Sensitisation, asthma, and a modified Th2 response in children exposed to cat allergen: a population-based cross-sectional study

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Summary

Background Although asthma is strongly associated with immediate hypersensitivity to indoor allergens, several studies have suggested that a cat in the house can decrease the risk of asthma. We investigated the immune response to cat and mite allergens, and asthma among children with a wide range of allergen exposure.

Methods We did a population-based cross-sectional study of children (aged 12–14 years), some of whom had symptoms of asthma and bronchial hyper-reactivity. Antibodies to mite (Der f 1) and cat (Fel d 1) allergens measured by isotype (IgG and IgG4) specific radioimmunoprecipitation assays were compared with sensitisation and allergen concentrations in house dust.

Findings 226 children were recruited, 47 of whom had symptoms of asthma and bronchial hyper-reactivity. Increasing exposure to mite was associated with increased prevalence of sensitisation and IgG antibody to Der f 1. By contrast, the highest exposure to cat was associated with decreased sensitisation, but a higher prevalence of IgG antibody to Fel d 1. Thus, among children with high exposure, the odds of sensitisation to mite rather than cat was 4·0 (99% CI 1·49–10·00). Furthermore, 31 of 76 children with 23 μg Fel d 1 at home, who were not sensitised to cat allergen had >125 units of IgG antibody to Fel d 1. Antibodies to Fel d 1 of the IgG4 isotype were strongly correlated with IgG antibody in both allergic and non-allergic children (r=0·84 and r=0·66, respectively). Sensitisation to mite or cat allergens was the strongest independent risk factor for asthma ($p<0·001$).

Interpretation Exposure to cat allergen can produce an IgG and IgG4 antibody response without sensitisation or risk of asthma. This modified T-helper-2 cell response should be regarded as a form of tolerance and may be the correct objective of immunotherapy. The results may also explain the observation that animals in the house can decrease the risk of asthma.

Lancet 2001; **357:** 752–56
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Introduction

The fact that sensitisation of asthmatic children reflects the allergens found in different climatic areas has been taken as evidence that exposure to allergens plays an important part in the disease.[1–6] For dust-mite allergens there is good evidence for a dose-response link between exposure and both sensitisation and asthma.[1,2,7] By contrast, recent population-based studies have suggested that having a cat in the house could decrease the risk of sensitisation and asthma.[8–11] Simple explanations for this finding include the possibility that families affected by allergy avoid having animals in the house, or that the measurements of cat allergen do not adequately reflect exposure of the respiratory tract. An alternative explanation for the effect of animals in the home is that high amounts of animal products, such as antigens and endotoxins, can protect against the development of allergy. Thus, the effect of animals in the home could be seen as evidence for the hypothesis that increasing cleanliness has led to increased allergic disease because of a shift in the immune system from a T-helper-1 cell (Th1) to a T-helper-2 cell (Th2) bias.[12–15] However, research has shown that extended and high-dose exposure to occupational or injected allergens can induce an increase in IgG and IgG4 antibodies with a decrease in IgE antibodies,[16,17] and that expression of the gene for IgG4 can be induced by the Th2 cytokine interleukin 4 (IL-4).[18,19] Thus, a response including IgG4 without IgE antibody, could be regarded as a modified Th2 response. However, to date there has been no evidence that documents tolerance or any other form of immune response among non-allergic children with high exposure to one of the allergens associated with asthma.

In a population-based cross-sectional study of school children in the USA, we have previously reported that increasing concentrations of cat allergen (by contrast with mite allergen) did not increase the risk of sensitisation to cat allergen.[3,10,20] In that study the children were exposed to a very wide range of dust mite (Der f 1 and Der p 1) or cat (Fel d 1) allergens. Subsequent analysis of that data has shown that the significant difference in the response to cat allergen was a decreased risk of sensitisation among children exposed to greater than 20 μg Fel d 1/g dust. This finding meant that there were a large number of atopic and non-atopic children who had documented high exposure to cat allergen but who were not allergic. The current study was designed to answer whether these children showed serological evidence of an immune response to Fel d 1. The results for isotype specific antibodies of children in our study were related to evidence of sensitisation, exposure, and the risk of asthma.

