REVIEW

From allergen sources to reduction of allergen exposure

J.C. Bessot, F. de Blay, G. Pauli

From allergen sources to reduction of allergen exposure. J.C. Bessot, F. de Blay, G. Pauli. ©ERJ Journals Ltd. 1994.

ABSTRACT: Allergen exposure plays a major role in the development of sensitization, inflammation and symptomatic asthma. The development of new means of detecting allergens has enabled the evaluation of the effect of a reduction in allergen exposure to be better assessed. In order to prevent allergen exposure it is necessary to determine the allergen sources and reservoirs to which avoidance measures should be directed. The allergen sources and reservoirs of mite, cat, dog, cockroach, rodent, mould and pollen allergens are briefly reviewed.

The relationships between allergen exposure, sensitization, nonspecific bronchial hyperreactivity and asthma symptoms are discussed. Recognition of the risks, environmental control, and reduction in allergen loads should be among the objectives of asthma management.

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Numerous studies have demonstrated an increasing prevalence and severity of asthma in certain countries [1]. Reasons for this increase are not fully known, but an increased allergen load could be partially responsible. For genetically predisposed subjects, allergen exposure is a risk factor for sensitization because the relationship between allergen exposure and asthma has now been more accurately documented [2]. Recognition of this risk is essential. Allergens also play a major role in the development of inflammation and symptomatic asthma. The therapeutic implication is that allergen avoidance could prevent the onset of asthma in some atopic children, and could play an important role in the management of asthma.

The development of new means of detecting allergens (*i.e.* immunochemical assays involving monoclonal antibodies [3], quantitative and semiquantitative guanine measurements for mite allergens [4]), has made it possible to identify thresholds for the risk of sensitization and symptom expression for the major aeroallergens. Allergen sources and reservoirs, can now be more precisely determined, objective quantitative data concerning exposure can be obtained, and the effects of a reduction in allergen exposure better evaluated.

Allergen sources and reservoirs

Mite allergens

Two major groups of mite allergens have been identified. Group I allergens are 25 kD, thermolabile proteins, primarily found in mite faeces. Group II allergens

are essentially of somatic origin, have molecular weights of 15 kD, and are heat-resistant proteins. The ratio of Group I to Group II allergens in faeces ranges from 20–33 [5]. Group I and II mite allergens are proteolytic enzymes. Biochemical studies have demonstrated Der p I to be a cysteine protease [6], and Der p II a lysozyme. Moreover, amylase activity was found in extracts of Dp whole mite growth medium and correlated with Der p I concentrations [7]. Group III and Group IV allergens have also been reported. Der p III and Der f III have also been demonstrated to be proteinases [8], although belonging to the serine group of proteinases.

Mattresses are the major reservoir of house dust mite allergens. Their amounts are not dependent upon the type of mattress [9]. Carpets constitute another important allergen reservoir. DYBENDAL *et al.* [10] have shown that carpets and floors accumulated more dust, proteins and allergens per unit area than smooth floors. Pillows, blankets and all textile-based objects are also ecological niches occupied by mites. It has been suggested that concentrations of allergen measured in settled dust are less representative than concentrations of airborne allergens to which people are exposed [11]. In the air, Group I and Group II allergens are mainly carried by particles larger than 10 µm which only become airborne when disturbed *e.g.* by vacuum cleaning [12, 13].

Cat allergens

The major cat allergen (*i.e.* Fel d I) is found mainly in the sebaceous glands of the skin, but also in the salivary glands [14–16]. Fel d I is a tetrameric polypeptide,

with a molecular weight of 35–39 kD on size exclusion high-performance liquid chromatography (HPLC) and 17–18 kD in sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) [17]. It is especially abundant in dust from carpets and upholstered furniture. Cat allergens are so widespread that they are part of our daily environment; there is hardly a dwelling without Fel d I in its dust. In contrast with mite allergens, cat allergens remain suspended in the air for extended periods of time, even without disturbance. They are carried by different-sized particles, some of which have an aerodynamic diameter less than 2.5 μm [18, 19]. Various factors determine the concentrations and airborne behaviour of cat allergens. We were able to show that within 30 min of entering a 30 m³ clean room, a cat was found to increase airborne Fel d I by 30–90 ng·m⁻³ [19]. The presence of a carpet in a room results in a significant increase in the airborne concentrations of Fel d I, in comparison with the same room with a bare floor. Increased ventilation modifies the distribution of particles carrying Fel d I, favouring those with aerodynamic diameter greater than 10 μm [19].

