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## Immunogenicity and Tolerogenicity of a Major House Dust Mite Allergen, *Der p I* from *Dermatophagoides pteronyssinus*, in Mice and Rats

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**Abstract.** Primary immunization of different inbred strains of mice with the house dust mite allergen *Der p I* (from *Dermatophagoides pteronyssinus*) adsorbed to adjuvant revealed differences in IgE but not IgG responses. Thus, the CBA and C57 Black strains were shown to be high IgE responders in that persistent IgE was induced which lasted for several months. In contrast, the C3H, AKR and Balb/c strains were judged to be poor responders. Subsequent experiments showed that such responses were adjuvant-independent, refractory to irradiation (850 rad) and that they could be adoptively transferred using immune spleen cells in a dose-dependent manner. At sub-optimal doses, adoptively transferred IgE responses were initiated by either intraperitoneal or intranasal challenge of recipients with allergen in the absence of adjuvant. Allergen-specific IgE in rats was also shown to be strain-dependent, with Brown Norway rats, in contrast to Wistar Furth (WF) and Lou/M rats, being the highest responder strain studied. Repeated intranasal administration of soluble allergen induced IgE tolerance in rats but not mice. However, tolerance was restricted to the low IgE responder phenotype strain, WF.

### Introduction

Immediate hypersensitivity to the house dust mite (HDM) *Dermatophagoides pteronyssinus* has been shown to be strongly linked with asthmatic disease in both children and adults [12, 15, 21]. These observations have stimulated many studies devoted to both the characterization of the allergens involved in the initial sensitization and subsequent provocation, and in devising more efficient immunotherapeutic regimes. With regard to the latter, however, although it has been shown that, subject to stringent selection criteria, the technique is of value [1, 22], our lack of knowledge regarding the mechanisms involved in IgE regulation per se has precluded the optimization of such approaches in humans.

Attention has, therefore, been directed toward the development of animal models with which to dissect the regulatory pathways involved and devise manipulative procedures such that ongoing IgE responses may be dampened or abrogated. These studies have been invaluable but the majority, however, have either used experimental antigens which are of limited

clinical relevance or complex allergenic mixtures containing both clinically relevant allergens as well as irrelevant antigens. Very few studies have been performed using purified allergens of major importance to human allergic patients.

With regard to HDM, for example, crude mite extracts, shown on the basis of crossed immunoelectrophoretic studies to contain at least 19 allergens [9, 11, 18], have been used in several studies [10, 13], yet of the allergens and innumerable irrelevant antigen present in mite extracts only 4 appear to be of major clinical significance [9, 11, 18]. The immunobiology of purified major mite allergens has thus far not been studied in any detail either because they have yet to be purified, or alternatively because those that have, have been isolated in too small quantities to allow such studies.

Thus, the present experiments were designed to examine the nature of the humoral immune response, particularly those of IgE and IgG isotypes to the *D. pteronyssinus*-derived allergen *Der p I* in rats and mice as a prelude to further studies designed to examine and manipulate the immune response to clinically

relevant allergens at the molecular level. Specifically, we have studied the influence of genetic background and the role of adjuvant on IgG and IgE responses (and their duration) to the major mite allergen *Der p* I [3, 17] since it has been isolated in sufficient amounts in our laboratory. We present evidence which indicates that the purified allergen is tolerogenic in animals exposed to this allergen via a mucosal route, in particular the respiratory system. These data are consistent with those obtained using the experimental antigen ovalbumin reported previously from our laboratory [6–8].

## Materials and Methods

### Preparation of Mite Extract

Whole devoid of growth medium and spent growth medium (SGM) devoid of mites were kindly provided by Commonwealth Serum Laboratories (Parkville, Australia). The HDM were extracted by grinding at 0°C in 50 mmol/l of ammonium bicarbonate, pH 8.0, and the resulting extract clarified by centrifugation at 15,000 g for 15 min. The supernatant was removed and the insoluble material was extracted twice more in a similar manner. The supernatants were combined and dialyzed exhaustively against 50 mmol/l of ammonium bicarbonate, pH 8.0. The supernatant was then freeze-dried and stored desiccated at -20°C until required. An extract of SGM was prepared in a similar manner to that described for HDM.

