

The IgG subclass antibody response to an inhalant antigen (*Dermatophagoides pteronyssinus*) during the first year of life: evidence for early stimulation of the immune system following natural exposure

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Summary

This study investigates the early humoral immune response following natural exposure to an inhalant antigen (*Dermatophagoides pteronyssinus*) in 36 babies, from birth until 1 year of age. The total IgG and subclass 1 and 4 *D. pteronyssinus*-specific antibody levels were assayed in sera collected at 7 days, 3 and 12 months by ELISA. After an initial fall, due to the progressive loss of maternal antibodies, an IgG specific response to *D. pteronyssinus* was seen between 3 and 12 months. This was restricted to the IgG1 subclass when the values at 12 months were significantly higher than those detected at the third month ($P < 0.001$, paired *t* test). No *D. pteronyssinus*-specific IgG4 antibody was detected in any subject at any of the time points tested. The present study demonstrates that inhalant as well as food antigens are able to stimulate the immune system during the first year of life and that the antibodies produced are of the IgG1 subclass.

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Introduction

A number of recent studies have investigated the response of the immune system to food antigens in babies. Foods constitute a substantial antigenic load for the maturing immune system and certainly appear to be the most common cause of sensitization in the first 12 months of life [1–8].

However, the gut is not the only route by which antigens are able to enter the body and exposure to inhalant antigens, such as *D. pteronyssinus*, may constitute an important source of antigen for the respiratory tract mucosa later in life. Little is known about the humoral immune response to inhalant antigens in early infancy or its relevance to the subsequent development of allergic disease.

Some studies have detected an IgG1 and IgG4 subclass

antibody response following natural exposure to *D. pteronyssinus* both in adults and in children [9]. IgE and IgG specific for food and inhalant antigens have been detected in adults [8] and babies [1, 10] but thus far little is known about the early humoral response to inhalant antigens. The aim of the present study was to determine whether babies mount an IgG response to *D. pteronyssinus* during the first year of life and if so to identify the subclass of IgG involved.

Materials and methods

Subjects studied

The patients studied form part of a larger study previously described elsewhere [10]. For a defined period all mothers of newborn infants at Kings College and Dulwich Hospitals, London, were invited to participate in the study, 76% agreed. Ethical permission for this study was obtained. Blood samples were collected from all babies at 7 days and at 3 and 12 months. Sera were

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separated and stored at -20°C . Of the original sera only 36 were available in sufficient amounts for the present study.

Materials

Nunc immuno-I microtitre plates were purchased from Gibco Ltd (Paisley, Scotland, U.K.). Horse serum was purchased from Sera Lab. Ltd (Crawley, U.K.). Tween 20, alkaline phosphatase-labelled rabbit anti-mouse IgG and *p*-nitrophenyl phosphate were purchased from Sigma Ltd (Poole, Dorset, U.K.). IgG subclass-specific mouse monoclonal antibodies (clones 8a4-IgG, NL16-IgG1, RJ4-IgG4) were purchased from Oxoid Ltd (Bedford, U.K.). House dust mite freeze dried extract (*D. pteronyssinus*) was a kind gift from Dr Whal, Allergopharma, Reinbeck, Germany.

Methods

Specific total and subclasses IgG ELISA

Specific IgG, IgG1 and IgG4 antibodies to *D. pteronyssinus* were measured by ELISA [11–13]. *D. pteronyssinus* extract diluted to $10\text{ }\mu\text{g/ml}$ in pH 9.6 carbonate/bicarbonate buffer was used to coat the microtitre plates (Nunc maxisorb) at 4°C overnight. All incubations were with $100\text{ }\mu\text{l}$ at 4°C , except the last which was at room temperature and between each incubation there were three washes with phosphate-buffered saline (PBS, pH 7.4 0.1 M containing 0.05% Tween 20). The assay diluent was PBS containing 0.5% Tween 20 and 0.5% normal horse serum. The test sample (1:50) was added and after 2 hr the plates were washed and monoclonal anti-IgG or IgG4 added (1:1000). After 1 hr AP-anti-mouse IgG was added at a dilution of 1:500 and after a further 1 hr incubation and wash, the substrate *p*-nitrophenyl phosphate (1 mg/ml in pH 9.8 di-ethanolamine buffer) was added. After a 1 hr incubation at room temperature the colour reaction was stopped by the addition of $50\text{ }\mu\text{l}$ of 3 M sodium hydroxide. The optical density was read in a Titertek multiskan plate reader (Flow Labs, Rickmansworth, U.K.) at 405 nm. Results were expressed as arbitrary units/ml by reference to a standard curve generated by a series of different dilutions of an in house high positive reference serum pool, with an IgG pan-reactive monoclonal (8a4) antibody, as described elsewhere [11].