After removal of the cat from the home, Fel d I levels in house dust remain high for a long period [20]. Even following regular routine cleaning of a home, cat allergen levels take 12–16 weeks to fall below the levels associated with asthma [21].

Dog allergens

Dog allergens are found mainly in skin, but are also present in serum and saliva [22]. A major allergen, Can f I, has been purified [23]. Can f I is a polypeptide with a molecular weight of 22–25 kD. Albumin is another allergen of some importance, the molecule is considered responsible for cross reaction between cat and dog extracts [24]. Assays based on monoclonal or monospecific antibodies have shown Can f I levels to be one order of magnitude above those of Fel d I *i.e.* up to 10 ng·g⁻¹ of dust. The concentration of the allergens may vary as much within as between breeds. It has been shown that dog allergens could be present in high concentrations even where dogs have no access, for instance in schools [25]. On average, levels of dog allergens are 100 times greater in dust from homes with a dog than those without [26].

Cockroach allergens

Cockroach allergens are important sensitizing agents, especially in populations with asthma living in large economically deprived urban areas [27, 28]. This allergen seems to have a less important role in Europe than in the USA. The prevalence of cutaneous sensitization to cockroach in atopic patients is about 10% in Europe, 53% in the USA and 46% in Asia [29]. Two major allergens have been identified: Blg I and Blg II.

Cockroach allergens are found mainly in the exoskeleton as well as in faeces [30–32]. The highest concentrations of cockroach allergens are present in

dust from kitchen floors and cupboards [33, 34]. The size and type of the particles carrying cockroach allergens are still unknown.

Rodent allergens

In rodents, the major allergen sources are urine, fur, saliva and skin [35–37]. The major allergens in rat and mouse urine have been identified [38]. Rat n IA is a prealbumin with a molecular weight of 21 kD. Rat n IB is an alpha-2-globulin with a molecular weight of 16 kD. The major allergen from mouse is Mus m I, a prealbumin with a molecular weight of 18–19 kD. A second important allergen is Mus m II, a glycoprotein with a molecular weight of 16–21 kD. Significant allergen levels have been demonstrated in both litter and floor dust. Rodent allergens are carried by particles with aerodynamic diameters ranging from 0.8–5 μm [39, 40].

Mould allergens

Moulds are part of the allergens found in the indoor and outdoor environments. The abundance and variety of household moulds contrast with the small number of fungal allergens known and tested [41]. The major allergens of *Alternaria* (Alt a I), *Cladosporium* and *Aspergillus* have been identified [42, 43]. Moulds are capable of growing on the surface or inside most substrates of plant or animal origin. Air itself is not a habitat for moulds, but most moulds rely on dispersal by air currents. Warm and humid spells permit optimal mould growth.

Pollen allergens

Although fungal spores are far more frequent than pollen allergens, pollen allergens are more clearly implicated in allergic diseases. Occasionally, they may be a component of house dust. In subjects sensitized to pollen, there appears to be a clear relationship between pollen levels and symptoms. However, the part played by pollen in provoking asthma has been a subject of wide debate, since the sizes of most airborne pollens range from 20–60 μm . It is now known that pollen allergens may be carried by submicronic particles. Studies by SOLOMON *et al.* [44] on *Ambrosia* and by SPIEKMA *et al.* [45] on *Gramineae* have confirmed the presence of airborne allergenic determinants in fractions of submicronic dimensions. SUPHIOGLU *et al.* [46] investigated the mechanism by which rye grass pollen causes asthma, and concluded that starch granules released from rye-grass pollen are micronic particles, and that these particles cause asthma.

Relationship between allergen exposure, sensitization, nonspecific bronchial hyperreactivity and symptoms

The role of allergens in sensitization

The presence of allergens in the environment is an obvious and necessary requisite for sensitization to occur.