The allergen *Der p* I [the new designation for allergens P<sub>1</sub>, Dpt 12 and Dp 42 previously described by several groups [see ref. 3], was isolated by chromatofocussing from SGM in a manner similar to that described earlier [17]. However, the ion exchange matrix and the buffer used in this study were the anion exchanger DE53 (Whatman, UK), and the chromatofocussing buffer system described by Hearn and Lyttle [4]. A crude Dpt 12 allergen preparation was obtained from an aqueous solution of SGM by precipitation with 50% saturated ammonium sulphate. *Der p* I was then partially purified by gel filtration on Sephacryl S-300 (Pharmacia South Seas Pty., Australia) and those fractions containing the allergen were pooled, concentrated and dialyzed exhaustively against 50 mmol/l Tris buffer, pH 7.5. This preparation was then applied to a DE 53 column pre-equilibrated with the same buffer. After removal of non-binding material, the allergen was eluted using the chromatofocussing buffer adjusted to pH 5.0 with 1 mol/l HCl. Those fractions containing the allergen (pI 6.9–5.8) were pooled, dialyzed against 50 mmol/l ammonium bicarbonate, pH 8.0, and freeze-dried. The allergen preparation was finally purified by gel filtration on a P-60 column (Bio Rad Laboratories, Australia) equilibrated with 50 mmol/l ammonium bicarbonate, pH 8.0. Fractions containing the allergen were pooled and freeze-dried.

### Animals

All animals were obtained from the Animal Resource Centre, Murdoch University, Western Australia. They were maintained on a diet of acidified water and food pellets supplied ad libitum. All animals were aged between 8 and 12 weeks at the beginning of the

experiments. Strains employed were Balb/c, C57 Black, C3H/HeJ, AKR, CBA, Brown Norway (BN), Lou/M and Wistar Furth (WF).

### Cell Preparation

Cells were prepared as described previously [7]. Briefly, spleens were finely minced in chilled mouse buffered balanced salt solution (MBBSS) and sieved. After removal of dead cells by centrifugation, the cells were resuspended in MBBSS and viable counts performed. The cells were then pelleted and resuspended in MBBSS containing 20% v/v fetal calf serum (FCS; Flow Laboratories, Alexandria, USA) at a concentration of  $2.5 \times 10^8$  viable cells/ml.

### Adoptive Transfer

Recipient mice were X-irradiated (850 rad) 24 h before transfer was effected by intravenous inoculation of spleen cells (SPC) in FCS-supplemented MBBSS. The standard protocol comprised an inoculum of either 1, 2 or  $5 \times 10^7$  SPC in 0.2 ml of MBBSS containing 20% v/v FCS [7]. The recipients were bled from the tail vein for determination of antibody levels.

### Immunization and Parenteral Challenge

Mice and rats were immunized intraperitoneally with *Der p* I adsorbed to alum (Wyeth, Australia) unless otherwise stated. Mice received 0.01, 0.1, 1 or 10 µg adsorbed to 4 mg alum, whereas rats received either 10 or 100 µg adsorbed to 20 mg of alum. Intranasal immunization was performed by instilling 12.5 µl of antigen dissolved in 0.01 mol/l phosphate buffer containing 0.15 mol/l NaCl (PBS) into each nostril of an anaesthetized animal.

