

Blocking activity of mite-specific IgG antibodies studied by skin tests

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The aim of the study was to determine whether IgG antibodies from patients on specific immunotherapy could inhibit skin test reactivity of allergens when mixed with them prior to testing. In a preliminary double-blind study, mite extracts were incubated with glycerol-saline solution or non-specific IgG and used in testing 15 patients. Wheal and flare diameters produced with the two mite extracts did not differ significantly. In a second double-blind study with 15 other patients, skin tests using mite extracts incubated with either non-specific IgG or specific IgG showed significantly decreased diameters when mite extracts were preincubated with specific IgG. This result, *in vivo*, confirms the antigen neutralizing capacity of specific IgG demonstrated *in vitro*.

Key words: blocking activity; mites; skin tests; specific IgG.

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CLINICAL ASPECTS

The blocking activity of mite-specific IgG versus non-specific IgG was demonstrated in a double blind study using skin prick tests in allergic patients. The therapeutical value of these IgG remains to be demonstrated.

The mechanisms of specific immunotherapy (SI) are still unclear, although its efficacy has been proven in pollen, mite and venom allergies (14, 15, 20). SI induces both humoral and cellular changes (22). Production of specific IgG antibodies, mainly IgG₁ and IgG₄ subclasses, has been demonstrated many times (4, 26). These antibodies may act as blocking factors, as demonstrated *in vitro* by inhibition of histamine release (13, 25) or degranulation of human basophils (12).

The antigen neutralizing capacity (ANC) of the serum is well correlated with specific IgG₄ antibodies in patients undergoing rush-specific immunotherapy with mite extract (18).

The aim of the present work was to study the ANC of specific IgG (SP-IgG) and non-specific IgG (nSP IgG) *in vivo*, i.e. by skin testing sensitive patients with mite extracts preincubated either with SP IgG or nSP-IgG. The work was conducted in two double-blind protocols. In protocol A the ANC of nSP IgG was studied versus that of a glycerol-saline solution (GS) in 15 patients. In protocol B, the ANC of SP IgG and nSP IgG were compared in 15 other patients.

PATIENTS

All patients gave their informed consent. The inclusion criteria were: positive history of mite allergy; positive prick test (wheal ≥ 6 mm, flare ≥ 15 mm); RAST class 3 or 4; no SI in the last 3 years.

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Antihistaminics and corticosteroids were withdrawn at least 1 week before the tests. No patients had been treated with long-acting antihistamines or corticosteroids for at least 6 weeks.

The 15 patients taking part in protocol A were 10 men and 5 women (18 to 62 years) 14 had asthma and one rhinitis. Protocol B comprised 8 men and 7 women (21 to 52 years) 12 with asthma and 3 rhinitis.

MATERIAL AND METHODS

Mite extracts

The *Dermatophagoides pteronyssinus* (DP) and *Dermatophagoides farinae* (DF) used were standardized freeze-dried commercial allergenic preparations (Laboratoire des Stallergènes, 94260 Fresnes, France), reconstituted in GS at a concentration of 1000, 100 and 10 IR/ml (biological units expressed as the indice of cutaneous reactivity) (2, 8). The extracts were prepared on the same day as skin testing.

IgG preparation

The IgG fractions were obtained by plasmapheresis in volunteers (Centre National de Transfusion Sanguine des Armées "Jean Julliard"). The plasma collected by plasmapheresis was determined to be HBs and HIV free.

IgG separation required two chromatographic columns: one column of Trisacryl GF 05 large scale (LS) and one column of DEAE Trisacryl LS (6, 23). The clear human plasma was frozen at -30°C and then thawed in a cold room at 4°C . The insoluble cryoglobulins were totally separated by centrifugation at 4°C . The supernatant was then acidified at pH 5.2 by adding cold acetic acid drop by drop. After overnight agitation, the acidic precipitate was eliminated by centrifugation at 4°C , and the clear supernatant was then filtered through a fibrous medium (decreasing pore size 1.2; 0.8 and $0.4\ \mu\text{m}$).

After addition of aprotinin (5 U/ml) the clear solution was injected directly onto a column of

Trisacryl GF 05 LS preequilibrated in 0.05 M Tris-HCl buffer, pH 8.4. The purpose of this gel filtration was to buffer equilibrate the plasma proteins (with regard to pH and ionic strength) and to eliminate the natural salts. This step also removed a fraction composed of unidentified low molecular weight products that absorb at 280 nm. The desalted proteins were then injected on a column of DEAE-Trisacryl LS, preequilibrated in the same buffer. Only IgG were eluted by 0.05 M Tris-HCl, 0.015 M NaCl, pH 8.4 buffer and collected directly in the eluate at a concentration of approx. 5 g/l.

