High prevalence of IgE sensitization against house dust mites in pregnant women

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Abstract

There is an increase in prevalence and financial burden of childhood atopic disorders in recent years. Understanding allergic conditions of pregnant women is important for developing strategies for prevention and management of allergy-related diseases. However, little is currently known about the atopic conditions in pregnant women.

The sera from 46 pregnant women were analyzed for allergen-specific IgE antibodies using the Optigen assay and SDS-PAGE immunoblot analysis.

Results from the Optigen assay showed that 20 (43%) of the 46 serum samples analyzed demonstrated IgE reactivity against mite p (\textit{Dermatophagoides pteronyssinus}) (95%), mite f (\textit{D farinae}) (95%), house dust (60%), cat (25%), shrimp (20%), crab (15%), cockroach (10%), dog (5%), latex (5%), willow black (5%), and timothy grass (5%). Nineteen of the 20 Optigen-positive sera demonstrated IgE reactivity against both the house dust mites \textit{D pteronyssinus} and \textit{D farinae}, with 10 of them having a high IgE CLA class value of 4. IgE reactivity to the house dust mite \textit{D pteronyssinus} was confirmed in SDS-PAGE-immunoblots, which correlated well with the intensity of IgE-binding to the 15-kDa \textit{D pteronyssinus} component and to the purified recombinant Der p 2 major house dust mite allergen.

A high prevalence of IgE sensitization against house dust mites during pregnancy is noted, which is worthy of clinical attention. Children of IgE-sensitized mothers should be closely monitored for development of allergenic disorders.

Abbreviations: \textit{D. farinae} = \textit{Dermatophagoides farinae}, \textit{D. pteronyssinus} = \textit{Dermatophagoides pteronyssinus}, \textit{rDer p 2} = recombinant Der p 2, \textit{idDer p 7} = recombinant Der p 7, SDS-PAGE = sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

Keywords: atopic sensitisation, Der p 2, house dust mites, IgE, pregnant women

1. Introduction

Asthma is among the most prevailing chronic disorders in children.\textsuperscript{1} In recent decades, an increase in the prevalence of allergic diseases, including atopic asthma, has been observed, which leads to a significant disease burden.\textsuperscript{11-4} In Taipei, Taiwan, the prevalence of childhood asthma increased from 1.3% in 1974 to 5.0% in 1985.\textsuperscript{17} Notably, an increase in asthma prevalence was observed in 6- to 7-year-old Taipei schoolchildren in 1994.\textsuperscript{18} A survey in 2002 on 13- to 14-year-old schoolchildren in Taipei further disclosed that the prevalence of symptoms of allergic rhinitis, asthma and atopic eczema was increased by 51%, 37% and 193%, respectively, in the 12-month period of survey.\textsuperscript{1,4} In 2007, a survey of 24,999 first-grade students from 153 Taipei elementary schools indicated that the proportion of children who experienced nocturnal cough and wheezing in a 12-month period increased significantly when compared with results for 1994 and 2002, and the prevalence of diagnosed asthma, rhinitis, and eczema in Taipei was 13%, 33.7% and 29.8%, respectively.\textsuperscript{9} The persistent elevation of prevalence of such allergic ailments has made it important to understand the origins and risk factors, especially of atopic asthma, for the development of effective clinical preventive and management strategies.\textsuperscript{2,10}

Both genetic and environmental factors contribute to the development of atopic asthma in childhood.\textsuperscript{15,11,12} About one-third of patients suffering from asthma have a family history of asthma, which may be added to the list of predictors of asthma risk.\textsuperscript{11,12-15} In addition, maternal history of asthma was found to confer a greater risk for asthma in infancy.\textsuperscript{112-13} It was found that persistently sensitized children with an asthmatic mother were at 10 times the risk of having current asthma at the age of 7 years in comparison with persistently sensitized children from asthma-free mothers.\textsuperscript{112} Furthermore, infants from atopic mothers were also found to be significantly more often associated with sensitization to common food, such as eggs, when compared with infants from nonatopic mothers.\textsuperscript{16} Since atopic sensitization plays a significant role in the development of asthma,\textsuperscript{17} about two-third of asthmatic patients are minimally sensitized to...
at least one inhalant allergen. Therefore, determination of IgE reactivity against common environment allergens in pregnant mothers may be beneficial in developing significant preventative strategies against childhood atopic disorders. However, little is currently known about the atopic condition in pregnant women.

