

# Newly generated IgE antibodies to *Dermatophagoides pteronyssinus* in children are directed against components distinct from *Der p I* and *Der p II*

Peter G. Calkhoven, PhD, Marja Aalbers, BSc, Piet P. M. Schilte, MD,\*  
Bart-Jan L. Yntema, MD,\* Rupino W. Griffioen, MD,\*\*  
Jan C. van Nierop, MD,\*\* Arnold P. Oranje, MD,\*\*\* and  
Rob C. Aalberse, PhD Amsterdam, Alkmaar, and Rotterdam, The Netherlands

*The specificity of newly generated IgE antibodies (Abs) to the house dust mite, Dermatophagoides pteronyssinus, in longitudinal serum samples from 18 young children with an increased risk for IgE-mediated allergy was studied. The first IgE Ab response to house dust mite was detected early in life (mean age, 32 months; range, 11 to 60 months). For 83% of the children, more than half of the newly generated IgE Ab response to house dust mite was directed against components distinct from the major allergens, Der p I (P<sub>1</sub>) and Der p II (DpX). These results suggest that the early IgE Ab response to house dust mite is induced by components distinct from the major allergens, Der p I and Der p II. (J ALLERGY CLIN IMMUNOL 1991;88:265-71.)*

**Key words:** House dust mite, *Dermatophagoides pteronyssinus*, IgE-mediated allergy, *Der p I*, *Der p II*

Allergy to house dust mites is an important cause of asthma and allergic rhinitis. Many investigations have been performed to characterize the allergens present in house dust mites.<sup>1-8</sup> Some of these allergens were designated major allergens, for example, *Der p I* and *Der p II* in the house dust mite, *Dermatophagoides pteronyssinus*.

In a previous study, van der Zee et al.<sup>9</sup> analyzed the relative contribution of IgE Abs against *Der p I* and *Der p II* to the total IgE Ab response against house dust mite with monoclonal antibodies. For some patients, *Der p I* and *Der p II* represented >80% of the allergenic activity of the mites. However, for other

## Abbreviations used

*Der p I*: *Dermatophagoides pteronyssinus* antigen 1 (P<sub>1</sub>)  
*Der p II*: *Dermatophagoides pteronyssinus* antigen 2 (DpX)  
Ab: Antibody

From the Central Laboratory of The Netherlands Red Cross Blood Transfusion Service and Laboratory for Experimental and Clinical Immunology, University of Amsterdam; \*Medical Center Alkmaar; \*\*Emma Children's Hospital, Amsterdam, and \*\*\* University Hospital Rotterdam/Sophia Children's Hospital, Rotterdam, The Netherlands.

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Reprint requests: R. C. Aalberse, PhD, c/o Publication Secretariat, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, PO Box 9406, 1006 AK Amsterdam, The Netherlands.

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patients, the contribution of these two allergens to the total IgE response against mite was <20%. The results clearly indicated that, in addition to *Der p I* and *Der p II*, other allergens must be important. Furthermore, these results indicated the existence of marked differences in the patient's fine specificity of the IgE Ab response to house dust mite.

One explanation for these observed differences might be related to differences in the duration of the sensitized period. Some patients presumably had been sensitized many years before the blood sample was obtained, whereas other patients might have become mite positive very recently. Perhaps IgE Abs were more easily induced to a quantitatively dominant component like *Der p I*; consequently, Abs would appear early in the antimite response (and perhaps also disappear more quickly on continuous exposure). There-