The massive introduction or transplantation of new plant species into regions previously lacking them has resulted in new sensitizations: this happened with Ambrosiaceae in the Turin region, Hungary, Romania and Yugoslavia, and Cupressaceae in the Mediterranean region. The sudden increase in asthma in Kuwait, which started in the 1950s, has been attributed to the import of *Prosopis*, the pollen of this tree being considered responsible for numerous sensitizations [47].

The percentage of positive skin tests to *Dermatophagoides* is lowest at high altitude, where the content of major mite allergens is also less [48]. It has been shown [49] that levels of specific immunoglobulin E (IgE) against mites are higher if the environmental levels of major *Dermatophagoides* allergens are greater than $2 \mu\text{g}\cdot\text{g}^{-1}$ of dust.

Studies performed prior to the 1950s had shown that of all mammalian allergens, those of horses and cattle were mainly responsible for sensitization, whereas allergies to cats, dogs and other household pets (such as various rodents) were infrequent. In 1960, RYSSING [50] was still able to show that 25% of 460 asthmatic children had positive skin tests to horse. The situation has reversed (at least in urban settings), due to a doubling presence of household pets over a 20 year period, their presence in confined spaces, and the increase in their allergen density.

The earlier the exposure, the greater seems the risk of sensitization. The first year of life appears to be an especially vulnerable period, as has been suggested by studies relating sensitization to date of birth [51]. WARNER *et al.* [52] have demonstrated a correlation in infants between concentrations of major Group I mite allergen and major cat allergen (*i.e.* Fel d I) and the presence of positive skin test and specific IgE to mites and cat, respectively.

A combination of genetic and environmental factors contributes to sensitization and to the phenotypic expression of respiratory allergy. In one controlled study, involving 70 mite-sensitized patients presenting with asthma and/or rhinitis and 113 control subjects, 90% of house dust samples had major mite allergen levels greater than $2 \mu\text{g}\cdot\text{g}^{-1}$, which is considered to be a threshold level for sensitization [53]. The absence of significant differences between patients and controls underlines the importance of the genetic background. In a case-control study, WICKMANN *et al.* [54] showed a significant difference in terms of mite allergen exposure levels, between mite-sensitized asthmatic children and atopic asthmatic children not sensitized to mites. YOUNG *et al.* [55] have demonstrated that for siblings, the levels of major *Dermatophagoides* Der p I allergens in mattress dust determined the development of mite allergy only in those with an atopic background.

The role of allergens in nonspecific bronchial hyperreactivity (NSBHR) and asthmatic symptoms

In 1970, ALTOUNYAN [56] showed that in patients allergic to grass pollen NSBHR increased during the pollen

season and diminished with its end. Similarly, one observes an increased NSBHR following a specific-allergen bronchial provocation test [57]. SEARS *et al.* [58] have shown that NSBHR due to histamine varied according to the degree of allergen sensitization. Thus, sensitization to animal allergens (*i.e.* cat, dog) or to *Aspergillus fumigatus* resulted in a relative risk of NSBHR >10 . The role played by mites in triggering asthma or rhinitis is not always easily demonstrated. CHARPIN *et al.* [48] found a decreased prevalence of mite asthma and rhinitis when previous exposure to their allergens had been less pronounced. A classic example of the impact of allergen pressure is the increase from 0.1 to 7.3% in the prevalence of asthma in certain regions of Papua; regions with a greater prevalence of asthma also had a greater number of mites- g^{-1} house dust [59]. A prospective study involving 67 children has shown that in 19 out of 20 who still had asthma at the age of 10 yrs, exposure levels of Der p I during the first year of life had been greater than $10 \mu\text{g}\cdot\text{g}^{-1}$ of dust [60]. Certain mite-allergic asthmatics exhibit asthma in one dwelling, whereas they are asymptomatic in another. A study with patients meeting these selection criteria, has allowed us to show that symptom occurrence in a particular dwelling depended upon major mite allergen levels, as indirectly evaluated by a guanine assay [61]. Several authors [62, 63] have shown that inhalation of Fel d I levels between 8 and 80 ng by cat-sensitized asthmatic subjects was likely to provoke bronchospasm. Similarly, high concentrations of major cockroach allergens (*i.e.* Bla g I, Bla g II) are found in the dust from homes of cockroach-sensitized patients with asthma and perennial rhinitis [34].