Methods

Children in grades seven and eight (age 12–13 years) of three middle schools were tested for lung function and answered a questionnaire in school. The schools were in Los Alamos, New Mexico; Albemarle County, Virginia; and Charlottesville, Virginia; and were chosen because they represent a wide range of climatic and socioeconomic conditions. On the basis of this screening children with symptoms or a history of asthma were identified.[3,10,20] All

the children identified as symptomatic by questionnaire, and an equal number of random controls from the same cohort were skin tested and challenged with histamine to define bronchial hyper-reactivity (BHR) as described previously.[20] Children were considered hyper-responsive if at the maximal cumulative dose of 3·9 μmol histamine or before the forced expiratory volume in 1 s (FEV_1) decreased by 20% of the post-saline value. Skin testing was carried out in a medical clinic by the prick technique with lancets, with extracts of *Dermatophagoides pteronyssinus*, *D farinae*, cat dander, mixed cockroach extract, grass pollen, and ragweed pollen.[3,20] A wheal size of 4×4 mm was regarded as positive.[3,10,20] Asthma was defined as symptomatic BHR.[1–3,20] Written consent was obtained from the parents for skin testing, blood, and bronchial provocation. The studies were approved by the Human Investigation Committee of the University of Virginia. Dust samples were obtained from four different areas within the children's homes and assayed for dust mite (Group 1 mite allergen) and cat allergen (Fel d 1) as described previously.[3,20,21] In accordance with the international workshop report, the values were expressed as μg/g and the highest value found in the home for each allergen was taken as the index of exposure.[22]

Serum assays

IgE antibodies to cat and dust mite were measured by means of a quantitative radioallergosorbent test (RAST). Values ≥40 RAST units were considered positive and one RAST unit had been estimated to be ~0·1 ng IgE antibody.[6,20] For the analysis a child was defined as sensitised when either they had a skin test ≥4 mm or a RAST ≥40 units/mL.[3,20] IgG antibodies to the purified allergens, Der f 1 and Fel d 1, were measured with a radioimmunoprecipitation assay.[17,23] Briefly, 6 ng of Fel d 1 (or Der f 1) radiolabelled with ^{125}I was added to 0·1 mL of serum diluted 1/25 or 1/50. After 4 h, 0·1 mL of polyspecific goat antibodies to human IgG (diluted 1:4) was added. The precipitate was allowed to form overnight and washed three times by centrifugation. Each assay was carried out in parallel with a control curve with serum containing 4100 units with binding activity for Fel d 1.[21,23] The goat antibodies to human IgG bind each of the IgG isotypes equally, so the results reflect the total IgG antibodies. IgE antibodies to Fel d 1 were assayed by a similar technique using IgE myeloma serum (PS) as a source of carrier IgE and goat antibody to human IgE to precipitate IgE.[23] Antibodies specific for Fel d 1 of the IgG4 isotype (a subset of the IgG antibodies) were measured by a double antibody modification of the IgG assay, by means of a mouse monoclonal antibody to human IgG4 (Lot # BYAS 1121, Clone # HP 6025 [Accurate Chemical and Scientific Co, Westburg, NY, USA]). The antibodies were precipitated using goat antibody to mouse IgG, which had been repeatedly absorbed over human IgG bound to activated Sepharose (Pharmacia, Kalamazoo, MI, USA).[17] The units for IgG antibody and IgG4 antibody to Fel d 1 used here were estimated to represent 0·1 ng and 0·03 ng of Fel d 1 binding activity/mL. In preliminary experiments precipitating antibodies were tested for by the Ouchterlony technique using cat extract (1:10 weight to volume; Hollister-Stier, Spokane, WA, USA) containing 16 μg Fel d 1/mL and rabbit antibody to Fel d 1 as a positive control. None of the serum samples tested, including the 40 samples with the highest titres of IgG antibody to Fel d 1, had detectable precipitins.