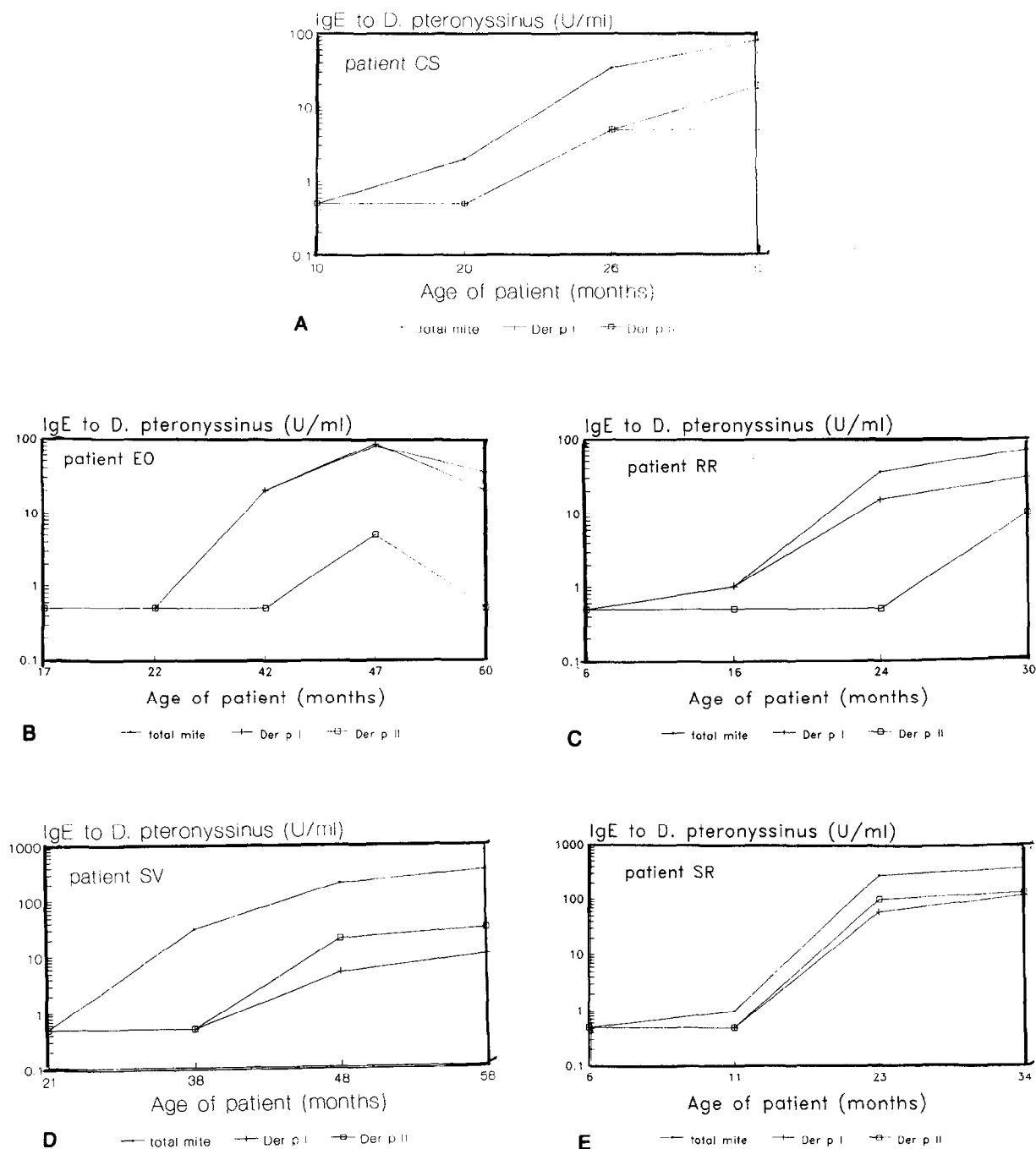
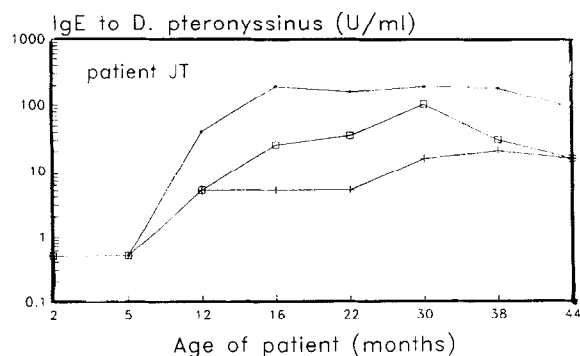


**FIG. 1A. A-E, Patients C. S., E. O., R. R., S. V., and S. R. Development of IgE Abs to house dust-mite allergens (total mite, *Der p I* and *Der p II*) in longitudinal serum samples from 5 high-risk children. Results expressed as units per milliliter related to the local reference serum.**

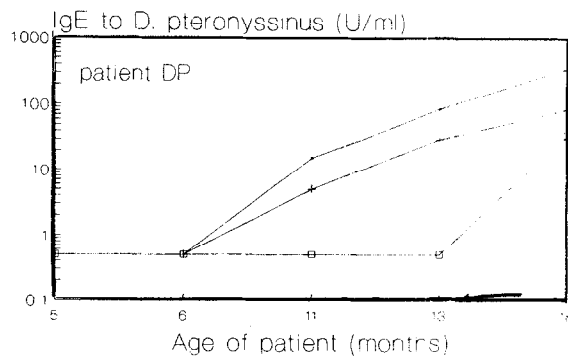
fore, it appeared to be of interest to study the fine specificity of recently induced mite-specific IgE Abs.