Among the various environmental allergens, house dust mites Dermatophagoides pteronyssinus (D. pteronyssinus) and D. farinae are important causative agents of atopic asthma. [18–20] Since crude allergen extracts may contain various components and are difficult to be standardized, well characterized and purified recombinant allergen proteins have been used in diagnostic tests. [21] Currently, at least 23 different mite allergens are known. [18,22] Der p 2 and Der p 7, with molecular masses of 15 and 30/31 kDa, respectively, have been identified as 2 major and important mite allergens with frequencies of IgE-binding of 80% to 100% (Der p 2) and 40% (Der p 7), in mite-sensitized asthmatic patients. [22,23]

In the present study, the presence and specificities of serum IgE antibodies against a range of known allergens and purified mite allergens in pregnant women were analyzed. Results obtained would provide an important basis for prevention and management of childhood atopic disorders.

2. Methods

2.1. Serum samples

In this study, serum samples from 46 randomly recruited term pregnant women, aged 28 to 46 years old were collected after obtaining informed consent. Serum samples were stored in aliquots at −20°C until use. This study was approved by the Institutional Review Board of the Taipei Veterans General Hospital, Taipei.

2.2. Determination of serum IgE antibodies by Optigen assay

IgE reactivity against 36 known allergens in the serum samples was analyzed by the Optigen assay (Optigen Universal Panel 36, Taiwan Panel, Hitachi Chemical Diagnostics, Inc., Mountain View, CA) according to manufacturer’s instructions. The 36 allergens included belong to categories of pollens, food, animals, molds, and dust mites and others as shown in Table 1. CLA class values were as measured in the CLA-1™ Lumimeter, which registers light emission from the Optigen Pette, following manufacturer’s instructions. CLA class values were assigned from 0 to 4 based on the amount of light emitted by the individual allergens in the test chamber. CLA class 0 represents an absence, or nondetectable levels, of allergen-specific IgE antibodies. CLA class values of 1 to 4 represent progressively increasing concentrations of allergen-specific IgE antibodies.

2.3. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)-immunoblot analysis

Serum IgE antibodies against components of the house dust mite D. pteronyssinus, purified recombinant major Der p 2 (rDer p 2) [22] (kindly provided by Prof. J.J. Tsai, Taichung Veterans General Hospital, Taichung, Taiwan) and rDer p 7 [22,23] (prepared in our laboratories), were detected by SDS-PAGE immunoblot analysis. Proteins in the crude D. pteronyssinus extracts (GREER, Lenoir, NC), or the purified recombinant mite allergens, were separated by SDS-PAGE then transferred electrophoretically onto 0.45-μm polyvinylidene difluoride membranes (Millipore, Bedford, MS). The membranes were blocked with 1% skimmed milk and incubated with serum samples for 16 hours at 4°C. The membranes were washed and incubated with alkaline phosphatase-conjugated monoclonal anti-human IgE antibodies (Pharmingen, San Diego, CA); reactivity was determined by developing the membranes with enzyme substrates as described, [23,24] and the relative intensities of the 15-kDa component and rDer p 2, rDer p 7 bands were determined by the public domain algorithm Image J, and were arbitrarily scored as – for non-detectable to + to ++++ indicating increasing densities. Serum from a Fusarium-sensitized atopic individual was used as a negative control.