The cat allergen (Fel d 1) was purified from cat extract by affinity chromatography, with the monoclonal antibody FdIA.[21] The antigen eluted at pH 3·9 was further purified by size exclusion chromatography. Dust-mite allergen Der f 1 was purified from extract of *D farinae* by means of affinity chromatography with the monoclonal antibody 4C1B8.[22]

Statistical analyses

χ^2 tests for trend were used to analyse links between exposure to dust mite or cat allergen in the home and sensitisation or antibody responses. Exposure groups were formed by dividing the patient sample into tertiles or sextiles. Univariable and multivariable logistic regression analyses were used to distinguish associations with asthma. Logistic regression was also used to model the binary response of mite sensitisation relative to cat sensitisation and their interaction. The Huber-White method was used to adjust variances to correct for correlated responses for mite and cat allergen coming from the same house.[24] Associations between IgG and IgG4 responses to Fel d 1 were assessed with Spearman rank correlations. All analyses were done with S-PLUS 4.5 (MathSoft, Seattle, WA, USA).

Results

226 children were recruited to the study (117 boys, 109 girls), of whom 49 were African American and 47 had symptoms and BHR. Serum samples were available from all of them. Assessment of sensitisation to cat or mite allergen was based on RAST or skin tests because we wished to identify all the children who were allergic. In accordance with previous results, sensitisation to mite increased with increasing exposure[1,7,22] (table 1). On examination the results and the published data we found that the difference between the response to the two allergens was primarily due to a decreased number of children with evidence of sensitisation to cat in the high-exposure group (table 1). With logistic regression to model the sensitisation by two allergen types, three exposure groups and their interactions showed that the odds of being sensitised to mite compared with cat was significantly increased in the high-exposure group (odds ratio 4·0 [99% CI 1·49-10·00]). By contrast, the odds of being sensitised to mite or cat was not significantly different among children with low (1·63 [0·58–4·61]) or moderate exposure (1·36 [0·58–3·2]). We found no qualitative difference in logistic regression results when we adjusted variances to correct for correlated responses coming from the same school rather than from the same student's house. Thus, the dose-response link between cat exposure and

House-dust mite	Group 1 mite allergen (μg/g dust)*			p
	0–0·92 (n=76)	0·92–14·4 (n=76)	14·4–129 (n=74)	
Sensitised to mite†	18	24	28	0·06
IgG-positive Der f 1‡	8	16	24	0·02
IgG positive/sensitised	3	11	21	<0·0001
IgG positive/not sensitised	5	5	3	ns
Atopic	47	36	39	ns
Cat	**Cat allergen (Fel d 1 μg/g dust)‡**			**p**
	0–1·7 (n=75)	1·7–23·0 (n=75)	23·0–3840 (n=76)	
Sensitised to cat†	12	19	10	ns
IgG-positive Fel d 1‡	13	24	41	<0·0001
IgG positive/sensitised	10	6	10	ns
IgG positive/not sensitised	3	18	31	<0·0001
Atopic	39	41	42	ns

*Group 1 mite allergen is the highest concentration of Der f 1 plus Der p 1 found in the home, Fel d 1 is the highest concentration of cat allergen found in the home.
†RAST ≥40 units and/or skin test ≥4 mm weal. ‡≥200 units for Der f 1 and ≥125 units for Fel d 1. NS=not significant.