The IgG were identified by electrophoresis on cellulose acetate (Cellogel®) and by immunoelectrophoresis (Immunofilm®), which shows only IgG, according to the manufacturer's instructions.

The purity was verified by gel filtration on Ultragel ACA 34 (IBF) and by FPLC (fast protein liquid chromatography, Pharmacia).

This solution was then concentrated 10-fold by ultrafiltration and equilibrated in NaCl (2g/l) and glycine (1g/l) and heated for 10 h at 60°C after addition of heating protectors: all these conditions in order to inactivate possible viruses.

Two pools of IgG fractions were isolated under the same conditions:

- specific IgG pool (SP IgG) obtained by plasmapheresis carried out in 30 asthmatics treated by rush SI with DP and DF for at least 6 months.
- non-specific IgG pool (nSP IgG) obtained by plasmapheresis in normal subjects.

Allergen incubation

For protocol A, 10 μl of DP or DF at 1,000, 100 and 10 IR were added to 90 μl of nSP IgG or GS, thus giving a final concentration of 100, 10 and 1 IR, respectively. nSP IgG and GS were obtained from coded tubes (double-blind). For protocol B, the same method was used for nSP IgG and SP IgG.

The mixtures were then incubated at 37°C for 1 h and used for prick tests within 1 h. GS

alone and codeine phosphate (9% in GS) were used as negative and positive controls, respectively.

Prick tests

The prick tests were performed on the anterior part of both forearms using a disposable poly-metacrylate needle (Stallerpoint® laboratoire des Stallergènes). Tests were performed in the same sequence on each arm. The tests were read 20 min later. The wheal and flare contours were transferred to a recording sheet by means of adhesive transparent tape which was pressed on the skin at the site of each encircled wheal and flare. The largest diameter and the perpendicular diameter were measured to obtain mean diameters of wheal and flare.

Statistical analysis

All results were tested by analysis of variance (one factor with repeated measures) and the non-parametric Wilcoxon test for paired series was used when F was significant.

RESULTS

Skin tests with control solutions were negative and positive as expected. As shown in Fig. 1, the preincubation of mite extracts with nSP IgG was without effect on skin tests (protocol A). However, the cutaneous responses to DP and DF were significantly decreased when mite extracts were preincubated with SP IgG (protocol B) (Fig. 2).

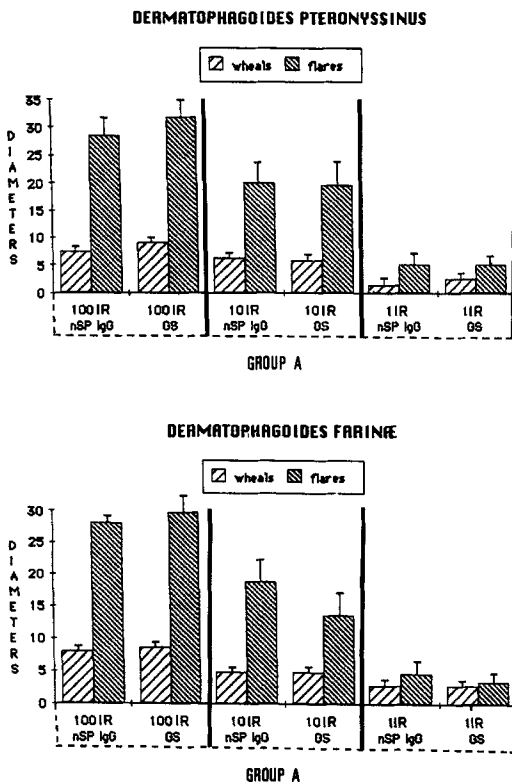


Fig. 1. Results of skin tests (wheal and flare $\pm$ SEM) obtained with 3 concentrations of mites (100, 10 and 1 IR) preincubated with either non-specific IgG (nSP IgG) or glycerol-saline (GS).