Another reason we are interested in the fine specificity of the newly generated IgE Ab response is related to our hypothesis that a full-blown allergic sensitization might consist of two phases subsequent to sensitization toward foods:

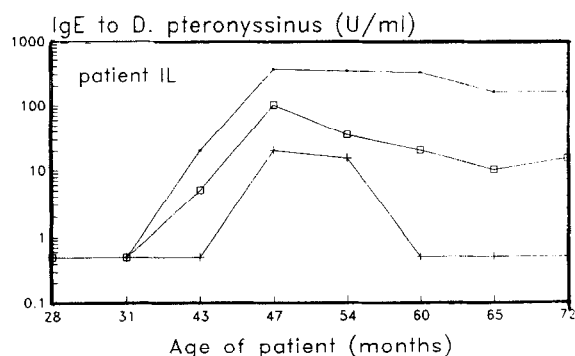
1. IgE Abs toward a "primary" allergen are generated, possibly facilitated by cross-reactivity between the primary allergen and an antigen in food.
2. IgE Abs against other, "secondary" allergens, present on the airborne particle that carried the primary allergen into the airways, are formed; the assumption made here is that the inflammatory reaction,



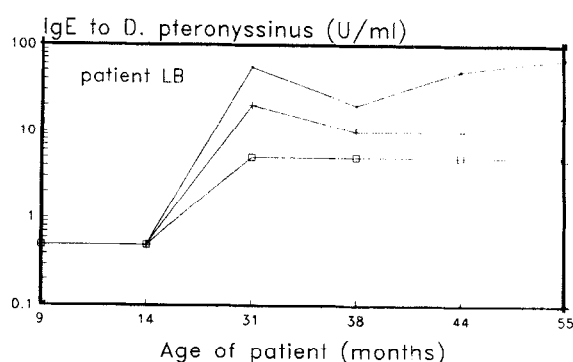
**F** —•— total mite —+— Der p I —□— Der p II



**G** —•— total mite —+— Der p I —□— Der p II



**H** —•— total mite —+— Der p I —□— Der p II



**I** —•— total mite —+— Der p I —□— Der p II

**FIG. 1B. F-I, Patients J. T., D. P., I. L., and L. B. Development of IgE Abs to house dust-mite allergens (total mite, *Der p I* and *Der p II*) in longitudinal serum samples from four high-risk children. Results expressed as units per milliliter related to the local reference serum.**

caused by the interaction between IgE and the primary allergen, facilitates the induction of Abs toward other allergens that happen to be present.

To investigate the first phase, it would thus be important to analyze the specificity of the very first IgE Abs to house dust mite. We analyzed sera from young children who participated in a follow-up study.<sup>10</sup> At the start of the study, these children were negative in the RAST for house dust mite; during the follow-up, the children developed IgE Abs to *D. pteronyssinus*. To establish whether the early mite-specific IgE Ab response was restricted to a single allergen (possibly *Der p I*), we analyzed the fine specificity of these recently induced mite-specific IgE Abs.

## MATERIAL AND METHODS

### Population

Young children were studied in a follow-up project.<sup>10</sup> The parents of the children were informed about the objectives of this study and gave their consent.

The children were selected by the pediatricians in our

group. The parents were asked to allow their child to participate if their child has (1) a positive family history of atopy and/or (2) atopic eczema, and/or (3) IgE Abs to foods in serum.<sup>10</sup> Furthermore, for the present study, another criterion was (4) a negative RAST for house dust mite.

During the period of follow-up, 23 children developed IgE Abs to the house dust mite *D. pteronyssinus*. Testing the first serum sample collected, all 23 children were RAST negative for house dust mite. The amount of serum available permitted us to study samples from 18 children in detail. Fourteen of these children had (a history of) atopic eczema.

