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Specific IgG and IgE Responses to *Dermatophagoides pteronyssinus* in Sprague-Dawley Rats*

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A. GILABERT, J. SALGADO, A. FRANCH, J. QUERALT and A. TORRALBA. *Specific IgG and IgE Responses to* Dermatophagoides pteronyssinus *in Sprague-Dawley Rats.* Rev. esp. Fisiol., 46 (4), 353-358, 1990.

Specific IgG and IgE antibodies against the mite *Dermatophagoides pteronyssinus* (Dp) have been determined in the serum of Sprague Dawley (SD) rats by enzyme linked immunosorbent assay (ELISA) and passive cutaneous anaphylaxis (PCA), respectively. Immunization of rats with Dp produces a rapid increase of serum specific IgE antibody levels which decrease when specific IgG antibodies start rising. The results indicate that the SD rat is a good model to induce a humoral immune response to allergen Dp.

Key words: *Dermatophagoides pteromyssinus*, ELISA, IgE, IgG, Passive cutaneous anaphylaxis.

The house dust mite, *Dermatophagoides pteronyssinus* (Dp), is well known as one of the most important inhalant allergens in respiratory allergy diseases such as asthma and allergic rhinitis (1, 12, 16, 17).

There is evidence that IgE antibodies are often elevated in atopic respiratory diseases (10). Also, during allergen specific immunotherapy several changes in specific IgG and IgE antibodies may be observed, such as a rise in the concentration of allergen specific IgG antibodies and a fall in the concentration of allergen specific IgE in serum (4). So, detection of serum specific IgG and IgE antibodies should be useful in the assessment of a hypothetical treatment by allergen specific immunotherapy. Determination of Dp specific IgG and IgE antibodies have been previously reported in human serum by different techniques (2, 5, 7, 13, 14).

In this work we evaluated the changes in Dp specific IgG and IgE antibody levels in the serum of rats which have been previously immunized with Dp. Specific IgG antibodies have been quantified by enzyme linked immunosorbent assay (ELISA), and specific IgE antibodies de-

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tected by passive cutaneous anaphylaxis (PCA).

Materials and Methods

Animals. — Female Sprague-Dawley (SD) rats weighing 180-220 g were used. Rats were fed on standard diet (UAR-A-04) and kept in constant climatic conditions (22 °C). Food and drinking water were available *ad libitum*.

Immunization. — Rats were immunized by subcutaneous injection of 175 PNU/100 g of Dp extract (Merk-Igoda S.A. Div. Alergia; Barcelona) suspended in aluminum hydroxide. *Bordetella pertussis* suspension (9e + 7bact/100 g) (Difco) was administered also, intraperitoneally, as adjuvant. Twenty two days later, rats were boosted with a subcutaneous injection of 35 PNU/100 g of Dp solution (3).

Antisera. — Antisera were obtained from the jugular vein every 4-6 days. Blood samples were allowed to coagulate for about 1 h at room temperature and then centrifuged at 15,000 *g* (Eppendorf 54/14S) during 5 min. The serum was decanted and stored in conveniently sized aliquots at -20 °C.

Determination of specific IgG by ELISA. — In order to detect Dp specific IgG an indirect non-competitive ELISA was developed. All steps were made in polystyrene microplates (Dinatech M-129-B). The microtitre plates were coated overnight at 4 °C with 100 µl/well of Dp extract containing 35 PNU/ml in PBS (6). Excess adsorption sites were blocked for 1 h at room temperature with 200 µl/well of bovine serum albumin (BSA), 10 mg/ml in 0.05 % Tween-20 in PBS (P-T-BSA) (3). The plates were washed three times with PBS and once with 0.05 % Tween-20 in PBS. Then, 100 µl/well of different concentrations of a positive and control serum (dilutions from 1/50 to 1/800) in P-T-BSA were added, followed by incubation for 3 h at room temperature in a moist chamber (15). The plates were washed again and incubated with 100 µl/well of rabbit anti-rat IgG-Peroxidase (Miles-Yeda Ltd.) (dilutions from 1/800 to 1/25600 in P-T-BSA) 17 h at 4 °C in a moist chamber. After discarding supernatants, plates were washed and DMAB (3-dimethyl-aminobenzoic) - MBTH (3-methyl-2-benzothiazolidone hydrazone) solution was added and they were incubated again for 20 min with shaking at room temperature. After stopping the enzymatic reaction with 50 µl/well of 2 M sulfuric acid, the absorbance at 60 nm was measured in a Titertek Uniskan (Flow Lab., Helsinki, Finland).

