Early immune responses to *Dermatophagoides pteronyssinus* and atopic predisposition

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Abstract

Responses to the house dust mite during infancy may be important determinants of asthma in susceptible individuals. This study assessed early IgG subclass antibody responses to *Dermatophagoides pteronyssinus* in children of atopic parents. Sixteen atopic and 15 non-atopic children were selected from a birth cohort, and atopic status was established according to follow up over the first two years. IgG₁ and IgG₄ antibodies to *D pteronyssinus* were measured by enzyme linked immunosorbent assay at 7 days and 3, 6, 12, and 24 months. In all children *D pteronyssinus* IgG₁ fell at 3 months (indicating maternal antibody loss), rose progressively to 12 months, and waned at 24 months. *D pteronyssinus* IgG₄ was only detectable at 7 days. Children who were atopic by 2 years and therefore at greater risk of asthma, tended to have higher *D pteronyssinus* IgG₁ at 6 and 12 months. These data suggest greater exposure or responsiveness to dust mite during infancy than in the second year.

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House dust mite sensitivity has long been associated with asthma.¹ A causal relationship has been inferred by the influence of the degree of mite exposure on the prevalence of asthma.²³ Exposure during infancy may be particularly important in determining subsequent sensitisation to aeroallergens.⁴ Children with mite sensitivity are more likely to have been born in a season when mite counts were high,^{5 6} and higher levels of mite exposure in infancy have recently been reported among children who have persistent wheezing at 11 years.⁷

While much research has concentrated on measuring mite exposure, it is the individual response to this 'allergen load' that determines whether sensitisation ultimately occurs. There may be threshold levels of exposure for sensitisation¹ but individuals with a similar genetic predisposition to atopy need not produce analogous immune responses to the same allergen load.

During the first year of life children are capable of mounting IgE antibody responses but they are usually to ingested allergens.^{8 9} IgE and IgG responses to aeroallergens are reported to be comparatively uncommon before the second year of life.⁸ It would be difficult to reconcile the importance of mite exposure during infancy with an apparent lack of immune responsiveness to the allergen at this time. We have recently demonstrated IgG₁ subclass anti-

bodies to the dust mite, *Dermatophagoides pteronyssinus*, during the first year in most members of a group of 'unselected' children.¹⁰ The discrepancy with previous findings may be explained by our use of an enzyme linked immunosorbent assay (ELISA) with whole *D pteronyssinus* extract instead of a radioimmunoassay for antibody to *D pteronyssinus* I.⁸ The latter assay was probably less sensitive as antibodies to *D pteronyssinus* I may not represent the majority of dust mite antibodies at this age; they apparently constitute less than half of newly generated IgE antibodies to *D pteronyssinus* in children.¹¹

The present study aimed to measure IgG subclass antibody responses to whole *D pteronyssinus* by ELISA in the first two years in subjects selected from a large cohort of children with a predisposition to atopy. The responses were compared between children with the most obvious evidence of atopy during the first two years, who were at greater risk of subsequent asthma,¹² and those who were most clearly non-atopic despite their predisposition.

Subjects and methods

COHORT DETAILS

All cohort members had been term neonates born between September 1987 and July 1989 in the Camberwell Health Authority. They had been identified by a history of atopic disease in both parents on antenatal questionnaires. If informed parental consent was obtained and atopy could be confirmed in at least one parent by a positive skin prick test, the infants were enrolled.¹³

They were assessed initially on day 7 when a blood sample was taken, and then at 3, 6, 12, and 24 months to document atopic disease, perform skin prick tests, and obtain further blood samples. Additional examinations were made when coughs or suspicious rashes were reported.

Eczema diagnosed according to the guidelines of Hanifin and Rajka¹⁴ but modified for infants,¹⁵ wheezing on at least two separate occasions, and a history of recurrent immediate food reactions were each regarded as evidence of atopic disease.

Skin prick tests to dust mite (*D pteronyssinus*), cows' milk, hens' eggs, mixed grass pollens, cat fur (Bencard), mixed moulds (Dome/Hollister-Stier), and histamine 1 mg/ml and diluent control solutions (Bencard) were performed on the back in children and the forearm in parents. A weal after 15 minutes at least 2 mm in children, or 3 mm in adults, greater than that

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caused by the negative control was considered positive.

Sera were stored at -20°C and subsequently analysed for total serum IgE antibody by an ultrasensitive ELISA¹⁶ in 7 day sera and by conventional two site ELISA¹⁷ in all other samples. Undetectable concentrations were assigned a value of 0.01 IU/ml and the geometric mean and SD were calculated for each age group, although 7 day concentrations continued to have a very skewed distribution after log transformation.

The ethical committee of King's College Hospital had approved the method of follow up and all procedures.

SUBJECT DETAILS

Eighty of the original cohort completed the two year follow up. The first 16 (eight girls) classed as atopic and the first 15 (seven girls) considered non-atopic were selected for the study. The number of subjects was limited so that all samples from each age could be assayed together on a single microtitre plate thus eliminating interassay variability.