Table 1: **Relationship of sensitisation-specific IgG antibody, and exposure**

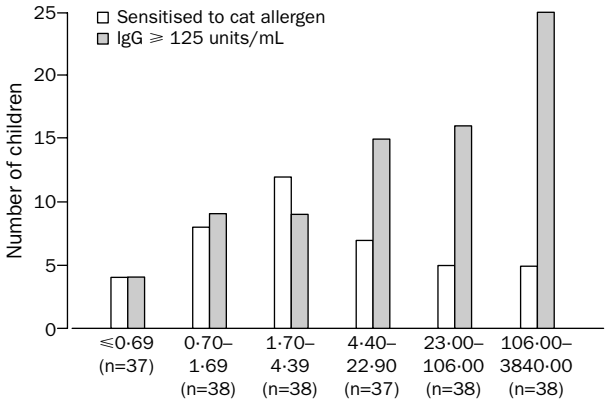

Figure 1: **Prevalence of sensitisation to cat allergens and of IgG antibody to Fel d 1 ⩾125 units/mL for six equal-exposure groups for cat allergen**

The range of exposure to Fel d 1 in μg Fel d 1 μg and the number of children in each group is shown. The number of atopic children in each group starting with the lowest exposure group was 17, 22, 23, 18, 22, and 20.

sensitisation was not the same as the link between mite exposure and sensitisation.

The results for IgG antibodies to Fel d 1 or Der f 1 for children in different allergen exposure groups were compared with results for sensitisation to mite or cat (table 1). The prevalence of IgG antibody to Der f 1 increased with exposure in parallel with sensitisation. There were 13 children who had IgG antibody to Der f 1 who were not sensitised to mite allergen and these children were present in each exposure group. Serum IgG to Fel d 1 also increased with exposure. However, the prevalence of sensitisation to cat allergen was decreased in the highest exposure group, and in this group there were 31 children who had IgG antibody to Fel d 1 without evidence of sensitisation. The prevalence of atopy amongst children was similar in each of the three exposure groups (for both of the allergens; table 1). The results for IgG antibodies to Fel d 1 were further analysed in relation to sensitisation to cat allergen for six exposure groups (figure 1). The correlation between exposure to cat allergen and the prevalence of IgG was strong (χ^2 test for trend, p<0·001 for each group). In the three highest exposure groups there were 41 children who had IgG antibody to Fel d 1 but were not sensitised to cat allergen; of these, 21 were atopic as

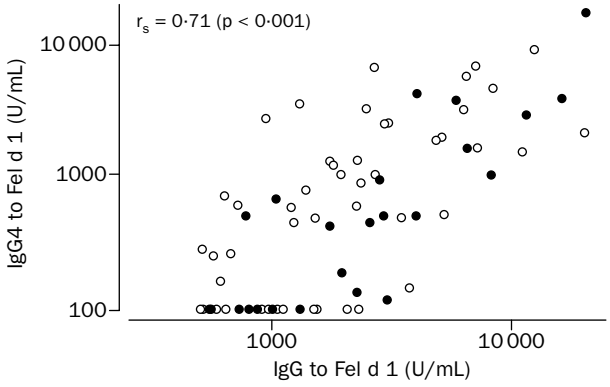

Figure 2: **Correlation between IgG and IgG4 antibodies to Fel d 1 serum samples from sensitised (closed circles) and non-sensitised (open circles) individuals**

The values for IgG are a measurement of the total IgG antibodies to Fel d 1 and the unit 0·1 ng Fel d 1 bound/mL. The units for IgG4 are 0·03 ng Fel d 1 bound/mL, Spearman rank correlation between IgG and IgG4 for serum samples with >125 units IgG/mL was 0·7; p=0·001.

Category	Asthma (n=47)	No asthma (n=179)	Odds ratio (99% CI)
Sensitisation			
Dust mite*	30	40	6·1 (2·5–15·2)
Cat*	18	23	4·2 (1·6–11·0)
Dust mite or cat*	34	49	6·9 (2·7–17·8)
Serum IgG antibody			
IgG+ to Der f 1†	21	27	4·5 (1·8–11·5)
IgG+ to Fel d 1†	18	60	1·2 (0·5–2·9)
IgG+ to Der f 1 or Fel d 1†	31	73	2·8 (1·2–6·8)
Total IgE ⩾100 IU/mL	33‡	69	4·0 (1·6–10·3)
Quantity of allergen			
Dust mite ⩾2 μg/g§	30	96	1·5 (0·6–3·6)
Cat ⩾8 μg/g‖	18	79	0·8 (0·3–1·9)
Dust mite ⩾2 μg/g or cat ⩾8 μg/g	35	131	1·1 (0·4–2·8)