Determination of specific IgE by PCA. — Serum antigen specific IgE levels were determined by passive cutaneous anaphylaxis (PCA) according to HARADA *et al.* (8). Briefly, serum was diluted 1:4 in saline and 0.1 ml was intradermally injected into the shaved back of male SD rats. After 48 hours, rats were intravenously challenged with 700 PNU/100 g of Dp extract plus 2 mg/100 g Evans blue dye. After 30 minutes, the animals were sacrificed and the amount of extravasated dye was determined spectrophotometrically.

Statistical analysis. — The results obtained from the same rat on different days were compared using the Student's paired t-test.

Results

An indirect microELISA technique, able to measure Dp specific IgG antibodies in rats immunized by Dp, was developed by us. The optimum experimental conditions were 35 PNU/ml of Dp to coat the microplates, test serum diluted at 1/

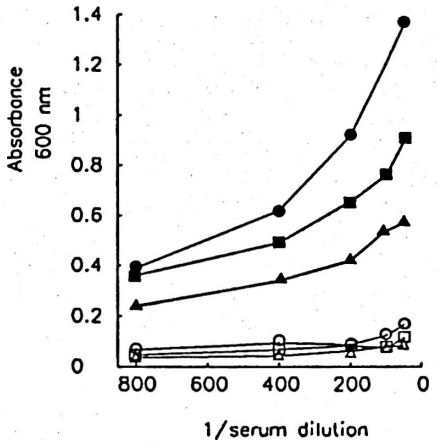

Fig. 1. *Determination of optimum anti-rat PO-anti-rat IgG dilution and adequate rat serum dilution to measure Dp specific IgG by indirect ELISA.*
PO anti-rat IgG dilutions: negative serum, 1/6400 (○), 1/12800 (□) and 1/25600 (△); positive serum, 1/6400 (●), 1/12800 (■) and 1/25600 (▲).

100 and 1/200, and a 1/6400 dilution of the PO-anti-rat IgG (fig. 1).

The sensitivity of the method is defined as the highest serum dilution that still shows significant differences with its previous and next serum dilutions and obviously, with the control serum. The sensitivity was 1/6400. Reproducibility was determined by two laboratory controls. The intra-assay variation coefficient ranged between 2.6-4 % and the inter-assay coefficient was 8.5 %. Once the optimal ELISA conditions, were established, the Dp specific IgG changes in immunized SD rats were evaluated individually (fig. 2).

Fig. 2 shows the time course of specific anti-Dp IgG antibodies. On day 16 specific IgG antibodies increased significantly (0.396 ± 0.147) in relation to values of day 0 (0.058 ± 0.003) ($p = 0.0188$). The specific IgG antibodies reached a maximum on day 28 (0.960 ± 0.097) ($p = 0.35e-3$) which remained during the rest of the studied period (53 days).

The time course of anti-Dp IgG antibodies, measured as μg of Evans blue extravasated through the skin during PCA test, can be seen in figure 3. On day 11 the anti-Dp IgE antibody levels showed a maximum in relation to values of day 0

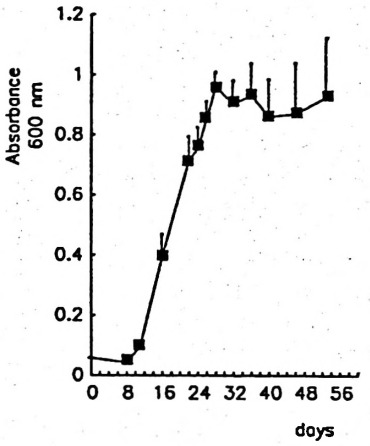

Fig. 2. *Time course of anti Dp IgG antibodies in SD rats serum after immunization with 175 PNU/100 g of Dp and a boosted secondary dose of 35 PNU/100 g on day 22.*
Each point represents the arithmetic mean ± SEM.