At the time of entry into this study, the mean age of the 18 children was 17 months (range, 2 to 35 months). The mean length of the follow-up was 28 months (range, 12 to 47 months).

For comparison, the RAST results for sera from 41 adult patients with IgE antihouse dust mite were used. The latter patients, suffering from rhinitis and/or asthma, participated in a study described by van der Zee et al.<sup>9</sup>

### Local reference serum

The local reference serum was obtained by plasmapheresis from a male adult suffering from house dust-mite al-

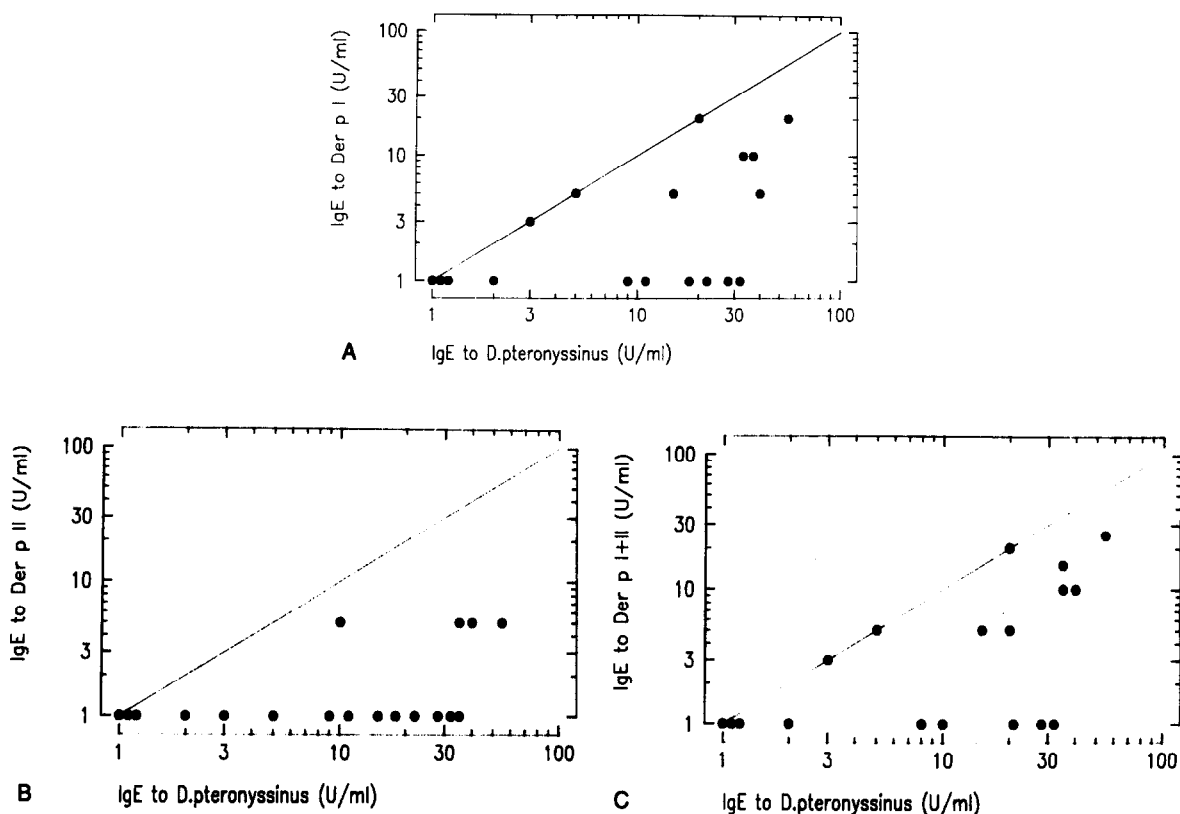


**FIG. 2.** A, Contribution of IgE antibodies to *Der p* I, B, to *Der p* II, C, to *Der p* I plus *Der p* II to the total IgE Ab response against house dust mite in the IgE seroconversion samples from 18 high-risk children. Results expressed as units per milliliter related to the local reference serum.

lergy. The contribution of IgE to *Der p* I and of IgE to *Der p* II to the total IgE Abs to house dust mite was determined as previously described.<sup>9</sup> In the local reference serum, 70% of the IgE Abs to house dust mite were directed against the major allergen *Der p* I, whereas 20% were directed against the major allergen *Der p* II.