When the causes of sudden and severe asthma attacks are listed, exposure to perennial or seasonal allergens is seldom included. However, O'HOLLAREN *et al.* [64] concluded, in a retrospective study of 11 asthmatics, aged 11–25 yrs, who had suffered respiratory arrest during the *Alternaria* sporulation season, that exposure to *Alternaria* resulted in a 200 fold greater risk of respiratory arrest. This work refers to several other studies from Argentina, New Orleans and Northern California, where there has been a marked correlation between the number of hospital emergency visits by asthmatics and local climatological conditions, including mould spore counts. GELBER *et al.* [65] have investigated the role of indoor allergens in 114 adult asthmatic patients presenting to an emergency room. Thirty eight percent of the asthmatics, but only 8% of the control subjects, were allergic to one of three indoor allergens (dust mite, cat and cockroach allergens) and had high levels of the relevant allergens in their homes.

Both the occurrence of asthma epidemics and the decrease in the number of cases once the incriminated allergen has been identified and eliminated, confirm the influence of allergen pollution upon asthma, and the need to reduce levels. Indeed, an epidemic of soybean dust-related asthma in Barcelona from 1981 to 1987 resulted in 958 emergency room admissions, and over 15 deaths [66, 67]. Since the soybean dust-related asthma outbreak was reported in Barcelona, similar situations have been found in other harbours [68].

If allergens favour sensitization, symptom development and severity, as well as the increase in NSBHR, their reduction should have the opposite effect. Residence at high-altitude represents proven therapy for mite asthma. Following a 9 month stay at high altitude, VERVLOET *et al.* [69] found a parallel between the clinical improvement and a significant drop in serum IgE specifically directed against Dermatophagoides. Allergen eradication is likely to result in diminished NSBHR which is proved to be lower 4 months after the grass pollen season than it is during the season [70, 71]. NSBHR also decreases in mite-allergic asthmatics after a stay at high altitude or in an environment equally poor in mite allergens (72–75). When allergen eradication is possible, it should be performed at an early stage. ARSHAD *et al.* [76] have found that reduced exposure of infants to allergens in house dust lowered the frequency of allergic disorders in the first year of life.

Almost every foreign protein that accumulates in a home can give rise to sensitization and asthma. However, allergy to domestic mites has a central role in the development of asthma and the maintenance of NSBHR. Mite avoidance appears to be a therapeutic option in patients at risk. Complete eradication of dust mites and their allergens is up to now impossible [77]. However, mite allergen concentrations can be reduced by a combination of methods using acaricides [78, 79], with methods to separate mites from the sleeper by encasing mattresses, and further systems for decreasing humidity in the indoor environment [80]. Domestic pets, especially cats, are also a leading cause of asthma. It is clear that atopic patients should avoid keeping domestic pets. Airborne cat allergens can also be dramatically reduced by combined measures: washing the cat, reducing furnishing, vacuum cleaning and air filtration [19].

In conclusion, it is necessary to determine allergen sources and reservoirs, which are the main target of avoidance measures. The problem is a difficult one, but it is not insurmountable. The example provided by occupational asthma shows that despite eradication, over half the subjects still exhibit bronchial hyperreactivity and symptoms. This unfavourable evolution may be imputed to an excessively long exposure. One can certainly try to influence the sometimes unfavourable evolution by accurate aetiological diagnosis. Although the need for such a diagnosis is not questioned in occupational asthma, its value in extrinsic asthma to indoor or outdoor allergens is not always recognized, at a time when one is more concerned with treating the resulting inflammation than with identifying and eliminating the cause. Recognition of the risks, environmental control and reductions in allergen loads should remain part of the therapeutic goal in the management of asthma.