3. Results

3.1. Determination of serum IgE antibodies by Optigen assay

The Optigen Universal Panel 36 used in this work is composed of 36 allergens that include pollens, food, animals, molds, dust, mites, and others (Table 1). IgE antibodies of sera of 46 randomly recruited pregnant women were first determined by the Optigen assay. The presence of allergen-specific IgE antibodies (CLA class ≥1) was detected in 20 (43%) of the 46 serum samples (Table 2), indicating a relative high ratio of pregnant women being sensitized to one or more of the common allergens. The results also showed that IgE antibodies in the 20 Optigen-positive sera reacted against 11 of the allergens tested (Table 3). In the positive sera, the number of IgE-reactive allergens detected ranged from 2 to 6, in different pregnant women (Table 4). In terms of the nature of the allergens (Table 3), 19 (95%) sera were reactive to mites f (D. farinae) and p (D. pteronyssinus) and 12 (60%) were reactive to house dust (Tables 2 and 3), echoing the reported common reactivity to these allergens in asthmatic patients. [18–20] In addition, ten of the 19 mite-positive samples showing a high CLA Class of 4 against both mites p and f (Tables 2 and 5).

It is noteworthy that 41.3% of the pregnant women were IgE-positive for the mites and 26.1% was reactive to house dust (Table 3, last column). Furthermore, 5 (25% of reactive and 10.9% of all pregnant women) sera were positive for cat, which should caution pregnant women on having cats as pets during pregnancy. The frequencies of IgE reactivity against shrimp, crab, cockroach and others were below 20% (Table 3). Four sera showed individual IgE reactivity against latex, black willow, timothy grass and dog, while serum 14 was unreactive against...
house dust and the mites but reacted positively against black willow, shrimp and crab (Table 2).

As noted above, besides serum sample 14 which was IgE-reactive to shrimp, crab and willow black, the 19 remaining serum samples were IgE reactive against mites f and p (Tables 2 and 3). Amongst these, 13 (65%) of the positive serum sample, or 28.3% of the pregnant women tested, were also co-reactive with 1 to 4 other allergens, in particular house dust (Table 4). The clinical significance of multivariate patterns of IgE co-reactivity remains to be elucidated.

In this study, the 46 randomly recruited term-pregnant women were aged between 28 to 46 years old (y.o.). The mean age was 33.8 ± 3.2 y.o. for the 19 mite p IgE-positive women (Table 2), and that of the 27 mite p IgE-nondetectable women was 35.37 ± 4.55 y.o. (data not shown). Among the positive subjects, the numbers for CLA class values of 1 to 4 were 7, 1, 1, and 10, respectively. Combining the CLA class values of 1 to 3 due to the small numbers, the mean age was 35.00 ± 2.96 y.o. for the 9 mite p IgE-positive women, and that of the 10 positive women with a CLA class value of 4 was 32.80 ± 3.22 y.o. Although the mean age of CLA class 4 appeared to be lower than those of the mite p IgE-nondetectable and the CLA class 1 to 3 groups, the age-related difference was, however, not statistically significant when analyzed by the One-way Anova test (*P* < .05). A larger study sample size will need to be examined to determine if mite p IgE-positivity and levels is age-associated.

### 3.2. Determination of IgE-binding against mite allergens by SDS-PAGE immunoblot analysis

Since mite p and mite f are the more important and common causative agents of atopic asthma, the presence of IgE antibodies in the sera of the 46 pregnant women against components of the house dust mite *D. pteronyssinus* in crude *D. pteronyssinus* extracts was further analyzed by SDS-PAGE immunoblot analysis (Fig. 1A and Table 5). In the blot, the 15-kDa band was Der p 2 and the 30/31-kDa bands corresponded to Der p 7.[22,23] The band intensities were also quantified as described in Section 2. For control, a serum sample from a fungal *Fusarium*-sensitized atopic individual demonstrated negative IgE-binding (Fig. 1, strip no. 21). The 26 of the 46 serum samples that were negative in the Optigen assay also showed negative IgE immunoblot results against components of *D. pteronyssinus* (data not shown). In the Optigen-positive cases, with the exception of sera 14 and 17, 18 of the serum samples, which represented 39.0% of the 46 sera of the pregnant women tested, that showed positive IgE-immunoblotting were also CLA classes ≥1 (Fig. 1A). Serum no. 14 with CLA class 0 against mite p

### Table 2

Allergen-specific IgE reactivity in positive serum samples of pregnant women.