### Chemicals

All chemicals were obtained from Merck (Darmstadt, Germany), unless indicated otherwise.

### Extracts

The (immobilized) extracts used in this study were prepared and described previously.<sup>11</sup>

### Determination of total and mite-specific IgE Abs

Total IgE was determined with a competitive radioimmunoassay with Sepharose-bound IgE and <sup>125</sup>I-labeled sheep antihuman IgE Abs,<sup>12</sup> as previously described.<sup>13</sup>

IgE antihouse dust mite was determined with the RAST, as previously described.<sup>11</sup>

Immobilization of *Der p* I and *Der p* II for use in the RAST was performed as follows: murine MAbs were produced against *Der p* I and *Der p* II.<sup>9</sup> Anti-*Der p* I or anti-*Der p* II (100  $\mu$ l of ascites) was coupled to 100 mg of

CNBr-activated Sepharose. Next, the immunosorbents were incubated overnight with an excess of 2% (wt/vol) *D. pteronyssinus* extract and washed extensively before use. Immobilized murine MAbs (ascites) to dog dander were used as a control for IgE binding not related to house dust mites.

The detection limits of the RASTs for total *D. pteronyssinus*, *Der p* I and *Der p* II, related to the local reference serum, were 0.10, 0.07, and 0.02 U/ml, respectively.

### RESULTS

Serum samples from children who developed IgE Abs to house dust mite during the follow-up were studied in the RAST. The RAST was performed for total *D. pteronyssinus* extract as well as for the purified components, *Der p* I and *Der p* II.

RAST results were expressed as percentage of radioactivity bound or as units per milliliter (related to the local reference serum).

High levels of IgE Abs to house dust mite were detected already in very young children. The mean age at which the IgE seroconversion to house dust mite was observed was 32 months (range, 11 to 60 months). The mean intensity of that newly generated antimitte IgE Ab response corresponded to 18 U/ml (range, 1 to 55 U/ml).