References

1. Woolcock A.J. Evolution de la morbidité et de la mortalité de l'asthme dans le monde. Explication de l'évolution. *Bull Union Int Tuberc Mal Resp* 1991; 66: 91–96.
2. Sporik R, Chapman M, Platts-Mills T. House dust mite exposure as a cause of asthma. *Clin Exp Allergy* 1992; 2: 897–906.
3. Chapman M, Heymann P, Wilkins S, Brown, Platts-Mills T. Monoclonal immunoassays for major dust mite (Dermatophagoides) allergens, Der p I and Der f I and quantitative analysis of the allergen content of mite and house dust extracts. *J Allergy Clin Immunol* 1987; 80: 184–194.
4. Pauli G, Hoyet C, Tenabene A, le Mao J, Thierry R, Bessot JC. Guanine and mite allergenicity in house dust. *Clin Allergy* 1988; 18: 383–392.
5. Report of the Second International Workshop. Dust mite allergens and asthma. *J Allergy Clin Immunol* 1992; 89: 1040–1060.
6. Chua K, Stewart G, Thomas W, Simpson R, Dilworth R, Plozza T. Sequence analysis of cDNA coding for a major house dust mite allergen, Der p I: homology with cysteine proteases. *J Exp Med* 1988; 167: 175–182.
7. Lake F, Ward L, Simpson R, Thompson P, Stewart G. House dust mite-derived amylase: allergenicity and physicochemical characterization. *J Allergy Clin Immunol* 1991; 87: 1035–1042.
8. Stewart G, Thompson P, Simpson R. Protease antigens from house dust mite (Letter). *Lancet* 1989; ii: 154–155.
9. van Bronswijk E. House dust biology. Thesis. The Netherlands, NIB Publishers. 1981; pp. 105–129.
10. Dybendal T, Wedberg N, Elsayed S. Dust from carpeted and smooth floors. *Allergy* 1991; 46: 427–435.
11. Price J, Pollock I, Little S, Longbottom J, Warner J. Measurement of airborne mite antigen in homes of asthmatic children. *Lancet* 1990; 336: 895–897.
12. Tovey E, Chapman MD, Platts-Mills T. Mite faeces are a major source of house dust allergens. *Nature* 1981; 289: 592–593.
13. de Blay F, Heymann P, Chapman MD, Platts-Mills T. Airborne dust mite allergens: comparison of group II allergens with group I mite allergen and cat-allergen Fel d I. *J Allergy Clin Immunol* 1991; 88: 919–926.
14. Ohman JL, Lowell FC, Bloch KJ. Allergens of mammalian origin. III. Properties of a major feline allergen. *J Immunol* 1974; 113: 1668–1677.
15. Bartholome K, Kissler W, Baer H, Kopietz-Schulte E, Wahn U. Where does cat allergen I come from? *J Allergy Clin Immunol* 1985; 76: 503–506.
16. Charpin C, Mata P, Charpin D, Lavaut M, Allasia C, Vervloet D. Fel d I allergen distribution in cat fur and skin. *J Allergy Clin Immunol* 1991; 88: 77–82.
17. Duffort O, Carreira J, Nitti G, Polo F, Lombardero M. Studies on the biochemical structure of the major cat allergen *Felis domesticus* I. *Mol Immunol* 1991; 28: 301–309.
18. Luczynska C, Li Y, Chapman M, Platts-Mills T. Airborne concentrations and particle size distribution of allergen derived from domestic cats (*Felis domesticus*). *Am Rev Respir Dis* 1990; 141: 361–367.
19. de Blay F, Chapman M, Platts-Mills T. Airborne cat allergen (Fel d I): environmental control with the cat *in situ*. *Am Rev Respir Dis* 1991; 143: 1334–1339.
20. van der Brempt X, Charpin D, Haddi F, Da Mata P, Vervloet D. Cat removal and Fel d I levels in mattresses. *J Allergy Clin Immunol* 1991; 87: 595–596.
21. Wood R, Chapman M, Adkinson N, Eggleston P. The effect of cat removal on Fel d I content in household dust samples. *J Allergy Clin Immunol* 1989; 83: 730–734.