### Determination of Antibody Titres

**Homocytotropic Antibody.** IgE antibody titres were determined via the passive cutaneous anaphylaxis (PCA) reaction. Serum samples were diluted in PBS and 50 µl aliquots inoculated intradermally on the shaved backs of female Sprague Dawley rats at marked sites. Twenty-four h later, the rats were challenged intravenously with either SGM or HDM extract in 0.5 ml of 1% (v/v) Evans blue in PBS at concentrations shown in preliminary experiments to be optimal for eliciting PCA reactions (i.e., 20 mg SGM or 4 mg HDM). Thirty min later, the rats were sacrificed, and the relevant skin areas excised for examination of the lesions. The reciprocal of the highest serum dilution giving a flare or 'blueing reaction' of 5 mm diameter was taken as the PCA titre.

SGM was used in PCA assays designed to measure IgE responses to *Der p* I because of the limited availability of the homologous antigen. The presence of antigen in the SGM was confirmed by single radial immunodiffusion using specific anti-*Der p* I antiserum [17].

**IgG Antibody.** IgG antibody responses to *Der p* I were determined using an enzyme-linked immunosorbent assay (ELISA) essentially as described [20]. In brief, 100 µl of serum diluted 1:200 in PBS containing 0.1% (v/v) Tween 20 was added to ELISA plates (EIA plates; Flow Laboratories, USA) optimally coated with *Der p* I (2 µg/ml) and incubated for 2 h at room temperature. The plates were washed and incubated with either alkaline phosphatase-conjugated sheep anti-mouse IgG or sheep anti-rat IgG at a dilution of 1:1,000 for 2 h at room temperature. The plates were washed and incubated with *p*-nitrophenol substrate (1 mg/ml; Sigma Chemical, St. Louis, Mo., USA). The data were expressed as optical densities at 405 nm (OD<sub>405</sub>) after deduction of the OD<sub>405</sub> obtained with a similar dilution of normal mouse or rat serum.

**Table I.** Primary anti-*Der p* I-specific IgE and IgG antibody responses in inbred mice

| Strain                                  | <i>Der p</i> I IgE PCA titre |                  |                  |                |                            | <i>Der p</i> I IgG ELISA OD <sub>405</sub> |                  |                  |                  |                  |
|-----------------------------------------|------------------------------|------------------|------------------|----------------|----------------------------|--------------------------------------------|------------------|------------------|------------------|------------------|
|                                         | day 7                        | 14               | 21               | 49             | 120                        | day 7                                      | 14               | 21               | 49               | 120              |
| CBA<br>(H-2 <sup>k</sup> )              | 0                            | 562<br>(320-640) | 323<br>(160-640) | 79<br>(40-160) | 0                          | 0.052<br>(0.014)                           | 0.048<br>(0.016) | 0.106<br>(0.034) | 0.198<br>(0.049) | 0.399<br>(0.330) |
| C57Bl<br>(H-2 <sup>b</sup> )            | 9<br>(0-80)                  | 79<br>(0-160)    | 282<br>(160-320) | 22<br>(0-160)  | 0                          | 0.080<br>(0.018)                           | 0.068<br>(0.022) | 0.218<br>(0.062) | 0.139<br>(0.045) | 0.360<br>(0.088) |
| C <sub>3</sub> H<br>(H-2 <sup>k</sup> ) | 0                            | 66<br>(0-640)    | 29<br>(0-160)    | 6<br>(0-80)    | 63<br>(0-80 <sup>a</sup> ) | 0.36<br>(0.015)                            | 0.140<br>(0.065) | 0.073<br>(0.029) | 0.106<br>(0.02)  | 0.313<br>(0.069) |
| AKR<br>(H-2 <sup>k</sup> )              | 0                            | 6.6<br>(0-160)   | 52<br>(40-80)    | 13<br>(0-40)   | 0                          | 0.058<br>(0.012)                           | 0.020<br>(0.009) | 0.003<br>(0.003) | 0.185<br>(0.050) | 0.174<br>(0.025) |
| Balb/c<br>(H-2 <sup>d</sup> )           | 0                            | 10<br>(0-80)     | 33<br>(40-160)   | 19<br>(0-80)   | 2<br>(0-40 <sup>b</sup> )  | 0.090<br>(0.017)                           | 0.068<br>(0.026) | 0.122<br>(0.036) | 0.229<br>(0.050) | 0.108<br>(0.000) |