Inhibition ELISA

To test the specificity of the assays, free antigen ($10\text{ }\mu\text{g/ml}$) was pre-incubated with the sample (1:50) dil for 1 hr at room temperature and then added to the microtitre plate

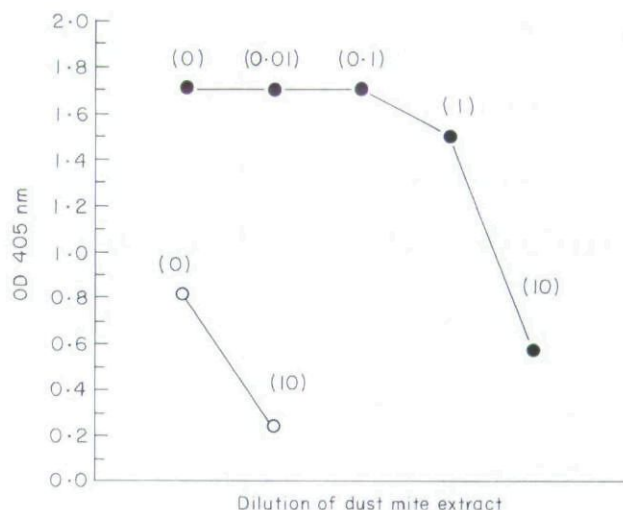


Fig. 1. (a) *D. pteronyssinus*-specific IgG level with and without pre-incubation with $10\text{ }\mu\text{g/ml}$ of free antigen (geometric mean of 24 samples). (b) Inhibition curve of the reference serum pre-incubated with 0, 0.01, 0.1, 1 and $10\text{ }\mu\text{g/ml}$ of free antigen. ●, adult. ○, baby.

and the assay completed as above. Different dilutions of free antigen were used in pre-incubation with the reference serum to plot the extinction curve. All inhibition assays used the pan-IgG subclass-reactive monoclonal antibody 8a4. Results were compared to wells to which no free antigen was included.

Statistical tests

Comparison between groups was by a two tailed paired *t* test and performed using Statview 512+ on an Apple Macintosh computer.

Results

Optimization and specificity of the assay

The assay was fully optimized and the conditions described in materials and methods used. IgG antibody to *D. pteronyssinus* was found in the sera of 3 month and 1-year-old babies and the specificity of the assay checked with the inhibition ELISA. The results are shown in Fig. 1. At a concentration of $10\text{ }\mu\text{g/ml}$ it was possible to completely inhibit the binding of IgG antibody in the sera of the babies ($P < 0.0001$, two tailed paired *t* test) but it was not possible to completely inhibit the reference serum, which contained a higher titre of antibody, with this amount of antigen.

D. pteronyssinus-specific total IgG and subclass specific IgG antibodies

Total specific IgG antibody levels to *D. pteronyssinus* were measured at 7 days, 3 and 12 months and the results expressed in arbitrary units/ml [11]. The 7 day level