22. Schou C. Defining allergens of mammalian origin. *Clin Exp Allergy* 1993; 23: 7–14.
23. Schou C, Svendsen U, Loewenstein H. Purification and characterization of the major dog allergen, Can f I. *Clin Exp Immunol* 1991; 21: 321–328.
24. Boutin Y, Hebert H, Vrancken E, Mourad W. Allergenicity and cross-reactivity of cat and dog allergenic extracts. *Clin Allergy* 1988; 18: 287–293.
25. Dybendal T, Vik H, Elsayed S. Dust from carpeted and smooth floors. II. Antigenic and allergenic content of dust vacuumed from carpeted and smooth floors in schools under routine cleaning schedules. *Allergy* 1989; 44: 401–411.
26. Schou C, Hansen G, Lintner T, Loewenstein H. Assay for the major dog allergen, Can f I: Investigation of house dust samples and commercial dog extracts. *J Allergy Clin Immunol* 1991; 88: 847–853.
27. Kang B, Jones J, Johnson J, Kang I. Analysis of indoor environment and atopic allergy in urban population with bronchial asthma. *Ann Allergy* 1989; 62: 30–34.
28. Lan J, Lee D, Wu C, Chang C, Yeh C. Cockroach hypersensitivity: preliminary study of allergic cockroach-asthma in Taiwan. *J Allergy Clin Immunol* 1988; 82: 736–740.
29. Bidat E, Chevalier MC, Croisier C, Guerin L, Guerin B, Scheinmann P. L'apparition de la blatte dans la poussière de maison. *Rev Fr Allergol* 1993; 33: 22–29.
30. Richman PG, Khan HA, Turkeltau P, Malveaux F, Baer H. The important sources of German cockroach allergens as determined by RAST analysis. *J Allergy Clin Immunol* 1984; 73: 590–595.
31. Lehrer S, Horner W, Menon P, Stankus R. Comparison of cockroach allergenic activity in whole body and fecal extracts. *J Allergy Clin Immunol* 1991; 87: 574–580.
32. Zwick H, Popp W, Sertl K, Rauscher H, Wanke T. Allergenic structures in cockroach hypersensitivity. *J Allergy Clin Immunol* 1991; 87: 626–630.
33. Pollarts S, Smith T, Morris F, Gelber L, Platts-Mills T, Chapman M. Environmental exposure to cockroach allergens: analysis with monoclonal antibody based enzyme immunoassays. *J Allergy Clin Immunol* 1991; 87: 505–510.
34. de Blay F, Kassel O, Ott M, Verot A, Pauli G. Mise en évidence des allergènes majeurs des blattes (Blattella germanica) par test ELISA dans la poussière domestique d'asthmatiques allergiques aux blattes. *Presse Med* 1992; 35: 1685.
35. Lutsky I, Fink J, Kidd J, Dahlberg M, Yunginger J. Allergic properties of rat urine and pelt extracts. *J Allergy Clin Immunol* 1985; 75: 279–284.
36. Walls A, Newman Taylor A, Longbottom J. Allergy to guinea-pigs: I. Allergenic activities of extracts derived from the pelt, saliva, urine and other sources. *Clin Allergy* 1985; 15: 241–251.
37. Ohman J, Lowell F, Bloch K. Allergens of mammalian origin. II. Characterization of allergens extracted from rat, mouse, guinea-pig and rabbit pelt. *J Allergy Clin Immunol* 1975; 55: 16–24.
38. Price J, Longbottom J. Allergy to mice. I. Identification of two major mouse allergens (Ag 1 and Ag 3) and investigation of their possible origin. *Clin Allergy* 1987; 17: 43–53.
39. Platts-Mills T, Heymann P, Longbottom J, Walkins S. Airborne allergens associated with asthma: particle sizes carrying dust mite and rat allergens measured with a cascade impactor. *J Allergy Clin Immunol* 1986; 77: 850–857.
40. Swanson M, Agarwal M, Yunginger J, Reed C. Guinea-pig-derived allergens. Clinicoimmunologic studies, characterization, airborne quantitation and size distribution. *Am Rev Respir Dis* 1984; 129: 844–849.