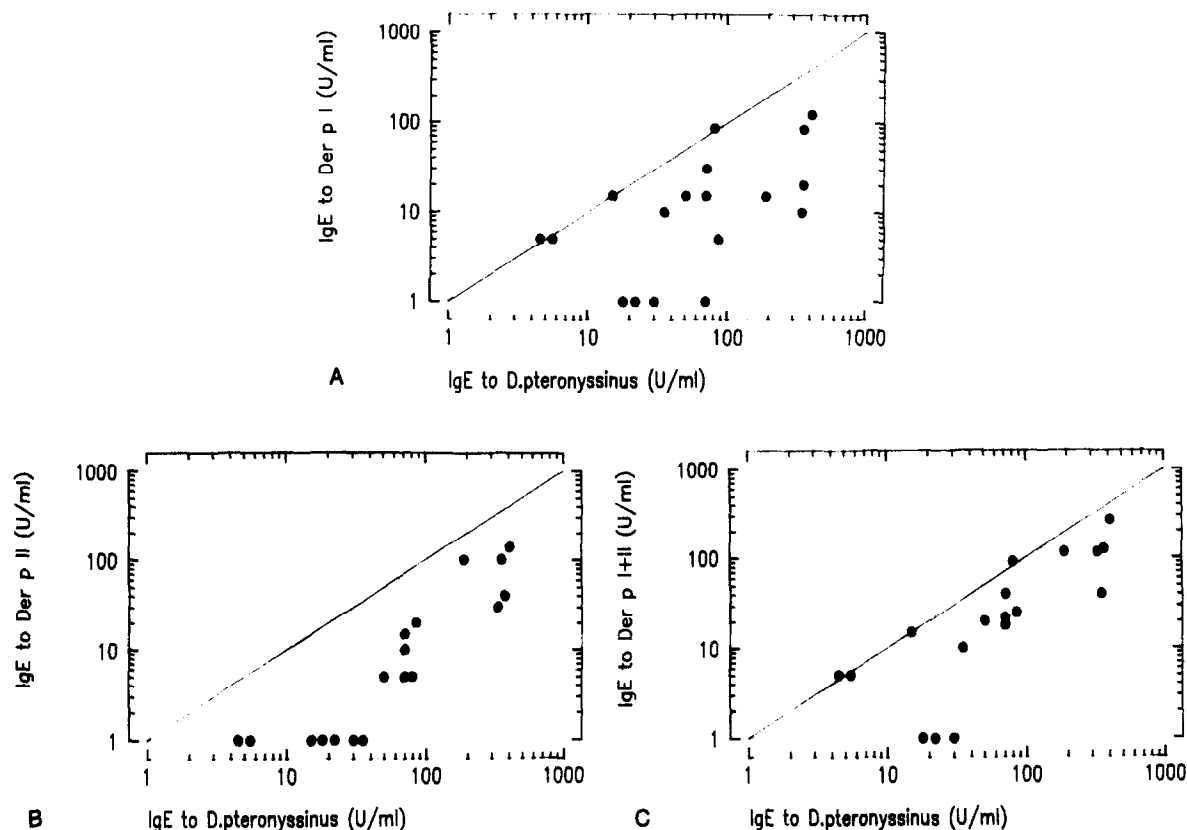


**FIG. 3.** A, Contribution of IgE antibodies to *Der p I*, B, *Der p II*, and C, *Der p I* plus *Der p II* to the total IgE Ab response against house dust mite in serum samples containing the highest levels of mite-specific IgE Abs from 18 high-risk children. Results expressed as units per milliliter related to the local reference serum.

In Figs. 1A and 1B, the development of the mite-specific IgE Ab response in nine children is presented. In these children, high levels of IgE Abs to *D. pteronyssinus* were detected.

For 15 of the 18 children (83%), the highest levels of IgE antihouse dust mite were detected in serum samples drawn in the second half of the year (mainly in September). In contrast, with respect to the first detectable mite-specific IgE Abs, no season effect was observed.

The IgE Ab response to the major allergens *Der p I* and *Der p II* of the house dust mite *D. pteronyssinus* was investigated.

In Fig. 2, A to C, the contribution of the IgE anti-*Der p I* and *Der p II* to the total IgE Abs to house dust mite, present in the seroconversion samples from the 18 young children, is illustrated. In these serum samples, containing the first detectable IgE Abs to house dust mite, both IgE to *Der p I* and IgE to *Der p II* were often not detectable. This finding indicates that IgE Abs to other allergens are important in the early antimitic response.

In the sequentially drawn serum samples containing the highest levels of IgE antihouse dust mite, again both IgE to *Der p I* and IgE to *Der p II* were often not detectable (Fig. 3, A to C). Therefore, it is likely that in the present high-risk group, a major part of the early IgE Ab response to house dust mite is directed against components distinct from *Der p I* and *Der p II*.

Although the study was based on a limited number of children, neither the contribution of IgE anti-*Der p I* nor the contribution of IgE anti-*Der p II* to the total mite-specific IgE Ab response was significantly different between children with ( $N = 14$ ) and without ( $N = 4$ ) a history of atopic eczema.

The RAST results of the sera from the present 18 children were compared to results of a panel of 41 mite RAST-positive sera from adult patients suffering from rhinitis and/or asthma.<sup>9</sup> In that study, the contribution of IgE Abs to *Der p I* and *Der p II* to the total IgE antimitic response was determined as well.

As illustrated in Fig. 4, for 21 of 41 adult patients, the contribution of IgE Abs to *Der p I* and *Der p II*

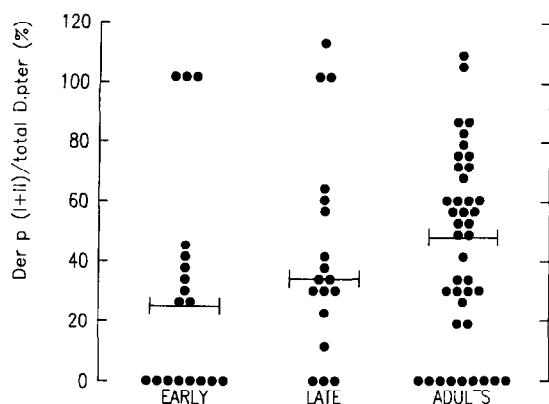


**FIG. 4.** Contribution of IgE antibodies to *Der p I* plus *Der p II* to the total IgE Ab response against house dust mite in serum samples from 41 adults suffering from rhinitis and/or asthma<sup>17</sup> (left panel); IgE seroconversion samples from 18 high-risk children (right panel); serum samples containing the highest levels of mite-specific IgE Abs from these 18 high-risk children (center panel). Results expressed as percentage of total IgE antihouse dust mite. For each panel, median values are indicated with a horizontal bar.

