

Natural exposure to *Alternaria* spores induces allergic rhinitis symptoms in sensitized children

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Allergic rhinitis is associated with exposure to inhaled allergens. Allergic sensitization to the fungus *Alternaria* is common, especially in warm climates, but the relevance of sensitization to *Alternaria* in the generation of rhinitis has not been elucidated. The aim of this study was to determine whether natural exposure to *Alternaria* induces rhinoconjunctivitis symptoms in *Alternaria*-sensitized children. Symptoms of rhinoconjunctivitis in 132 atopic (measured by skin prick tests) children (aged 7–12 years) in inland New South Wales Australia were measured using parent-completed questionnaires in summer and winter 1998. Airborne concentrations of *Alternaria* spores and grass pollen were measured throughout the study period using a Burkard volumetric trap. The relation between airborne concentrations of *Alternaria* spores and symptoms of rhinoconjunctivitis was examined using Wilcoxon's matched pairs signed ranked test and generalized estimating equations. The proportion of children atopic to *Alternaria* reporting symptoms of rhinitis was significantly higher in the summer, when airborne concentrations of *Alternaria* were high, than in the winter, when airborne concentrations were low (66.2% vs. 38.2% for nasal symptoms at night, $p = 0.0001$, 70.6% vs. 51.52% for nasal symptoms during the day, $p = 0.02$). The proportion of children using anti-allergic treatment was also higher in summer than in winter (anti-allergic medication for the nose 39.7% vs. 17.7%, $p = 0.0003$). A strong correlation between airborne concentrations of grass pollen and *Alternaria* was found ($R = 0.94$, $p < 0.001$). However, when all children atopic to ryegrass were excluded from the analyses, significant associations between symptoms and season remained. We conclude that exposure to *Alternaria* spores may be an important cause of allergic rhinoconjunctivitis.

**Morgan Andersson¹, Sara Downs²,
Teresa Mitakakis², Jörg Leuppi²
and Guy Marks²**

¹Department of Otorhinolaryngology, University Hospital, Lund, Sweden and ²Institute of Respiratory Medicine, Sydney University, Sydney, NSW, Australia

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Morgan Andersson, Department of Otorhinolaryngology, Head and Neck Surgery, University Hospital, S-221 85 Lund, Sweden
Tel.: +46 46 171705
Fax: +46 46 211 09 68
E-mail: Morgan.Andersson@onh.lu.se

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Symptoms of allergic rhinitis are generated by inhalation of allergens that attach to the nasal mucosal surface. Mucosal allergic reactions and associated nasal symptoms are then initiated by cross-linking of specific IgE with allergens on the surface of inflammatory cells residing in the airway mucosa (1). A causative relationship between allergic sensitization and propagation of rhinitis symptoms is well established for allergens from various pollens, animal dander and house dust mites (2), but the importance of

fungal spores for inducing allergic nose and eye symptoms in sensitized individuals during natural disease conditions has not been clarified.

Alternaria is a wide spread fungus found in both urban and rural areas, and is particularly common in regions with a warm climate. The fungus grows on plants and therefore the source of exposure is outside (3), although significant exposure to *Alternaria* may also occur inside peoples' homes (4). The prevalence of allergic sensitization to *Alternaria* appears to vary with

climate. For instance, nearly 50% of children in Arizona, USA (2, 5), are sensitized to the fungus compared with less than 1% of children in Austria (3, 6). Sensitization to *Alternaria* has been associated with asthma diagnosis (5, 7–9) and a relation has recently been demonstrated between high levels of *Alternaria* spores in ambient air and changes in level of airway hyperresponsiveness in *Alternaria*-sensitized children (10). Few studies, however, have addressed the question whether sensitization to *Alternaria* is associated with rhinoconjunctivitis (5), and none have investigated whether natural exposure may induce nose and eye symptoms.

The aim of the present study was to investigate whether exposure to *Alternaria* during natural conditions in an inland rural region in south-eastern Australia, could cause rhinoconjunctivitis symptoms in children sensitized to this fungus.

Methods

Study population and design

A prospective cohort study of school children aged 7–12 years was conducted in Wagga Wagga; an inland rural town in New South Wales Australia. The study was approved by the Human Ethics' Committee at the University of Sydney, and consent was obtained from the New South Wales Department of School Education, the Catholic Education Office, Principals of the schools from which the children were selected and the children's parents. Details of the study design and methods have already been published (10).