41. van Bronswijk J, Rijckaert G, van de Lustgraaf B. Indoor fungi, distribution and allergenicity. *Acta Bot Neerl* 1986; 35: 329–345.
42. Yunginger J, Jones R, Nesheim M, Geller M. Studies on Alternaria allergens. III. Isolation of major allergenic fraction (ALT. I). *J Allergy Clin Immunol* 1980; 66: 138–147.
43. Paris S, Debeaupuis JP, Prevost MC, Casotto M, Latge JP. The 31 Kd major allergen ALT a I of *alternaria alternata*. *J Allergy Clin Immunol* 1991; 88: 902–908.
44. Solomon W, Burge H, Muilenberg F. Allergen carriage by atmospheric aerosol. I. Ragweed pollen determinants in smaller micronic fractions. *J Allergy Clin Immunol* 1983; 72: 443–447.
45. Spielsma F, Kramps J, van der Linden A, et al. Evidence of grass-pollen allergenic activity in the smaller micronic atmospheric aerosol fraction. *Clin Exp Allergy* 1990; 20: 273–280.
46. Suphioglu C, Singh M, Taylor P, et al. Mechanism of grass-pollen-induced asthma. *Lancet* 1992; 339: 569–572.
47. Davies RJ. Environmental allergy. *Allergy* 1988; 43: 23–25.
48. Charpin D, Birnbaum J, Haddi E, et al. Altitude and allergy to house dust mites. A paradigm on the influence of environmental exposure on allergic sensitization. *Am Rev Respir Dis* 1991; 143: 983–986.
49. Lau S, Falkenhorst G, Weber A, Werthmann I, Buttner-Cotz P, Wahn V. High mite-allergen exposure increases the risk of sensitization in atopic children and young adults. *J Allergy Clin Immunol* 1989; 84: 718–725.
50. Ryssing E. Allergy to horse dander in asthmatic children. *Acta Allerg (Kbh)* 1960; 15: 315–318.
51. Quoix E, Bessot JC, Kopferschmitt-Kubler MC, Fraisse Ph, Pauli G. Positive skin tests to aeroallergens and month of birth. *Allergy* 1988; 43: 127–131.
52. Warner J, Little S, Pollock I, Longbottom J, Warner J. The influence of exposure to house dust mite, cat, pollen and fungal allergens in the home on primary sensitization in asthma. *Pediatr Allergy Immunol* 1991; 1: 79–86.
53. Pauli G, Quoix E, Hedelin G, Bessot JC, Ott M, Dietemann A. Mite allergen content in mattress dust of Dermatophagoides-allergic asthmatics/rhinitics and matched controls. *Clin Exp Allergy* 1993; 23: 516–522.
54. Wickman M, Nordvall S, Pershagen G, Sundell J, Schwartz B. House dust mite sensitization in children and residential characteristics in a temperate region. *J Allergy Clin Immunol* 1991; 88: 89–95.
55. Young RP, Hart BJ, Merret TG, Read AF, Hopkin JM. House dust mite sensitivity: interaction of genetics and allergen dosage. *Clin Exp Allergy* 1992; 22: 205–211.
56. Altounyan REC. Changes in histamine and atropine responsiveness as a guide to diagnosis and evaluation of therapy in obstructive airways disease. In: Pepys J, Frankland AW, eds. Disodium Cromoglycate in allergic airways disease. London, Butterworth, 1970; pp. 47–53.
57. Cockcroft DW, Ruffin RE, Dolovitch J, Hargreave FE. Allergen-induced increase in non-allergic bronchial reactivity. *Clin Allergy* 1977; 7: 503–513.
58. Sears MR, Herbison GP, Holdaway MD, Hewitt CJ, Flannery EM, Silva PA. The relative risks of sensitivity to grass pollen, house dust mite and cat dander in the development of childhood asthma. *Clin Exp Allergy* 1989; 84: 718–725.
59. Turner KJ, Stewart GA, Woolcock AJ, Green W, Alpers