| Serum Age | No. of IgE-reactive allergens |
|-----------|-----------------------------|
| Latex 2 | 4 |
| Willow 2 | 4 |
| Black 4 | 4 |
| Timothy Grass 2 | 4 |
| Shrimp 2 | 4 |
| Crab 2 | 4 |
| Cat 2 | 4 |
| Dog 2 | 4 |
| House dust 2 | 4 |
| Cockroach 2 | 4 |
| Mite f 2 | 4 |
| Mite p 2 | 4 |

*Allergen-specific IgE reactivity was determined by the Optigen assay, and IgE reactivity was evaluated by CLA class values. CLA class 0 represents an absence, or non-detectable levels, of allergen-specific IgE antibodies. CLA class values of 1 to 4 represent progressively increasing concentrations of the allergen-specific IgE antibodies.*

### Table 3

Frequency of IgE reactivity against allergens in IgE-positive samples and in pregnant women.

| Allergen | No. of IgE-positive samples | IgE +ve samples (n = 20) | Pregnant women (n = 46) |
|----------|-----------------------------|-------------------------|-------------------------|
| Latex    | 1                           | 5                       | 2.2                     |
| Willow, black | 1                           | 5                       | 2.2                     |
| Timothy grass | 1                           | 5                       | 2.2                     |
| Shrimp   | 4                           | 20                      | 8.7                     |
| Crab     | 3                           | 15                      | 6.5                     |
| Cat      | 5                           | 25                      | 10.9                    |
| Dog      | 1                           | 5                       | 2.2                     |
| House dust | 12                          | 60                      | 26.1                    |
| Cockroach | 2                           | 10                      | 4.3                     |
| Mite f   | 19                          | 95                      | 41.3                    |
| Mite p   | 19                          | 95                      | 41.3                    |

*Frequency (%) in the Table was calculated by dividing the number of IgE-positive samples for each allergen by the number of serum samples (n = 20) and by dividing the number of positive serum samples for each allergen by the number of pregnant women (n = 46).*
showed negative IgE-immunoblot (Fig. 1A, strip no. 14), as expected. However, serum 17 with a positive CLA class value of 2 against mite p also showed negative IgE-immunoblot (Fig. 1A, strip no. 17).

More than 15 IgE-binding *D. pteronyssinus* components with molecular masses of 15 kDa to greater than 66 kDa could be identified in the SDS-PAGE-immunoblot (Fig. 1A). Except for serum no. 5, the 15-kDa Der p 2 component bound IgE antibodies in 17 (94%) of the 18 IgE-immunoblot positive sera. In addition, 5 (serum no. 3, 6, 8, 10, 11) of the 18 positive sera also demonstrated an IgE reactivity against the 30/31-kDa *D. pteronyssinus* components (Fig. 1A).

The IgE-binding reactivity was confirmed using purified recombinant Der p 2 (rDer p 2) and Der p 7 (rDer p 7) in the 46 serum samples also by SDS-PAGE-immunoblot (Fig. 1B and Table 5). The 26 Optigen assay negative samples were also negative in IgE-binding against rDer p 2 and rDer p 7 (data not shown). Sera 5, 14, 17, and 18 were negative in IgE-binding against rDer p 2 (Fig. 1B and Table 5), correlating well with the non- or low-binding data of the 15-kDa (Der p 2) band in the total mite extract blots (Fig. 1A and Table 5). On the other hand, 7 (serum no. 3, 6, 8, 10, 11, 13, 19) of the Optigen assay-positive samples showed IgE-binding against the purified rDer p 7 allergen (Fig. 1B and Table 5). Five of these samples (serum no. 3, 6, 8, 10, 11) also had a strong rDer p 7 IgE reactivity (Fig. 1B and Table 5), 5 (serum no. 3, 6, 8, 10, 11) of which that had a strong rDer p 7 IgE reactivity were also reactive against the 30/31-kDa *D. pteronyssinus* components (Fig. 1A) and purified rDer p 2 (Fig. 1B and Table 5). These samples also showed a high CLA class of 4 against mite p in the Optigen assay (Table 3), suggesting that most pregnant women that were sensitized to Der p 7 were also high IgE responders to Der p 2.