Subjects

The children recruited into the study were from five primary schools in Wagga Wagga selected at random in July 1997. All children whose parents consented to their child's participation in the study were tested for atopy (514, response rate 67%) and their parents completed a Children's respiratory symptom questionnaire. All children who were classified as sensitized (atopic by skin prick test) to *Alternaria* (103) and a random sample of 103 of the 118 other children who were sensitized to other allergens were selected for the study. Six children withdrew soon after the study started in 1997, leaving 100 children in each atopic category. The questions about rhinitis symptoms were administered in parent-completed questionnaires in December 1998 (summer) and June 1999 (winter). Parents of 132 children (66%) answered questionnaires in both seasons

and parents of 150 children answered questionnaires in at least one season. There were no differences in the distribution of age, gender, respiratory symptoms and atopic status between the children in the original sample selected in 1997 to the sample whose parents answered questionnaires in 1998/99.

Skin prick test

Sensitization to relevant allergens was assessed by skin prick tests on the children's forearms (11). The panel included the house dust mite *Dermatophagoides pteronyssinus*, rye grass pollen *Lolium perenne*, cat dander, the fungi: *Alternaria tenuis*, *Cladosporium homodendrum*, and *Helminthosporium interseminatum*, Australian Pine/She-Oak pollen *Casuarinaglauca* and Cypress Arizona pollen *Cupressus arizonica*. All allergens were purchased through Hollister-Stier (Bayer Corp., Spokane, WA, USA). Histamine (10 mg/ml) was used as the positive control and a normal saline/glycerol solution as the negative control. Skin wheal size was recorded at 15 min after the skin prick test as the mean of the length of the long axis to the wheal and the length of its perpendicular. A mean wheal size of 3 mm or more was coded as a positive response. Children were classified as atopic if they had a positive response to one or more of the allergens tested.

Assessment of *Alternaria* and pollen in ambient air

A Burkard volumetric trap (Burkard Manufacturing Co. Ltd, Hertford, UK) was used to measure concentrations of *Alternaria* spores and pollens in the ambient air in the town throughout the period of study (7). Spores and pollens were trapped onto adhesive tapes that were cut into sections representing one day. Each section was viewed by light microscopy and the number of trapped *Alternaria* spores, grass pollen grains and other pollen grains were counted. The daily concentration of spores and pollens per cubic meter of air was calculated. Exposure to *Alternaria* and grass pollen was defined as the mean daily concentration of spores or pollen grains per cubic meter of air measured in the 30 days preceding the mid-date of each data collection period (see Table 2).

Questionnaire

Questionnaires were distributed to children at the schools (10). One follow-up telephone call was made to parents whose child who had not

returned a questionnaire to the school, asking for the questionnaire's return. Only questionnaires dated as completed by the parents before the end of data collection period, which lasted between 7 and 10 days, were included in the analysis. The respiratory symptom questionnaire included the following questions about nose and eye symptoms and treatment:

- 1 Over the past 1 month, has your child had symptoms in the nose during the day not related to a cold or flu? (Like sneezing or an itchy, runny or blocked nose)
- 2 Over the past 1 month, has your child had symptoms in the nose at night not related to a cold or flu? (Like sneezing or an itchy, runny or blocked nose)
- 3 Over the past 1 month, has your child had symptoms in the eyes not related to an infection? (Like itchy, red or swollen eyes)
- 4 Over the past 1 month, has your child visited your GP for sneezing or an itchy, runny or blocked nose or itchy, red or swollen eyes?
- 5 Over the past 1 month, has your child used any anti-allergic medication for the nose?
- 6 Over the past 1 month, has your child used any anti-allergic eye drops?

Response alternatives for first three questions were: not at all, a few days, some days, most days, and every day. Not at all was coded as a no answer and a few days, some days, most days and every day were coded as yes. Possible responses to questions four, five and six were no or yes. Parents were also asked how long the child spent outside each day and whether the child had left the area for a period of time during the preceding month.

Statistical analyses

The analyses were undertaken using STATA (Stata Statistical Software Release 6.0., Stata Corp., College Station, TX USA). Univariate analyses were performed first to examine the distribution of outcome variables, concentrations of *Alternaria* and grass pollen, residence factors and meteorological factors. We then compared the proportion of children with symptoms of rhinoconjunctivitis between each of the time periods in the towns using Wilcoxon's matched-pairs signed ranks test. Finally we estimated the association between airborne concentrations of *Alternaria* spores and rhinoconjunctivitis symptoms using generalized estimating equation (GEE). The model was a random-effects regression robust to unbalanced data. It therefore

Table 1. Exposure factors for 1 month (30 days) preceding the date of each data collection period

Exposure factors	Summer	Winter
	8 December 98*	21 June 99*
<i>Alternaria</i> (mean spores/m ³ /day)	300.6	30.4
Grass pollen (mean grains/m ³ /day)	133.2	1.2
Temperature at 15.00 hours (mean °C)	24.1	13.4
Relative humidity at 15.00 hours (mean percentage)	31.5	67.4
Child spent more than 2 weeks outside the local area (%)	5.6	1.4
Child spent more than 3 h outside each weekday (%)	24.8	10.8

*Mid-date of each data collection period.

adjusted for repeated measures within children but also allowed us to include data from the 18 children for whom we only had data for one season.