Mice, in groups of 5, were immunized i.p. with 10  $\mu$ g *Der p* I adsorbed to alum. The figures in parentheses indicate the H-2 haplotype of the mouse strain studied. The PCA data shown are geometric-mean reciprocal titres with the range of titres in parentheses. The ELISA data are expressed as the mean OD<sub>405</sub> obtained with a 1:200 dilution of mouse serum. The figures in parentheses indicate the standard error of the mean.

<sup>a</sup> Two animals in this group died.

<sup>b</sup> Three animals in this group died.

#### Statistical Analyses

These were performed using the statistical package of the Western Michigan University.

## Results

### Primary Anti-*Der p* I IgG and IgE Antibody

Table I illustrates the primary immune response to the allergen *Der p* I adsorbed to alum and its longevity in selected inbred strains of mice. The data are ranked (top to bottom) with respect to the degree of specific IgE responsiveness on the basis of a Student *t* test analysis of data obtained using sera collected on days 14 and 21 post-immunization, the period of maximum response. The most responsive strain was CBA followed closely by C57Bl. The least responsive strains were Balb/c, C3H and AKR. Specific IgE was detected in sera from all strains up to 49 days after immunization but only in some sera from Balb/c and C3H animals at day 122. IgG titres expressed as OD<sub>405</sub> were low in sera from all strains at day 7, 14 and 21 but a trend towards rising titres was observed in sera obtained 49 and 120 days post immunization.

### Influence of Adjuvant on Anti-*Der p* I-Specific IgE

Table II shows the primary, secondary and tertiary IgE responses in C57Bl mice immunized with *Der p* I with or without adjuvant. These studies showed that IgE responses in both systems were dose-dependent. Maximum responses, in mice immunized with the allergen adsorbed to alum, were achieved with the dose of 10  $\mu$ g/mouse followed by 1  $\mu$ g/mouse. Responses were not detected with either the 0.1 or 0.01  $\mu$ g/mouse dose. With the former doses, repeated immunizations did not appear to influence greatly the IgE response. Without alum, boosterable IgE responses were obtained but only with 10  $\mu$ g/mouse. When the 0.1 and 0.01  $\mu$ g/mouse dose groups were challenged with 10  $\mu$ g *Der p* I adsorbed to alum the 1  $\mu$ g and 0.1  $\mu$ g/mouse dose groups responded, whereas the 0.01  $\mu$ g did not, suggesting tolerance in this latter group.

### Radiation Resistance of Persistent Anti-*Der p* I-Specific IgE in Mice

The radioresistance of the anti-*Der p* I-specific IgE response in CBA and C57Bl mice immunized with allergen adsorbed to alum was determined (table III).

**Table II.** The effect of adjuvant on the primary and secondary anti-Der p I IgE antibody responses in C57B1 mice

| Treatment<br>$\mu\text{g}$ Der p I | Days after immunization |       |     |       |       |       |     |                 |       |       |
|------------------------------------|-------------------------|-------|-----|-------|-------|-------|-----|-----------------|-------|-------|
|                                    | 0                       | 14    | 21  | 28    | 35    | 49    | 70  | 84              | 91    | 99    |
| <i>With alum</i>                   | 1.                      |       | 2.  |       |       |       |     | 3.              |       |       |
| 10                                 | 0                       | 1,280 | 640 | 1,280 | 1,280 | 320   | 640 | NT              | 2,560 | NT    |
| 1                                  | 0                       | 0     | 0   | 640   | 640   | 320   | 640 | NT              | 2,560 | NT    |
| 0.1                                | 0                       | 0     | 0   | 0     | 0     | 0     | 0   | NT              | 0     | NT    |
| 0.01                               | 0                       | 0     | 0   | 0     | 0     | 0     | 0   | NT              | 0     | NT    |
| <i>Without alum</i>                | 1.                      |       | 2.  |       | 3.    |       |     | 4. <sup>a</sup> |       |       |
| 10                                 | 0                       | 0     | 0   | 160   | 0     | 2,560 | 640 | NT              | 320   | NT    |
| 1                                  | 0                       | 0     | 0   | 0     | 0     | 0     | 0   | NT              | 2,560 | 2,560 |
| 0.1                                | 0                       | 0     | 0   | 0     | 0     | 0     | 0   | NT              | 0     | 2,560 |
| 0.01                               | 0                       | 0     | 0   | 0     | 0     | 0     | 0   | NT              | 0     | 0     |