*RAST ⩾40 units/mL and/or skin prick ⩾4 mm. †⩾200 units/mL for Der f 1 and ⩾125 units/mL for Fel d 1. ‡IgE for 46 or 47 children. §⩾2 mg group 1 mite allergen/g of dust.
‖⩾8 μg Fel d 1/g of dust.

Table 2: **Univariate logistic regression analysis of risk factors for asthma**

judged by the presence of a positive skin test to one of the other allergens tested. The serum samples were also assayed for IgE antibody to Fel d 1 and the results correlated strongly with the values for RAST to cat antigen (r=0·62; p<0·001).

To further investigate the response to cat allergen the serum samples with the highest titres of IgG antibodies to Fel d 1 were assayed for IgG4 antibody to Fel d 1 by radioimmunoassay. The correlation between IgG4 and IgG antibodies to Fel d 1 was similar whether the children were sensitised to cat or not (figure 2). The correlation between IgG4 and IgG was 0·84 for cat-sensitised children and 0·66 for non-sensitised children (p<0·001) for each group. With values for the quantity of Fel d 1 bound by the antibodies, we estimated the contribution of IgG4 antibodies to the total IgG antibodies. The quantity of IgG4 varied widely from less than 5% to greater than 50%. Among the 54 children with detectable IgG4 antibodies to Fel d 1, the IgG4 was on average 20% of the IgG antibodies to Fel d 1.

In this cohort, 47 of the 226 children had asthma, defined as symptomatic BHR. Sensitisation to cat or mite allergens was associated with asthma; odds ratios 4·2 and 6·1 respectively (table 2). The presence of IgG antibodies to mite allergens was also significantly associated with asthma. By contrast, IgG antibodies to Fel d 1 were not associated with asthma. In accordance with this, the presence of IgG antibodies to Fel d 1 in the absence of sensitisation was not associated with asthma, odds ratio 0·63 (0·25–1·50). The risk factors for asthma were analysed by multivariate logistic regression analysis, with three models (table 3). In the first model, sensitisation to

Category*	Model 1 (dust mite)	Model 2 (cat)	Model 3 (combination)
Sensitisation			
Dust mite	p<0·01	..	..
Cat	..	p=0·01	..
Dust mite or cat	..	..	p<0·001
Serum IgG antibody			
IgG+ to Der f 1	p=0·09	..	..
IgG+ to Fel d 1	..	p=0·63	..
IgG+ to Der f 1 or Fel d 1	..	..	p=0·25
Total serum IgE	p=0·10	p<0·01	p=0·31
Quantity of allergen			
Dust mite exposure ⩾2 μg/g	p=0·74	..	..
Cat exposure ⩾8 μg/g	..	p=0·72	..
High dust mite or cat exposure	..	..	p=0·83

*For cut-off values see table 2 footnotes.

Table 3: **Multivariate logistic regression analysis of risk factors for asthma**

mite was significant but IgG to Der f 1, total IgE, and mite allergen (2 μg) were not. In the second model, sensitisation to cat and total IgE were independent risk factors but neither IgG antibodies to Fel d 1, nor cat allergen exposure, were independently related to asthma. In the third model sensitisation to either allergen was compared with IgG antibody to either Fel d 1 or Der f 1, total serum IgE, and exposure to high concentrations of either allergen. In this model only sensitisation remained significant (p=0·0007).