Children classed as atopic had: (i) positive skin tests to any allergen and (ii) evidence of atopic disease. Children were considered non-atopic if they met all of the following three criteria: (i) negative skin tests, (ii) a total IgE concentration less than 0.5 IU/ml at 7 days and below the geometric mean plus 1 SD at any other age, and (iii) no evidence of atopic disease.

Of the 16 atopic children 11 had suffered with eczema (fig 1) and five had given positive skin tests to *D pteronyssinus*.

LABORATORY MATERIALS

Nunc Immuno (MaxiSorp F96) microtitre plates were purchased from Gibco Ltd, horse serum from Sera Lab Ltd, Tween 20, alkaline phosphatase labelled rabbit antimouse IgG and *p*-nitrophenyl phosphate (PNP) from Sigma Ltd and IgG subclass-specific mouse monoclonal antibodies (clones NL16-IgG1, RJ4-IgG4 and 8a4-IgG) from Oxoid Ltd. Freeze dried whole dust mite extract (*D pteronyssinus*) was a kind gift from Dr R Whal (Allergopharma, Reinbeck, Germany).

IgG SUBCLASS ASSAYS

IgG₁ and IgG₄ antibodies to *D pteronyssinus* were

Figure 1 Categorisation of the 16 atopic children by evidence of atopic disease.

measured by a two site ELISA¹⁸ in sera from 7 days, and 3, 6, 12, and 24 months in all 31 children. Incubation volumes were 50 μl and temperatures were 4°C unless stated otherwise. There were three washes with phosphate buffered saline (PBS) containing 0.05% Tween 20 between each incubation. The assay diluent was PBS containing 0.5% Tween 20 and 0.5% normal horse serum. Briefly, the microtitre plates were coated overnight with 10 $\mu\text{g/ml}$ *D pteronyssinus* extract in pH 9.6 carbonate/bicarbonate buffer. The test sera, diluted 1/50, were added, in duplicate and incubated for two hours. There followed in sequence one hour incubations with monoclonal IgG₁ or IgG₄ (1/1000) and alkaline phosphatase-labelled antimouse IgG (1/300). Finally, 100 μl of the substrate, PNP (1 mg/ml in pH 9.8 diethanolamine buffer) were added and after one hour at room temperature, the colour reaction was stopped with 50 μl 3M sodium hydroxide and the optical density read at 405 nm in a Titertek multiskan plate reader (Flow Labs). Antibody concentrations were derived from a standard curve obtained using an IgG panreactive monoclonal antibody (clone 8a4) with dilutions of a high positive reference serum which was assigned 10 000 arbitrary units/ml (AU/ml) when undiluted.¹⁸

STATISTICAL ANALYSIS

Even after log transformation *D pteronyssinus* IgG₁ antibody concentrations were not normally distributed at every age. However the log ratios of the concentrations at successive ages in each child did have normal distributions. Changes in *D pteronyssinus* IgG₁ concentrations between two ages were therefore given as the geometric mean ratio of the concentrations at the two ages with a 95% confidence interval. The corresponding one sample *t* tests gave the probability of the observed means if the true mean log ratio was zero (that is, if the ratio was 1); $p < 0.0125$ was taken as the level of significance to allow for multiple comparisons (Bonferroni method). The Mann-Whitney test was used to analyse differences within age groups. The probability level for a significant difference was adjusted to $p < 0.01$ to allow for five comparisons. Finally a sample size of 31 was theoretically able to have an 80% chance of detecting a significant difference at the 1% level of about 1.2 times the value of a SD. Bearing in mind that any SDs would be tentatively derived from actual study data with a less than perfect distribution, this meant that one might expect to detect differences down to 40–70 AU/ml, except at 7 days when variability was greatest.

Results

Every child had detectable concentrations of IgG₁ antibody to *D pteronyssinus* at all ages (fig 2). Concentrations were highest at 7 days, reached a nadir at 3 months, rose progressively up to 12 months but declined at 24 months. The 95% confidence intervals for the geometric mean ratio of concentrations at consecutive ages was calculated. One could be 95% sure that on average 12 month concentrations were between

Figure 2 Concentrations of IgG₁ antibody to *D pteronyssinus* in five atopic children with dust mite sensitivity, 11 other atopic children, and 15 non-atopic children during the first two years of life. Median concentrations in the atopic and non-atopic subgroups at each age are indicated by the horizontal bars.

1.43 to 2.40 times higher than concentrations at 24 months, with 1.86 times as the best estimate (table 1).

Atopic children tended to have higher *D pteronyssinus* IgG₁ concentrations than non-atopic counterparts at 6 and 12 months, with $0.01 < p < 0.05$ (table 2). A trend for higher concentrations in the five dust mite sensitive individuals compared with the other 26 children was only seen at 3 months, with $p = 0.03$ (fig 2). The six children who had recurrent wheezing had no tendency towards having higher values at any age.

IgG₄ antibody to *D pteronyssinus* was only found in 7 day sera and then in only three children. All three had mothers who had given florid skin reactions to *D pteronyssinus* with weals of 6 mm, 8 mm, and 11 mm in diameter respectively.