Mice, in groups of 5, were immunized intraperitoneally at the time points indicated with an appropriate amount of Der p I either in the presence or absence of alum. Data shown are the reciprocal PCA titres of pooled serum samples.

<sup>a</sup> Animals were immunized with 10  $\mu\text{g}$  Der p I adsorbed to alum.

NT = Not tested; 1.-4. = first to fourth challenge with Der p I, with or without alum.

**Table III.** Radiation resistance of persistent IgE-specific anti-Der p I responses in mice

| Mouse strain |   | Der p I PCA titre |        |                  |                |
|--------------|---|-------------------|--------|------------------|----------------|
|              |   | control group     |        | irradiated group |                |
|              |   | day 14            | day 21 | day 14           | day 21         |
| CBA          | 1 | 640               | 640    | 1,280            | 1,280          |
|              | 2 | 640               | 640    | 1,280            | 1,280          |
|              | 3 | 320               | 160    | 1,280            | 640            |
|              | 4 | 640               | 1,280  | 320              | 1,280          |
| C57B1        | 1 | 640               | 640    | 640              | 640            |
|              | 2 | 80                | 80     | 320              | - <sup>a</sup> |
|              | 3 | 40                | 40     | 160              | 320            |
|              | 4 | 320               | 160    | 320              | 80             |

Mice were immunized with 10  $\mu\text{g}$  Der p I adsorbed to alum on day 0 and were boosted using the same regime on day 33. On day 75, selected mice were irradiated (850 rad) and reconstituted with  $1 \times 10^7$  non-immune bone marrow cells i.v. Control and irradiated mice were bled on days 14 and 21 post-irradiation. Data shown are individual PCA titres.

<sup>a</sup> Animal died.

**Table IV.** Adoptive transfer of persistent IgE anti-Der p I specific responses by SPC in CBA mice

| Group               | Secondary challenge<br>$\mu\text{g}$ Der p I | Route | Der p I PCA titre<br>in recipients |
|---------------------|----------------------------------------------|-------|------------------------------------|
| $2 \times 10^7$ SPC | -                                            | -     | < 40                               |
|                     | 10                                           | i.p.  | 10,240                             |
|                     | 10                                           | i.n.  | 7,760                              |
|                     | 1                                            | i.p.  | 5,881                              |
|                     | 1                                            | i.n.  | 735                                |
| $5 \times 10^7$ SPC | -                                            | -     | 5,120                              |

Mice were immunized with 10  $\mu\text{g}$  Der p I adsorbed with alum 90 days prior to transfer. Recipient mice were irradiated 24 h (850 rad) prior to transfer and given an i.v. injection of  $2 \times 10^7$  or  $5 \times 10^7$  SPC and challenged either intraperitoneally (i.p) or intranasally (i.n.) with soluble allergen. Mice were bled 21 days after adoptive transfer. PCA data shown are the geometric-mean reciprocal PCA titres.