Discussion

The immune response to common inhalant allergens includes IgG and IgA antibodies, as well as the IgE antibodies that give rise to sensitisation.[23,25] In all epidemiological studies the aspect of this immune response that had been used to investigate the association with asthma is immediate hypersensitivity judged by skin tests or serum IgE antibodies. Extended exposure, either naturally or during immunotherapy, can progressively increase the expression of the IgG4 isotype.[16,17,26,27] Our results establish that a large proportion of children with high exposure to cat allergen make an IgG antibody response, including IgG4, without being allergic and without a risk of asthma. The quantity of IgG4 measured represents a large proportion of the IgG response as compared with results for allergic patients, total serum IgG4 values; and the IgG4 response to tetanus toxoid which is generally less than 1% of the IgG.[16] There are two features of the IgG4 isotype that are relevant to the interpretation of these results. First, the primary cytokine that induces the gene for IgG4 in human B cells is IL-4.[18,19] Thus, an antibody response with a high proportion of IgG4 should be regarded as similar to a Th2 response. Second, IgG4 antibodies do not give rise to precipitins because they are functionally monovalent.[28] Thus, the fact that none of the serum samples with high titre IgG antibody to Fel d 1 showed visible precipitins against cat extract is in keeping with the finding that a large proportion of the IgG antibody was IgG4.

In our cohort, most of the children with the lowest exposure to cat allergen had no detectable IgG antibodies. Furthermore, they did not report symptoms on exposure to cats, and they did not have any detectable skin-test response. The most likely explanation for these results is that these individuals had not made an immune response to cat allergens. Similarly, children raised in Los Alamos, New Mexico, who had very low, or no exposure to dust-mite allergens, and no IgG or IgE antibodies to mite allergens, had probably not made a response to this antigen. It is important to recognise that the measurement of current exposure is being used here as a surrogate for the measurement of cumulative or lifetime exposure. However, the strong correlation between increasing exposure and IgG supports the relevance of the exposure measurements. High prevalence of IgG antibodies to allergens has previously been reported among animal handlers and beekeepers.[16,17] In both situations extended high-dose exposure seems to produce an IgG and IgG4 antibody response. Among animal handlers, IgG antibody to rat urinary allergen without IgE can develop over a few years. Beekeepers who receive multiple stings may similarly develop IgG and IgG4 antibodies without IgE.[16,27]

The range of cat exposure in our cohort was wide (figure 1). We have previously reported that most houses with a cat have >8 μg Fel d 1 per g of dust, but that occasional houses without a cat have concentrations as high as 80 μg Fel d 1 per g of dust.[3] Concentrations of cat allergen as low as 0·6 μg per g are only common in a community where very few families keep a cat in the house.[4,6,20] The results establish that the dose-response link between exposure and sensitisation for cat allergen is different from the dose response for mite allergen. The results suggest that the maximum prevalence of sensitisation to cat occurred with moderate exposure. Previous results have shown that moderate exposure to cat allergen can result in sensitisation of a significant proportion of the population.[3,7,8,10] Previous reports such as that of the MAS study[7] appear to conflict with our results, since they observed a linear dose-response link between cat exposure and sensitisation.[7] However, the maximum concentrations of Fel d 1 they found in the homes they sampled were 1–10 μg/g dust. These values are not only much lower than our high exposure group, but also much lower than those reported from the UK and New Zealand.[29] Indeed, the results suggest that measurement of exposure in μg of Fel d 1 per g of dust is a better predictor of the immune response than the reported presence of a cat in the house. The possibility that allergic families had chosen not to keep cats is not supported by our finding that the proportion of atopic children was similar in each of the exposure groups shown in table 1 and figure 1.[8] The high prevalence of IgG antibody to Fel d 1 without sensitisation (almost 20% in our study), suggest that this non-allergic response is the explanation for the decreased prevalence of sensitisation to cat allergen that has now been observed in studies from Scandinavia and New Zealand, as well as the USA.[8–11] The results from the European Community Respiratory Health Survey (ECRHS)[9] provide another estimate of the scale of the phenomenon we are investigating. In the centres from New Zealand and Australia, 50% of the families reported owning a cat, but the prevalence of sensitisation to cat was only 10% compared with value of 30% to dust mite.[9] Thus, the prevalence of cat sensitisation was less than a third of the prevalence that would be predicted if the dose-response link for cats was the same as that for mite.