Table 1 Ratio of *D pteronyssinus* IgG₁ antibody concentrations between successive age groups

<i>D pteronyssinus</i> IgG ₁ concentration ratio	Geometric mean ratio	95% Confidence interval	p Value*
7 day:3 months	5.15	4.27 to 6.21	<0.0001
3 months:6 months	0.50	0.38 to 0.69	0.0001
6 months:12 months	0.70	0.56 to 0.89	0.0040
12 months:24 months	1.86	1.43 to 2.40	<0.0001

*One sample *t* test.

Table 2 Comparison of median (range) *D pteronyssinus* IgG₁ antibody concentrations between atopic and non-atopic children

Age	<i>D pteronyssinus</i> IgG ₁ antibody concentration (AU/ml)		p Value*
	Atopic children (n=16)	Non-atopic children (n=15)	
7 day	76.5 (32-914)	93 (29-344)	0.45
3 months	16 (12-71)	16 (11-62)	0.87
6 months	53.5 (15-210)	25 (15-68)	0.03
12 months	59 (27-283)	44 (17-197)	0.04
24 months	26 (12-149)	20 (13-119)	0.54

*Mann-Whitney test.

Discussion

The validity of this study depends on the specificity of the assay for IgG subclass antibodies to *D pteronyssinus*. IgG₁ antibody binding to *D pteronyssinus* could be substantially inhibited by preincubation of the children's sera with the *D pteronyssinus* extract and cross reactivity with a different antigen (ovalbumin) was minimal. This implied the antibodies had reasonable affinity and specificity. The assay was therefore regarded to measure genuine *D pteronyssinus* IgG₁ antibodies in the children's sera.

The pattern of change in *D pteronyssinus* IgG₁ concentrations parallels changes in total IgG up to one year. The fall in concentrations at 24 months, however, suggests a specific immune response. A possible explanation for the fall is a reduction in the time spent maximally exposed to the antigen, as actual mite counts are unlikely to have altered drastically in every child's environment between the first and second years. Alternatively it could imply a switch in the subclass of IgG after prolonged exposure. IgG₄ would be the obvious alternative subclass¹⁹ but no IgG₄ antibodies to *D pteronyssinus* were detected in any child at 24 months.

Measurements made at 3 months and before reflect maternal rather than the infants' own antibodies. Rises in specific IgG₁ do occur between 7 days and 3 months, such as in IgG₁ to casein,²⁰ indicating an infant response at this time. A similar response to the dust mite may be obscured by relatively high concentrations of maternal mite specific antibody in these children. By 6 months, and certainly by 12 months, most maternal antibody will have cleared. Measurements at these times are therefore a better indicator of the infant response. It may be that responsiveness to the dust mite is greatest in infancy and begins to wane thereafter.

IgG₄ antibodies to *D pteronyssinus* had been undetectable at all time points during the first year in the study of 'unselected' children.¹⁰ The present findings were similar. *D pteronyssinus* IgG₄ was only found in 7 day sera and every child with detectable concentrations had a mother who was allergic to the dust mite, reflecting the higher concentrations that might be expected in such mothers.²¹ These antibodies may take longer to appear. They are certainly not present in all adults.²¹

Prospective documentation of atopic manifestations in the large cohort of children allowed the present study to focus on the most obviously atopic and non-atopic individuals in an attempt to clarify possible differences in the response to the house dust mite.

Although the differences in *D pteronyssinus* IgG₁ concentrations between atopic and non-atopic children at 6 and 12 months gave $0.01 < p < 0.05$, the statistical significance of these concentrations was interpreted cautiously in the light of multiple comparisons. The power calculation, however, indicated that significant differences smaller than 40-70 AU/ml might not be picked up by a study of the present size. Secondly the measurements were analysed according to atopic status as a means of grouping the children into those who were most likely

and least likely to develop asthma. Differences in the *D pteronyssinus* antibody response may have been more apparent had it been possible to identify the future asthmatic children with greater precision.

It has been suggested that aeroallergen exposure in early infancy may represent a critical time in determining subsequent sensitisation and disease manifestation.²² The present data intimates that *D pteronyssinus* IgG₁ antibody responses may be maximal during infancy. The highest IgG₁ responses at 6 months and thereafter were not, however, seen in those who developed IgE sensitisation to the dust mite (fig 2). Dust mite sensitivity was based on data from the first two years of life. Out of 80 children in the cohort followed up to two years, only six had given positive skin prick tests to *D pteronyssinus* of which five were in the present study. Clearly, many more of the children are likely to develop dust mite sensitivity when they are older.

This study demonstrates that IgG antibody responses to *D pteronyssinus* during the first two years in children predisposed to atopic disease are predominantly in the IgG₁ subclass. Higher *D pteronyssinus* IgG₁ concentrations at one year than at two years may reflect greater exposure or responsiveness. Finally the tendency for higher *D pteronyssinus* IgG₁ concentrations at 6 and 12 months in children with atopic manifestation in the first two years may become more clear cut in later years when analyses are made according to the presence of asthma.

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