Table V. Primary and secondary IgE and IgG anti-*Der p I* responses in rats

| Dose<br>$\mu\text{g Der p I}$ | <i>Der p I</i> IgE PCA titre |                 | <i>Der p I</i> IgG ELISA OD <sub>405</sub> |                  |
|-------------------------------|------------------------------|-----------------|--------------------------------------------|------------------|
|                               | 1.                           | 2.              | 1.                                         | 2                |
| <i>BN strain</i>              |                              |                 |                                            |                  |
| 10                            | 557<br>(320-640)             | 70<br>(<20-160) | 0.086<br>(0.054)                           | 0.756<br>(0.448) |
| 100                           | 640<br>(40-2,560)            | 121<br>80-640   | 0.238<br>(0.145)                           | 0.544<br>(0.196) |
| <i>Lou/M strain</i>           |                              |                 |                                            |                  |
| 10                            | 61<br>(40-80)                | 28<br>(10-80)   | 0.012<br>(0.029)                           | 0.340<br>(0.145) |
| 100                           | 23<br>(10-80)                | NT              | 0.184<br>(0.119)                           | NT               |
| <i>WF strain</i>              |                              |                 |                                            |                  |
| 10                            | 46<br>(40-80)                | 28<br>(20-40)   | 0.071<br>(0.109)                           | 0.567<br>(0.079) |
| 100                           | < 20                         | NT              | 0.363<br>(0.349)                           | NT               |

Rats, in groups of 5, were immunized with *Der p I* absorbed to alum and bled 10 and 11 days after primary (1.) and secondary (2.) immunizations, respectively. The PCA data shown represent the geometric mean of the reciprocal titre and the figures in parentheses represent the range of PCA titres obtained. The ELISA data shown represent the mean OD<sub>405</sub> obtained with a 1:200 dilution of rat serum. The figures in parentheses represent the standard error of the mean.

NT = Not tested.

These studies showed that the persistent IgE response detected in sera from mice immunized 75 days prior to irradiation was subsequently maintained in both strains of mice treated with 850 rad.

#### *Adoptive Transfer of Anti-*Der p I*-Specific IgE in Mice*

Table IV shows that the persistent IgE response to *Der p I* can be adoptively transferred via immune SPC to irradiated mice. This response was shown to be dose-dependent in that injection of  $2 \times 10^7$  SPC failed to transfer the response, in contrast to the transfer of  $5 \times 10^7$  SPC. However, it can be seen (table IV) that mice adoptively transferred with  $2 \times 10^7$  immune SPC, can be stimulated to respond to challenge with allergen in the absence of adjuvant by both the intraperitoneal and intranasal routes.

#### *Induction of IgE-Specific Tolerance to *Der p I**

The ability of the intranasal administration of *Der p I* to both CBA and C57Bl mice to induce IgE-specific tolerance was investigated using a procedure

known to induce this state in mice using ovalbumin [6]. However, this procedure was insufficient (data not shown) to induce tolerance in either strain using a range of doses (0.0001-10  $\mu\text{g}/\text{mouse}$ ).

#### *Primary and Secondary Anti-*Der p I* IgE and IgG Responses in Rats*

Table V shows the primary and secondary anti-*Der p I* IgE and IgG responses in Lou/M, BN and WF rats. These studies showed that primary IgE responses were obtained in all three strains tested with both doses of 10  $\mu\text{g}$  and 100  $\mu\text{g}$  of allergen adsorbed to alum. The most responsive strain was shown to be BN followed by the Lou/M and WF strains. High IgE titres were detected in sera from BN rats immunized with either 10 or 100  $\mu\text{g}/\text{rat}$ . With both Lou/M and WF rats, 10  $\mu\text{g}/\text{rat}$  produced the highest titres. WF rats responded poorly to 100  $\mu\text{g}/\text{rat}$ . Further studies indicated that IgE responses to the allergen were not boosterable, since the geometric-mean titres obtained were lower than those observed for the primary response. All strains produced an IgG response to the