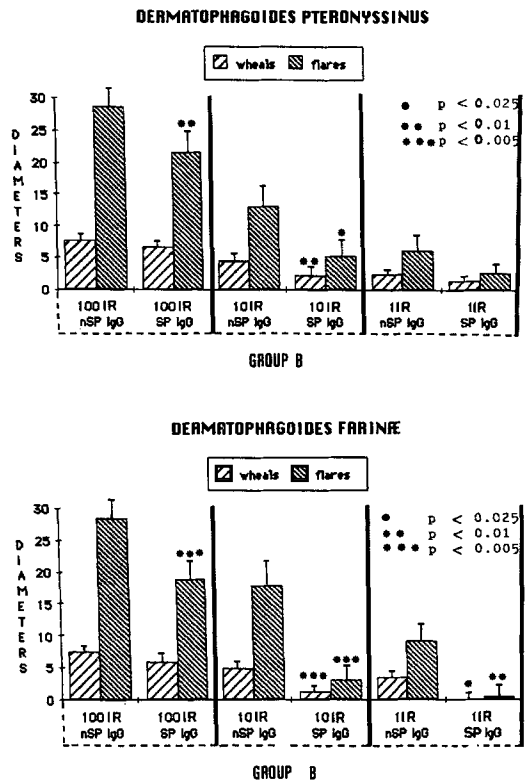


Fig. 2. Results of skin tests (wheal and flare $\pm$ SEM) obtained with 3 concentrations of mites (100, 10 and 1 IR) preincubated with either non-specific IgG (nSP-IgG) or specific IgG (SP IgG).

DISCUSSION

In 1935, Cooke (3) reported that the serum of patients who had received injections of pollen extract would neutralize pollen extract used in skin tests.

After specific immunotherapy a clear correlation between symptom scores and specific antibodies has yet to be established (20, 22). These antibodies have an antigen neutralizing capacity (ANC) *in vitro*, acting as blocking antibodies (14). This property was established by Lichtenstein & Osler in 1966 (13), using histamine release (HR). More recently it was demonstrated by Ottesen et al. (9, 10, 21) that blocking antibodies, mainly of IgG₄ subclass were naturally present in human filariasis and modulate hypersensitivity responses. In a recent work, using the inhibition of basophil degranulation (in place of HR), the synthesis of IgG blocking antibodies was demonstrated in patients undergoing rush immunotherapy with mite extracts (12). Since this work, we have shown, *in vitro*, that the main part of the ANC belongs to the IgG₄ subclass with a significant correlation between ANC and specific IgG₄ (18). In the present double-blind work conducted *in vivo*, the ANC of IgG obtained from desensitized patients was clearly confirmed: preincubation of mite extracts with specific IgG obtained from immunotherapy patients decreased the skin test response, whereas unspecific IgG, isolated under the same conditions, had no effect.

To our knowledge, the allergen neutralizing effects of IgG antibodies on skin prick test has not been previously reported.

Our results were obtained after 1 h of allergen incubation. As suggested by May (16) the ANC would be unable to protect against the immediate reaction of allergens with cells (< 15 min).

However, we have previously demonstrated *in vitro*, that ANC can occur without preincubation, i.e. when basophils were challenged with an immediate mixture of mite extract/SP IgG which had not been preincubated (12).

There is much discussion about the role of specific IgG in immunotherapy. For example,

Ito et al. (11) and Nakagawa et al. (19) consider specific IgG₄ useful, whereas Moss et al. (17) observed a significant correlation between symptom scores and specific IgG₁ but not IgG₄, after pollen immunotherapy. Moreover, Djurup et al. (5) consider an increase of IgG₄ early in the course of immunotherapy to be predictive of treatment failure.

An animal model (rabbits and guinea-pigs) of allergic asthma has clearly shown that specific IgG do have a protective effect against allergen inhalation (1, 24, 27).

It is tempting to speculate that the skin test results observed in the present work could be extrapolated to the bronchial tree. The ANC of serum after immunotherapy may play a protective role against allergen, particularly in the late asthmatic response, increasing the clearance of inhaled allergens.

However, in a double-blind study of 20 patients with allergic rhinitis to mites, we did not observe nasal spray, b.i.d., containing specific IgG to be more effective than non-specific IgG (diluted at 1/10) (unpublished results). It has been previously shown that local administration of hyperimmune-specific IgG has no clinical efficacy in ragweed hay fever (7). Thus, these contradictory results clearly demonstrate that further clinical studies of the therapeutical value of specific IgG are needed.

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