- MP. Relationship between mite sensitivities and the prevalence of asthma: comparative studies in two populations in the Eastern Highlands of Papua New Guinea. *Clin Allergy* 1988; 18: 331–340.
60. Sporik R, Holgate ST, Platts-Mills TAE, Cogswell J. Exposure to house dust mite allergen (Der p I) and the development of asthma in childhood: a prospective study. *N Engl J Med* 1990; 323: 502–507.
 61. de Blay F, Pauli G, Velten M, Bessot JC. Influence of mite exposure on symptoms of mite-sensitive asthmatics. *J Allergy Clin Immunol* (In press).
 62. Findlay SR, Stotsky E, Leiterman K, Hemady Z, Ohman JL. Allergens detected in association with airborne particles capable of penetrating into the peripheral lung. *Am Rev Respir Dis* 1983; 128: 1008–1012.
 63. van Metre T, Marsh D, Adkinson F, *et al.* Dose of cat (*Felis domesticus*) allergen I (Fel d I) that induces asthma. *J Allergy Clin Immunol* 1986; 78: 62–75.
 64. O'Holleran M, Yunginger J, Offord K, *et al.* Exposure to an aeroallergen as a possible precipitating factor in respiratory arrest in young patients with asthma. *N Engl J Med* 1991; 324: 359–363.
 65. Gelber L, Seltzer L, Bouzoukis J, Pollart S, Chapman M, Platts-Mills T. Sensitization and exposure to indoor allergens as risk factors for asthma among patients presenting to hospital. *Am Rev Respir Dis* 1993; 147: 573–578.
 66. Rodrigo M, Morell F, Helm R, *et al.* Identification and partial characterization of the soybean-dust allergens involved in the Barcelona asthma epidemic. *J Allergy Clin Immunol* 1990; 85: 778–784.
 67. Picado C. Barcelona asthma epidemics: clinical aspects and intriguing findings. *Thorax* 1992; 47: 197–200.
 68. Alvarez Dardet C, Belda J, Pena N, Nolasce A. Outbreak of asthma associated with soybean dust. *N Engl J Med* 1989; 321: 1127–1128.
 69. Vervloet D, Bongrand P, Arnaud A, Boutin C, Charpin J. Données objectives cliniques et immunologiques observées au cours d'une cure d'altitude à Briançon chez des enfants asthmatiques allergiques à la poussière de maison et à Dermatophagoides. *Rev mal Respir* 1979; 7: 19–27.
 70. Barbato A, Pisetta F, Ragusa A, Marcer G, Zacchello F. Modification of bronchial hyperreactivity during pollen season in children allergic to grass. *Ann Allergy* 1987; 58: 121–124.
 71. Prieto L, Berto JM, Lopez M, Peris A. Modifications of PC₂₀ and maximal degree of airway narrowing to methacholine after pollen season in pollen sensitive asthmatic patients. *Clin Exp Allergy* 1993; 23: 171–178.
 72. Kerrebijn K. Endogenous factors in childhood CNSLD. In: Orie NGM, van der Lende R, eds. *Bronchitis III*. Assen, The Netherlands, Van Gorcum, 1970; pp. 38–48.
 73. Platts-Mills TAE, Tovey E, Mitchell E, Moszoro H, Nock P, Wilkins S. Reduction of bronchial hyperreactivity during prolonged allergen avoidance. *Lancet* 1982; ii: 675–678.
 74. Dorward A, Colloff M, MacKay N, Sharry M, Thomson N. Effects of house dust mite avoidance measures on adult atopic asthma. *Thorax* 1988; 43: 98–102.
 75. Ehnert B, Lau-Schadendorf S, Weber A, Buettner P, Schou C, Wahn U. Reducing domestic exposure to dust mite allergen reduces bronchial hyperreactivity in sensitive children with asthma. *J Allergy Clin Immunol* 1992; 90: 135–138.
 76. Arshad S, Matthews S, Gant C, Hide D. Effect of allergen avoidance on development of allergic disorders in infancy. *Lancet* 1992; 339: 1493–1497.
 77. Gillies D, Littlewood J, Sarsfield J. Controlled trial of house dust mite avoidance in children with mild to moderate asthma. *Clin Allergy* 1987; 17: 105–111.
 78. Kniest F, Young E, van Praag M, *et al.* Clinical evaluation of a double-blind dust mite avoidance trial with mite-allergic rhinitic patients. *Clin Exp Allergy* 1991; 21: 39–45.
 79. Dietemann A, Bessot JC, Hoyet C, Ott M, Verot A, Pauli G. A double-blind placebo-controlled trial of solidified benzyl benzoate applied in dwellings of asthmatic patients sensitive to mites: clinical efficacy and effect on mite allergens. *J Allergy Clin Immunol* 1993; 91: 738–746.
 80. Pauli G, Bessot JC, Dietemann A, de Blay F. Prevention of mite asthma. In: Godard Ph, Bousquet J, Michel FB, eds. *Advances in Allergology and Clinical Immunology*, Vol. 1. New Jersey, USA, The Parthenon Publishing Group, 1992; pp. 425–425.