to the total antimitte IgE response was <50% (right panel). In most of the 18 young children, the contribution of IgE antibodies to *Der p I* and *Der p II* was <50% of the total IgE antimitte response: 15 of 18 children (the seroconversion samples) left panel; 12 of 18 children (samples with highest levels of mite-specific IgE response) center panel. There was no statistically significant difference between the three panels of sera, as determined with the Mann-Whitney U test ( $p > 0.05$ ).

## DISCUSSION

The specificity of IgE to house dust mite in serum samples from young children with an increased risk for IgE-mediated allergy ( $N = 18$ ) was studied in the RAST. Although *Der p I* ( $P_1^3$ ) and *Der p II* ( $DpX^6$ ) are known as major allergens of the house dust mite *D. pteronyssinus*, other mite allergens appeared important as well. In most of the young high-risk children, the contribution of IgE Abs to *Der p I* and *Der p II* was <50% of the total IgE antimitte response. Van der Zee et al.<sup>9</sup> reported that 20% of the mite-sensitive (adult) patients had no detectable IgE Abs to *Der p I* and/or *Der p II*. Moreover, in 21 of the 41 adults participating in that study, the contribution of IgE Abs to *Der p I* and *Der p II* was <50% of the total antimitte IgE response. As determined with the Mann-Whitney U test, there was no statistically significant difference between the sera of the high-risk children and the adult patients.

Because the detection limits of the RASTs for total

*D. pteronyssinus*, *Der p I* and *Der p II*, were 0.10, 0.07, and 0.02 U/ml, respectively (related to the local reference serum), the observed low contributions of *Der p I* and *Der p II* were not due to an inappropriate analytical sensitivity. Therefore, it is likely that, in the young children as well as in the adult patients, a substantial part of the early IgE Ab response to house dust mite is directed to components distinct from *Der p I* and *Der p II*.

These results are in agreement with studies that other mite components may be important allergens as well.<sup>1, 2, 7, 14, 15</sup>

In a preliminary sodium dodecyl sulfate-polyacrylamide gel electrophoresis immunoblotting experiment, analyzing the serum samples containing the first detectable IgE antihouse dust mite, no IgE Abs directed against discrete components with molecular weights between 10 and 100 kd distinct from *Der p I* or *Der p II* were detected. However, in subsequent serum samples (with higher antimitte titers), IgE Abs reacting with components > 40 kd were found (data not presented). These samples have yet to be analyzed in detail.

Carswell and Thompson<sup>16</sup> and Thompson et al.<sup>17</sup> distinguished IgE Abs to a mite-fecal antigen (*Der p I*) and IgE Abs to mite-body components, including *Der p II*. They reported that the concentration of IgE Abs to the mite-body antigens was higher in children with eczema than in children with asthma, whereas the concentrations of IgE Abs to the fecal allergen were higher in children with asthma. However, in our study, neither the contribution of IgE anti-*Der p II* (mite-body allergen) nor the contribution of the IgE anti-*Der p I* (mite-fecal allergen) to the total mite-specific IgE Ab response was significantly different between children at risk for allergic asthma with ( $N = 14$ ) and without ( $N = 4$ ) a history of atopic eczema. Therefore, our data did not support Carswell and Thompson's suggestion that sensitization to mite-body and mite-fecal particles may occur by different processes. The allergens of mite bodies may penetrate the skin, whereas fecal allergen may enter the body by other routes.

Our data clearly prove that for most children studied, neither *Der p I* nor *Der p II* qualify as a "primary allergen," as defined previously in this article. However, for three of 18 children (E. O., R. R., and D. P.) for *Der p I*, this possibility is still open. It is also obvious that the fine specificity of IgE Abs is quite variable, even in young children who recently generated IgE Abs to *D. pteronyssinus*. Moreover, allergens distinct from *Der p I* and *Der p II* substantially contribute to the allergic potential of house dust mites, possibly as "primary" allergens. Further study

on the allergens distinct from *Der p 1* and *Der p II* participating in the early IgE anti-mite response is needed.

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