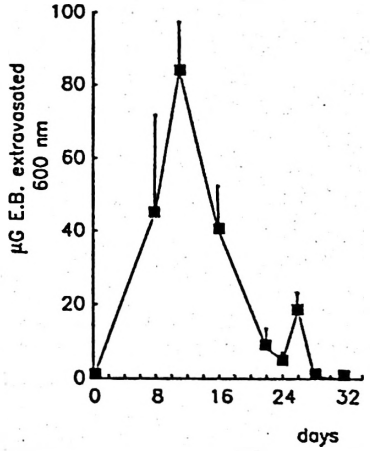

Fig. 3. *Time course of anti Dp IgE antibodies in SD rats serum after immunization with 175 PNU/100 g of Dp and a boosted secondary dose of 35 PNU/100 g on day 22.*
Each point represents the arithmetic mean ± SEM.

(p = 0.83e-2). Specific IgE antibody levels decreased thereafter to become undetectable on day 28. A secondary Dp administration produces an additional increase in specific IgE levels which are not significant.

Discussion

In the present study, specific IgG and IgE antibodies against the mite Dp have been determined in the serum of SD rats by means of the ELISA and PCA respectively.

The SD rats were first, immunized with the allergen Dp according with GILABERT *et al.* (6). Other Dp immunization guidelines have been reported in rabbit (2, 11).

Secondly, a high sensitive microELISA was developed to determine specific IgG antibodies against Dp in SD rat serum. Reproducibility was similar to other ELISAs for anti Dp specific IgG (7), with an intra-assay coefficient of variation (CV) between 2.6 and 4 % and interassay CV of 8.5 %. The amount of Dp incubated on polystirene plates was 7 PNU/well (20 µg) and optimal serum dilution lay between 1/100 and 1/200, which is in accordance with HARADA *et al.* (7). It allows the analysis of specific IgG antibodies in SD rat serum using a small serum sample.

Thirdly, a biological method, PCA, was used to measure anti Dp specific IgE levels. Contrary to what was expected, PCA showed a similar sensitivity to that of ELISA in detecting specific IgE antibodies (9). PCA avoids the interference of IgG antibodies existing in ELISA.

After immunization with Dp extract a rise in specific IgE antibody levels was observed in serum. These levels reach a maximum and rapidly decrease when specific IgG antibodies start rising. Specific IgE levels become undetectable when specific IgG reaches the plateau. These results show an inverse correlation between serum specific IgG and IgE antibodies, and suggest that our experimental model is a valid one to study the humoral response against Dp, in several experimental conditions.

This work has allowed us to determine the time course of the Dp specific humoral immune response in SD rats. Currently we are trying to elucidate how specific IgG and IgE levels change with the preadministration of the allergen Dp and its formulated allergoid.

Acknowledgements

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Resumen

Se determinan, mediante ELISA y PCA (anafilaxia cutánea pasiva), los niveles de anticuerpos específicos anti *Dermatophagoides pteronyssinus* (anti Dp) de tipo IgG e IgE, respectivamente, en el suero de ratas Sprague-Dawley (SD). La inmunización produce un incremento rápido de los niveles séricos de IgE específica anti Dp los cuales disminuyen a medida que aumentan los niveles séricos de IgG específica anti Dp. Los resultados obtenidos confirman que las ratas de la cepa SD son un buen modelo para inducir una respuesta inmune humoral frente al alergeno Dp.

Palabras clave: *Dermatophagoides pteronyssinus*, ELISA, IgE, IgG, Anafilaxia cutánea pasiva.

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