### 4. Discussion

This study reports a prevalence of 43% of IgE sensitization against common allergens, particularly house dust mites, in a cohort of pregnant women in Taiwan. Our results show that 95% of IgE-reactive serum samples showed reactivity against mites p and f (Table 3), in agreement with findings of 50% to 90% mite sensitization of asthmatics in different geographic regions. About half of the mite-sensitized serum samples in this work also demonstrated the highest CLA class value of 4 to the mites (Tables 2 and 5). Our data suggest that mothers of such allergic sensitization should be informed and properly advised to visit allergy clinics and to take proper actions to reduce levels of mite and other allergens in the environments. Newborns of IgE-reactive mothers should also be closely monitored and be placed under long-term care by pediatric allergy specialists to prevent atop allergic sensitization and for proper management should the disorders develop.

In addition, house dust mites are ubiquitous not only in subtropical regions such as Taiwan, the reported 50% to 90% mite sensitization of asthmatics in different geographic regions has led us to suggest that serum IgE antibodies against...
major allergens, including mite allergens, should be routinely screened during pregnancy. Data of this work, thus, provide an important reference for physicians and caretakers of multiple clinical fields, including obstetricians, pediatricians, allergy physicians, family and public health physicians, immunologists and government health officials.

In addition to house dust mites, some pregnant women also demonstrated IgE reactivity against cat and cockroach (Tables 2 and 3), correlating with the reported prevalence rates of 9% and 16% to 28%, respectively, to these 2 allergens in school children in Taiwan.[20,25] The pregnancy-childhood correlation may indicate high risks for children born of IgE-reactive mothers to develop the allergy. In a questionnaire-based survey of 30,018 individuals in Taiwan, about 7% were diagnosed as victims of allergies to seafood and farm products,[26] echoing our findings (Table 3). In addition, our finding showed that all of the allergen-specific IgE-positive sera demonstrated IgE sensitization against more than one (2–6) allergens (Table 4), which may be due to IgE cross-reactivity among allergens.[27] Hence, more accurate determination of specific allergen-reactivity should be developed and used to achieve proper management of atopic disorders.

Our immunoblot results against crude mite p extracts correlated well with the Optigen assay data (Table 3). The exception was serum no. 17 with CLA class 2 but showed negative IgE-immunoblot. The discrepancy may be explained by the absence of some mite component(s) in the crude *D. pteronyssinus* extracts obtained from GREER. Alternatively, the antigenicity of some IgE-reactive mite component(s) may have been inactivated due to denaturation by SDS and/or heating in the SDS-PAGE immunoblot procedure. Hence, conflicting results should be further examined by other procedures to accurately identify the proper IgE-reactive allergens.

Co-reactivity in immunoblots to Der p 2 and Der p 7 allergens reported here for pregnant women has previously been reported in children.[28] This work also showed that the intensity of the IgE immunoblot reactivity against the purified recombinant Der p 2 correlated well with that of the 15 kDa Der p 2 mite component in the crude extract and the CLA class values against mite p in the Optigen assay (Table 5). Since the Optigen assay, and other similar commercial assay kits, are costly, and crude allergenic extracts are currently still difficult to be standardized,[29] purified and well-characterized recombinant major mite allergens, such as rDer p 2 and rDer p 7 used in this work, are good options for use in rapid clinical screening of house dust mite and other allergies and for atopic sensitization. This option should also be applicable to other recombinant allergenic components.

5. Conclusions

A high prevalence of IgE sensitization against house dust mites in pregnancy was demonstrated in this study, which leads us to suggest that serum IgE antibodies against major allergens be routinely screened during pregnancy. With information on family history, gender and obesity of the patients, and taking into consideration of environmental factors,[2,5,11,30] predictive algorithms and preventive strategies, such as vitamin D supplementation[31] against allergenic sensitization, may be recommended during pregnancy to reduce childhood atopic
disorders. This study serves as an early warning to affected subjects by establishing and evaluation effectiveness of early warning and preventive strategies. The study also establishes an important platform for subsequent studies to monitor the IgE status of mothers and the offsprings of IgE-positive mothers to determine if the IgE status changes with age.

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