**Table VI.** Induction of IgE-specific tolerance to Der p I by intranasal administration of homologous antigen in rats

| Dose<br>$\mu\text{g}$ Der p I | Der p I IgE PCA titre |      |        |    | Der p I IgG OD <sub>405</sub> |                  |                  |                  |
|-------------------------------|-----------------------|------|--------|----|-------------------------------|------------------|------------------|------------------|
|                               | BN                    |      | WF     |    | BN                            |                  | WF               |                  |
|                               | day 10                | 20   | day 10 | 20 | day 10                        | 20               | day 10           | 20               |
| 10                            | 905                   | 113  | NT     | NT | 0.888<br>(0.068)              | 0.668<br>(0.111) | NT               | NT               |
| 1                             | 1,076                 | 34   | 0      | 2  | 0.287<br>(0.179)              | 0.295<br>(0.156) | 0.560<br>(0.38)  | 0.265<br>(0.038) |
| 0.1                           | 1,076                 | 160  | 2      | 2  | 0.030<br>(0.03)               | 0.093<br>(0.050) | 0.335<br>(0.186) | 0.575<br>(0.188) |
| 0.01                          | NT                    | NT   | 5      | 2  | NT                            | NT               | 0.240<br>(0.06)  | 0.547<br>(0.135) |
| Solvent control               | 761                   | 16.8 | 48     | 24 | 0.206<br>(0.019)              | 0.412<br>(0.015) | 0.312<br>(0.312) | 0.099<br>(0.099) |

Rats were given intranasal injections of Der p I weekly for 8 weeks and then challenged with 10  $\mu\text{g}$  Der p I adsorbed to alum i.p. They were bled 10 and 20 days after challenge. IgE titres shown are geometric-mean PCA titres; IgG titres are expressed as the mean OD<sub>405</sub> obtained with a 1:200 dilution of rat serum with the standard error of the mean in parentheses.

NT = Not tested.

allergen, but primary IgG responses were dose-dependent, with 100  $\mu\text{g}/\text{rat}$  clearly being better than 10  $\mu\text{g}/\text{rat}$ . Anamnestic responses were observed in those animals receiving secondary immunization with allergen adsorbed to alum.

#### Induction of IgE-Specific Tolerance to Der p I

The ability of intranasal administration of Der p I to produce IgE tolerance in BN and WF rats was studied. These studies showed (table VI) that 8 weekly doses of allergen were sufficient to induce a significant tolerance in the allergen-specific IgE response in WF rats but not in the high-responder BN rats as judged by the Student t test analysis of PCA titres obtained using sera taken on days 10 and 20 post-challenge ( $p < 0.05$  for solvent control versus each group of animals receiving allergen). The ability of WF rats to mount an IgG response to the allergen, however, was not impaired. Although the mean OD<sub>405</sub> obtained with sera from day 20 post-challenge were higher in those rats who had received the intranasal injection when compared with solvent controls, these differences were not significant. With BN rats, the mean OD<sub>405</sub> was higher ( $p < 0.05$ ) in day 10 sera from rats who had received 10  $\mu\text{g}/\text{rat}$  when compared with the mean OD<sub>405</sub> obtained with sera from solvent control animals. This difference was not apparent with the

day 20 bleeds, however. Those animals which received 0.1  $\mu\text{g}/\text{rat}$  had a significantly ( $p < 0.05$ ) lower mean OD<sub>405</sub> IgG response than that observed with either the other dosage groups or the solvent control.

#### Discussion

The studies described here indicated that the allergen Der p I behaved in a similar fashion to that exhibited by a diverse range of experimental antigens with regard to its capacity to induce antibody responses, particularly IgE and IgG, in experimental rodents. For example, the data presented showed that both rats and mice produced IgE and IgG antibodies on primary parenteral challenge with either allergen adsorbed to adjuvant or repeated stimulation with soluble allergen in the absence of adjuvant. These data are consistent with those previously described using the experimental allergen ovalbumin [8] and crude allergen mixtures such as rye grass pollen [5, 19].