Results

Airborne concentrations of *Alternaria* spores were higher in summer compared with winter in Wagga Wagga (Table 1). There was a close correlation between airborne concentrations of *Alternaria* spores and grass pollen. ($R = 0.94$, $p < 0.001$). For this reason we examined the relation between symptoms and airborne concentrations of *Alternaria* including and excluding children atopic to rye grass.

Consistently more children sensitized to *Alternaria* had nocturnal, nasal symptoms and eye symptoms in the summer than in the winter compared with children who were not sensitized to the fungus (Table 2). This association was observed even when children who were also atopic to rye grass were excluded from the analysis (Table 3).

No confounding could be observed by meteorological or residence factors when these were added to the GEE model. The model suggested that the association between *Alternaria* spore concentrations and symptoms of rhinoconjunctivitis was stronger in children sensitized to *Alternaria* than those not sensitized to the fungus although the differences in effect were not statistically significant (Table 4).

Discussion

In the present study we have for the first time demonstrated changes in rhinoconjunctivitis symptoms of *Alternaria*-sensitized children over the year coincide with alterations in ambient air concentrations of the fungus. These results

Table 2. Proportion (%) of children with positive responses to rhinoconjunctivitis questions stratified by sensitization to *Alternaria*

Question	Children sensitized to <i>Alternaria</i> % (n = 68)			Children not sensitized to <i>Alternaria</i> % (n = 64)		
	winter	summer	p-value	winter	summer	p-value
Nasal symptoms at night	38.2	66.2	0.0001	40.6	54.7	0.06
Nasal symptoms during the day	51.5	70.6	0.02	51.6	53.1	0.85
Eye symptoms	20.6	55.9	<0.0001	28.1	43.8	0.03
Visited GP	7.4	7.4	1.00	4.7	4.7	1.00
Anti-allergic medication for nose	17.7	39.7	0.0003	7.8	26.6	0.003
Anti-allergic medication for eyes	2.9	13.2	0.008	4.7	6.3	0.71

Table 3. Proportion (%) of children with positive responses to rhinoconjunctivitis questions sensitive to rye grass, stratified by sensitization to *Alternaria*

Question	Children sensitized to <i>Alternaria</i> but not to rye grass % (n = 23)			Children sensitized to <i>Alternaria</i> and to rye grass % (n = 45)		
	winter	summer	p-value*	winter	summer	p-value*
Nasal symptoms at night	34.8	56.5	0.03	40.0	71.1	0.001
Nasal symptoms during the day	47.8	60.9	0.32	53.3	75.6	0.02
Eye symptoms	17.4	47.8	0.02	22.2	60.0	0.0004
Visited GP	4.4	0	0.32	8.9	11.1	0.71
Anti-allergic medication for nose	13.0	30.4	0.05	20.0	44.4	0.002
Anti-allergic medication for eyes	4.4	8.7	0.32	15.6	2.2	0.01

*For difference between seasons within each sensitization category.

Table 4. Effect of increase in exposure by 50 *Alternaria* spores/m³/day over the preceding month (GEE analysis)

Symptoms during the previous month	Sensitized to <i>Alternaria</i>		Not sensitized to <i>Alternaria</i>		p-value for difference in effect
	Odds ratio	95% CI	Odds ratio	95% CI	
Symptoms in the nose a few days or more	1.17	(1.03–1.32)*	0.98	(0.87–1.10)	0.05
Symptoms in the nose a few nights or more	1.23	(1.11–1.37)†	1.08	(0.96–1.20)	0.07
Symptoms in the eyes a few days or more	1.29	(1.14–1.45)†	1.15	(1.03–1.28)	0.17
Visited GP for sneezing, itchy nose or eyes	1.08	(0.88–1.33)	0.96	(0.75–1.24)	0.50
Used anti-allergic medication for nose	1.24	(1.11–1.37)†	1.23	(1.05–1.44)*	0.96
Used anti-allergic medication for eyes	1.39	(1.09–1.75)*	1.00	(0.76–1.33)	0.09

*p < 0.05; †p < 0.001.