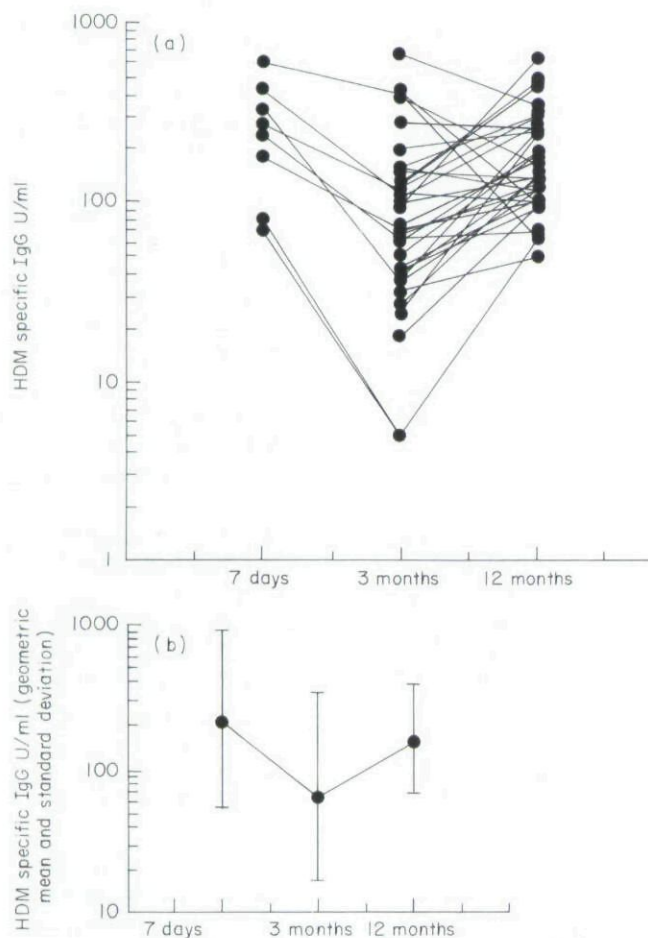


Fig. 2. *D. pteronyssinus*-specific total IgG. (a) Variations of the level in each subject. (b) Geometric mean $\pm$ s.d. for each group. $P < 0.005$ between 7 days and 3 months and $P < 0.001$ between 3 and 12 months (in the two tailed paired t test).

(measured in eight babies) is significantly higher than that registered at 3 months ($P < 0.005$, two tailed paired t test) but between 3 and 12 months the level increased ($P < 0.001$) in 31/36 babies (Fig. 2a). The geometric mean and s.d. of the three groups are shown in Fig. 2b.

All of the anti-*D. pteronyssinus* antibody activity could be accounted for by IgG1 (Fig. 3a and b). The changes in *D. pteronyssinus*-specific IgG1 antibody followed the same pattern as those seen with total IgG antibody with a fall at 3 months ($P < 0.001$) and a successive increase at 1 year ($P < 0.0001$). Although we attempted to measure IgG4 *D. pteronyssinus*-specific antibodies were unable to detect these at any age (Fig. 3b).

Discussion

Previous studies of the subclass of IgG produced during a specific immune response to ingested [8] or injected [14–