In particular, it was shown that mice immunized just once with allergen adsorbed to alum produced a persistent IgE response which not only lasted for several months but could also be transferred to irradiated recipients in a dose-dependent fashion without

secondary antigenic stimulation. The resulting IgE response in these mice was higher than that observed with either primary or secondary parenteral allergen challenge. These results are entirely consistent with those described for the transfer of persistent IgE responses to ovalbumin in Balb/c mice [7], which indicates the presence of a stable pool of allergen in spleen.

The transfer of low doses of SPC failed to spontaneously produce a persistent response, but expansion (and, therefore, expression of the IgE response) of the cell population transferred could be initiated by intraperitoneal challenge with allergen without adjuvant. Similar results were obtained by intranasal challenge, and the latter finding may be particularly pertinent with regard to immunotherapy in humans. For example, it is known that parenteral injection of allergen extracts containing irrelevant antigens during hypersensitization can stimulate the production of IgE antibodies to the latter [14]. It is thus conceivable that such responses could be boosted by exposure to the native aeroallergen via the oro-nasal route with concomitant clinical symptoms, provocation by what were once antigens of little clinical relevance.

The limited use of inbred strains of mice in our study indicated that some strains were good IgE responders to the allergen (CBA and C57Bl) whereas others (C3H, AKR and Balb/c) were poor. In the mouse, these observations indicate that IgE production to *Der p* I may be under immune response (IR) gene control, since strains such as Balb/c which responded poorly, in contrast to the high-responder CBA strain, are known to be good IgE responders to other antigens such as ovalbumin [8]. In the rat, where the BN strain was clearly superior in its capacity to produce IgE to the allergen, it is probable that this response reflects the high IgE responder status of this strain rather than any IR gene effect per se, since BN rats are known to respond equally well to a variety of antigens including ovalbumin [2, 16]. As seen in other studies with rats, IgE response, in contrast to the IgG antibody response, to *Der p* I on primary challenge was transient and refractory to subsequent secondary challenge [2, 16].

Many studies from our laboratory have shown that regular exposure of experimental rodents to aerosolized antigen over an extended period induces tolerance in allergen-specific IgE responses on parenteral challenge [6, 16]. It was thus of interest to repeat these studies using *Der p* I, but insufficient material pre-

cluded this approach. Therefore, experiments were performed using intranasal immunizations since other studies from this laboratory had previously shown that effects similar to those achieved by aerosolization could be obtained [6].

Exposure of WF rats in this manner, in contrast to BN rats, induced IgE tolerance; these data are consistent with those reported for the induction of tolerance in IgE responses to aerosolized ovalbumin. These data indicate that the induction of tolerance in a strain reflects its overall IgE responsiveness, i.e., tolerance is best achieved in those strains responding poorly to the antigen in question [6, 16]. For example, it is possible to induce IgE tolerance in low-responder rats with nanogram amounts of ovalbumin, yet 1,000-fold greater amounts are required to achieve the same effect in high-responder BN rats [16].

This explanation would account for our failure to induce IgE tolerance via the nasal route in CBA and C57Bl mice, the two most *Der p* I-responsive strains studied, and studies are underway using low-responder mouse strains to test this hypothesis. However, the observation [6] that exposure of high-responder mouse strains to ovalbumin in this fashion induces IgE tolerance suggests that other factors associated perhaps with the physicochemical characteristics of the antigen studied such as molecular weight and solubility may also play a role.

In conclusion, we have studied the IgE and IgG responsiveness of inbred strains of rats and mice to a major HDM allergen, *Der p* I. The data obtained indicated that, in general, the allergen behaved in a similar fashion to that exhibited by other experimental allergens. These data will form the basis for future studies designed to assess the importance of individual allergenic epitopes comprising the allergen, and will also be utilized in experiments on the mechanisms involved in an experimental model of intranasally induced IgE tolerance.

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