using each type of medium correlate not only with each other but also with numbers of conidia collected using the IOM personal air samplers (a non-viable method), indicates that no sampling bias was introduced by the culture methods.

We used the PAS staining technique on fixed Cytospin preparations of nasal lavage liquid. Limitations of this technique include the often difficult identification of unicellular conidia, hyphae, and fragments in samples containing small numbers of fungal propagules. In this study it was not possible to differentiate between *Aspergillus* and *Penicillium* conidia; culture-based methods reported more colonies of *Penicillium* than of any other unicellular spore-bearing species, however. The clinical significance of differentiating between *Penicillium* and *Aspergillus* exposure with regard to their effect as aeroallergen sources remains unknown. In these experiments the fact that unicellular conidia of these fungal genera were seen to be germinating was of primary interest, because of previous observations that germination is associated with greatly increased release of allergens.

We have demonstrated that germinating fungal conidia are commonly present in the nasal cavity of normal humans. Future studies investigating the in-vivo consequences of inhalation of viable fungal propagules will be required to confirm the existence of an increased allergenic effect because of inhalation of germinating and viable conidia.

5 Conclusions

Germinated and ungerminated conidia, and hyphal fragments, are commonly present in the human nasal cavity after natural environmental exposure. The numbers of these fungi present also depend on the nature of that exposure. This has profound implications for the study of allergic rhinitis, asthma, and fungal sinusitis.

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