Our results do not support the general recommendation that families should avoid having a cat in order to prevent sensitisation of their children. However, in our data, high exposure appears to be protective for some children and a risk factor for others, and it is possible that this difference is genetically controlled. The reasons why an IgG antibody response to Der f 1 without IgE antibodies to mite is much less common than for cat allergen are not clear. The concentrations of Der f 1 were lower than those for Fel d 1, however this difference alone is not sufficient to explain the results. Given the high concentrations of mite (and cat) allergens reported from New Zealand, one would expect to see some evidence for tolerance to mite allergens among children raised in that country.[9,29] The fact that it is possible to measure these allergens in absolute units (ie, μg) should not be taken to imply that the biological potency of 1 μg Fel d 1 is equal to 1 μg of Der f 1. Given the fact that cat allergen, unlike mite allergen, is persistently airborne in houses, the quantity of cat allergen inhaled is possibly far higher than the quantity of mite allergens inhaled. Alternatively, it is well established that this biochemical activity influences the humoral response to these proteins.[22,30] Understanding the mechanisms by which cat allergen can induce a "tolerant" immune response, or alternatively the reasons why dust-mite allergen does not, may be of great importance in understanding the factors that influence the prevalence of allergic disease in the more-developed world.

The observation that children exposed to cats or other animals in their houses are less likely to have asthma or a positive skin test has been interpreted as evidence supporting the cleanliness hypothesis. Our results could be

seen as evidence for that hypothesis in its most general form.[12] However, the hypothesis has been extended to suggest that the effect of decreased exposure to animal products and infections early in life is to bias the immune response towards a Th2 response, possibly secondary to decreased IL-12 production.[13–15,31] Our results are not in accord with this view because IgG4 antibodies are part of a Th2 response, and the effect we have seen appears to be antigen specific. Our results cast major doubts over whether changing prevalence of asthma and allergic disease can be explained simply by a shift in the balance of Th1 and Th2. These findings provide indirect evidence that it is the IgE part of the Th2 response that predisposes children to asthma. The mechanisms by which responses of the IgG4 isotype occur without IgE antibodies are not clear. However, there is good evidence from in-vitro studies that IL-10, together with IL-4, can enhance IgG4 production, while suppressing IgE.[32] A role for IL-10 has also been proposed in the mechanism of desensitisation to bee venom.[27] Although many of the so-called tolerant children have IgG and IgG4 to Fel d 1 the current data do not show whether these IgG antibodies play a part in blocking antigen or are simply markers of a different immune response. Our resuslts cast major doubts over whether changing prevalence of asthma and allergic disease can be explained simply by a shift in the balance of Th1 and Th2. Our results support the proposal that inducing an immune response in early childhood could be a target for preventing asthma. However, we would stress that the response seen here does not have the characteristics of a Th1 response. In accordance with this view, cat (and dust mite) allergens do not induce delayed hypersensitivity skin responses, or diseases such as hypersensitivity pneumonitis. Inducing Th1 responses to common allergens may not be an appropriate objective either of immunotherapy or as a preventive strategy.[33]

Contributors

T Platts-Mills planned the overall study and wrote the paper. J Vaughan and J Woodfolk designed and carried out assays for isotype specific antibodies. S Squillace planned and carried out and analysed the school studies in Virginia. R Sporik designed and supervised the school study in New Mexico, as well as contributing to the analyses of the data. All the authors contributed to the writing of the paper and the analysis of the data.

Acknowledgments

We thank Keven Blumenthal for help with purifying the antigens and Martin Chapman for advice in antigen purification. We thank Brent Evans for statistical assistance and Nancy Malone for preparing the paper. This research is supported by grants from the NIH: AI-20565 and NIEHS/NIAID AI-34607.

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