Number of observations = 150.

indicate that in regions with high concentrations of fungal spores, individuals allergic to *Alternaria* are at risk of allergic rhinoconjunctivitis or ‘hay fever’.

It was difficult to distinguish the possible effect from grass pollen exposure from the effect of *Alternaria* in Wagga Wagga because the pollen season coincides with the increase in

concentrations of *Alternaria* in summer. Hence some of the differences between the winter and the summer period in the proportion of children with *Alternaria*-negative skin prick tests could have been attributed to changes in grass pollen exposure in those children who were also sensitized to grass pollen. However, when all the rye grass-positive children were excluded, we could still demonstrate an increased proportion of symptoms associated with the fungus exposure in the *Alternaria*-positive group. This was true for nocturnal nose symptoms, and eye symptoms. In addition, a relation for the use of nasal medication was still observed. In contrast, no variations in symptoms and medication use were seen in the rye grass and *Alternaria*-negative group.

Around 70% of the *Alternaria*-positive children reported daytime nasal symptoms in the summer in Wagga Wagga, and when the rye grass-positive children were excluded, more than 60% of the *Alternaria*-positive children reported nasal symptoms day and night, suggesting that fungus exposure is an important cause of rhinitis symptoms. Sensitization to fungi is also prevalent in European children with an age-dependent distribution reaching maximum values at around 7 years of age (12).

The rhinoconjunctivitis symptoms may have been due, at least in part, to fungal exposure other than *Alternaria*. There is substantial cross-reactivity in allergenicity to different fungi (13) and it is possible that the measured effect relates to other fungi with a similar seasonal release as *Alternaria*. The seasonal distribution of airborne concentrations of *Cladosporium* spores in ambient air has, for example, been reported to be similar to that for *Alternaria* in some parts of Australia (14).

Measurements of spore and pollen concentrations in ambient air based on fixed point monitors are imprecise measures of personal exposure (15). The characteristics of allergic sensitization may vary between subjects, and, in addition, the characteristics of personal exposure to fungi are also likely to vary. If the misclassification of exposure has been random, the most likely effect from this bias is that the estimate of effect has been underestimated (16).

In the present study, symptoms in the children were measured by a parent-completed questionnaire. It would be useful to have an objective measure of nasal symptoms for children, similar to those used to measure lower airway function, but unfortunately no such devices are at present available (17). We depend upon the parents' perception of the child's

health status. We are of the view, however, that systematic bias in parental reports of symptoms was unlikely because there were no reports of airborne concentrations of *Alternaria* in the public domain. Furthermore, *Alternaria* had not been previously perceived as a risk factor for rhinoconjunctivitis.

There were no differences between seasons in the visits to the general practitioner for allergic rhinoconjunctivitis over the study period, although the children used medication for rhinoconjunctivitis symptoms. It is possible that the question measuring medication use was poorly designed. Parents may have visited a GP for lower airway symptoms, for example, and at the same time received treatment for eye and nose symptoms. Furthermore, parents may have treated their child using nonprescriptive medications products (18), for which we have no information. In addition, it is a well-known clinical experience that persons often seek medication well in advance or use medication prescribed from previous years. The relation between seeking medical prescriptions, and symptoms may be also be complex (19).

Some questions appeared to be more sensitive than others in measuring symptoms from *Alternaria* exposure. Nasal symptoms during the night seemed to be more important than symptoms during the day. Possibly this relates to mechanisms by which the fungus causes symptoms. It could also reflect changes in exposure although this seems unlikely as the principle sources of exposure to the fungus are outside and a child's exposure is likely to be highest during the day.

Alternaria spores are smaller in diameter than pollen, and range in size from 2 to 8 μm in diameter (3). The smaller size of *Alternaria* spores may allow the spores to pass more easily through the nasal airways into the lower airways than pollen. On the other hand, compared with other fungi such as *Cladosporium*, *Alternaria* spores are large and this could indicate that *Alternaria* is likely to be a more potent cause of rhinoconjunctivitis than other fungi.

In conclusion, we have demonstrated that the proportion of children sensitized to *Alternaria* who report symptoms of rhinoconjunctivitis and use anti-allergic medication for the upper airways, increase when *Alternaria* spore concentrations increase in the ambient air. The results indicate that children with an allergy to this fungus are at the risk of developing not only lower (10), but also upper respiratory tract symptoms when fungus spore levels are high in ambient air.

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