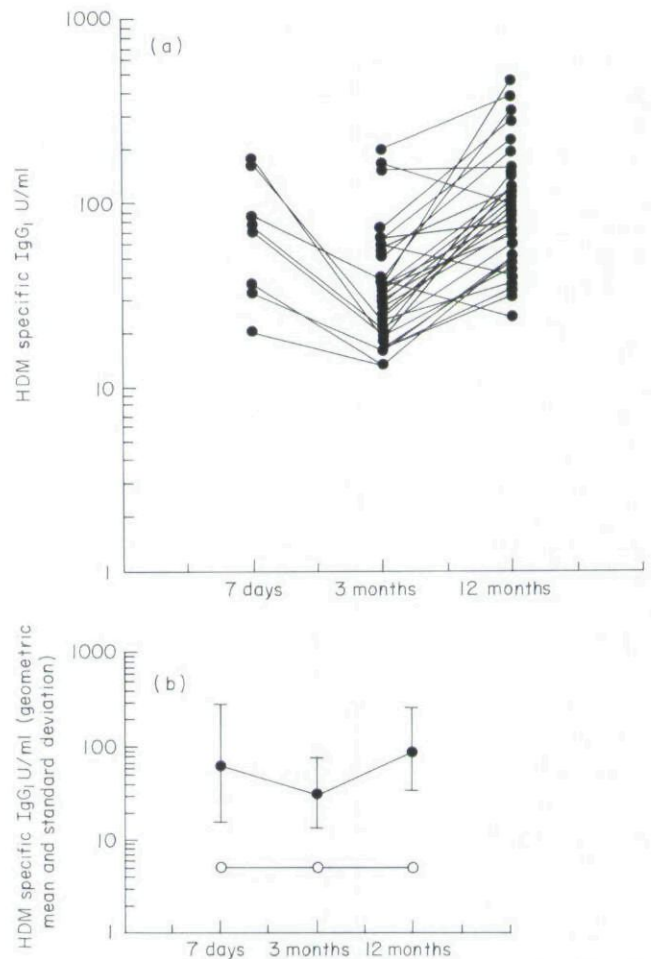


Fig. 3. *D. pteronyssinus*-specific IgG1. Variation of the level in each subject. (b) Geometric mean $\pm$ s.d.; the open symbols represent IgG4 antibody which was not detected and the closed symbols IgG1 antibody. $P < 0.001$ between 7 days and 3 months and $P < 0.0001$ between 3 and 12 months.

16] allergens have shown to occur first in subclass 1, the major IgG subtype. Later in life the subclass of IgG produced in atopic and in non-atopic individuals to antigens that are inhaled is largely confined to subclass 1 [9] as it is for some food antigens such as wheat gliadin [10]. This study shows that natural exposure to a common inhalant antigen (*D. pteronyssinus*) is able to evoke a specific IgG antibody response during the first year of life in most babies and that this occurs mainly in subclass 1. Using the same assay as was used in this study we have previously shown that IgG4 antibodies to *D. pteronyssinus* are present in dust mite allergic patients [9] and increase during immunotherapy [17].

Of central importance to the interpretation of the immune response to dust mite that we describe here is the question of whether the assay is actually measuring antibody to dust mite. We have shown in this paper that

IgG1 antibody binding to dust mite microtitre coated plates is completely inhibited by 10 µg/ml of free antigen. We do have reservations about the ability of some ELISAs to detect antibody. We have previously shown [9] that IgG binding to microtitre plates coated with dust mite growth medium (Ox liver extract) cannot be inhibited by the antigen used to coat the microtitre plate or indeed by any other antigen. Similarly in studies in which we have attempted to determine the functional affinity of IgG1 antibodies to ovalbumin we could not completely inhibit the binding of IgG1 antibodies to ovalbumin coated microtitre plates with free ovalbumin even at 10 mg/ml [25]. In addition, the non-specific binding of the 3 and 12 month sera to bee venom coated microtitre plates is low. Some sera do not have raised IgG1 antibody despite having a normal level of serum IgG1. While accepting that caution should be exercised in the interpretation of IgG antibody measurements by ELISA we believe that the specificity that we have demonstrated supports the view that genuine IgG1 antibodies to dust mite are normally produced during the first year of life. These might be useful when determining the efficacy of dust mite avoidance measures.

The IgG antibody to *D. pteronyssinus* present at 7 days would largely be of maternal origin [18–23] and by 3 months would be expected to have cleared from the circulation. The response that occurs at 12 months is believed to represent the babies own immune response and for IgG antibody to *D. pteronyssinus* occurred much earlier than that reported by Rowntree and his colleagues [1] who used a radioimmunoassay (RIA) with labelled *Der p* I. Such discrepancies between ELISA and RIA have previously been described [17] and are thought to be due to the differential ability of ELISA and RIA to detect antibodies of differing affinities. IgG1 antibodies induced during immunotherapy appear to have a lower functional affinity than comparable IgG4 antibodies [24,25].

We have shown that an unselected group of babies were able to produce a detectable immune response to *D. pteronyssinus* during the first year of life indicating that sufficient quantities of this antigen reach the infant immune system via this route to trigger an immune response. The response is produced in a high proportion of babies and precedes the formation of IgE antibodies which does not normally occur in atopic babies until greater than 2 years of age [1]. Similarly small quantities of inhaled antigen are known to provoke IgG antibody responses and to generate IgE-specific suppressor cells in low IgE responder strains of rat [26]. Although we have not been able to study T cell responses in this young age group it is likely that antigen-specific T cells would have been generated as *D. pteronyssinus* is a T dependent antigen. In the future with more sensitive techniques it is

to be hoped that the regulatory processes which govern the immune response in newborns can be studied further. What is clear from this study is that most babies do recognize and respond to inhaled antigens from a very early age.

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