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Intranasal immunotherapy with *Dermatophagoides* extract: *In vivo* and *in vitro* results of a double-blind placebo-controlled trial*

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SUMMARY

Intranasal immunotherapy (IT) has been proposed as a means to induce an effective immunity of the nasal mucosa in patients with allergic rhinitis, avoiding systemic side effects. In the present study 20 individuals with chronic allergic rhinitis, and skin prick test reactive to Dermatophagoides pteronyssinus (DP) only, were randomized and subjected to a three months' double-blind placebo-controlled trial of intranasal IT with DP extract. All patients received also sodium cromoglycate as pre-medication. Before and at the end of the treatment the patients performed specific nasal provocation tests, and samples of serum and nasal secretions were collected to measure total and specific IgE, levels of eosinophil cationic protein (ECP), and mast-cell-derived tryptase. A clinical score was computed by the symptoms indicated by the patients. The clinical score did not change in the two groups after the treatment, whereas a decrease in nasal reactivity was observed. Total IgE increased only in secretions from placebo-treated patients, but were not modified in sera. IgE to DP in sera and nasal secretions did not change significantly. Tryptase levels in nasal secretions decreased in both groups, while ECP was unchanged after IT. Serum ECP levels decreased more in actively treated patients than in the placebo group. The data suggest that changes of IgE and inflammatory mediators may be affected by the use of sodium cromoglycate in both groups, but some parameters change early in different directions in IT- and placebo-treated groups.

Key words: intranasal immunotherapy, chronic rhinitis, IgE, sodium cromoglycate, eosinophil cationic protein

INTRODUCTION

Allergic rhinitis is a disease characterized by rhinorrhoea and sneezing following the exposition to aero-allergens derived from pollen, house dust mites or mold spores. Mucosal oedema, increased serum levels of total and specific IgE, and an immediate response to skin and nasal provocation tests indicate the occurrence of type I allergy. Increased numbers of mast cells and eosinophils, and increased levels of inflammatory mediators are usually found in nasal secretions (Naclerio et al., 1983, 1985; Linder et al., 1987; Bentley et al., 1992). Eosinophil cationic protein (ECP) - a basic protein of 18-22 kD derived from eosinophils (Peterson et al., 1988; Rosenberg et al., 1989; Weller, 1991) - and tryptase - an 134-kD tetrameric serine protease derived from mast cells (Schwartz et al., 1989; Nadel et al., 1992) - have been found to be increased in sera and nasal fluids from type-I-allergic patients, particularly after allergen challenge (Dahl et al., 1978; Winqvist et al., 1981; Gomez et al., 1986; Linder et al., 1987; Castells et al., 1988; Juliusson et al., 1991; Sedgwick et al., 1991; Paganelli et al., 1991; Proud et al., 1992; Rasp et al., 1993).

Immunotherapy (IT) is used to decrease the sensitivity to a specific antigen, possibly by causing the development of immune responses limiting the production of IgE (Terr, 1969; Djurup, 1985). An increase in specific IgG in serum, mainly IgG_1 and IgG_4, has been reported in allergic patients after IT (Sondergaard et al., 1992; Einarsson et al., 1992; Peng et al.,

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1992). Recently, local intranasal IT has been proposed to afford more effective immunization of the nasal mucosa and to avoid systemic side effects (Taylor et al., 1972; Metha et al., 1975; Johansson et al., 1979; Nickelsen et al., 1981; Georgitis et al., 1986; Valverde et al., 1989; Andri et al., 1992, 1993). The beneficial clinical effects, usually observed within one year of therapy, are not generally accompanied by changes in serum levels of IgE or mediators.

We studied a group of allergic patients with chronic rhinitis before and after three months of intranasal IT for *Dermatophagoides pteronyssinus* extract in a double-blind placebo-controlled trial. Our aim was to evaluate the early effects of local IT on the production of immunoglobulins and mediators including ECP and tryptase, and to assess the relationship of our findings to clinical effects.

PATIENTS

Twenty patients with allergic rhinitis (11 males and 9 females, mean age (±SEM): 25.7±3.1 years), diagnosed by careful history taking, clinical examination and skin prick tests, were selected because of their skin reactivity to extracts of *Dermatophagoides pteronyssinus* (DP; Lofarma, Milano, Italy; and DHS Bayropharm, Milano, Italy) only. All other allergens tested (pollen of several grasses and weeds, mold spores, and animal skin) were negative.

After collection of whole blood samples and nasal secretions the patients were challenged with the allergen by means of a nasal provocation test (NPT) in anterior rhinomanometry (Markos, Mercury, Monza, Italy), considering as threshold value the maximum dilution of the extract able to induce an increase in the nasal resistance 50% over its baseline value.

Patients were randomized so that 10 of them received specific intranasal IT (Allerkin Dermatophagoides; Lofarma allergeni, Milano, Italy), and another group of 10 received a placebo. The extract and the placebo were placed into plastic capsules, coloured differently depending on the dose, to be put into a nasal nebulizer device. Both groups received the capsules three times per week for a period of 10 weeks, until reaching the maximal dose. The titer of allergen in IT, tested by the manufacturer in a RAST-inhibition assay, ranged from 2.5 to 240 AU (arbitrary units). The treatment was administered following the schedule of doubling the amount of allergen every week, alternating the nostrils on alternate days, and using a pre-medication with intranasal spray of disodium cromoglycate (Lomudal; Fisons). The patients were asked to indicate daily on a diary card the following symptoms: nose itching, rhinorrhoea, sneezings, nasal obstruction, conjunctivitis, coughing, and dyspnoea. They had to attribute to each symptom, according to severity or frequency, a value of "0" if absent, "1" if mild, and "2" if severe, in order to have a clinical score to evaluate.

After three months the patients were again subjected to collection of blood and nasal secretion, and a NPT was performed. Clinical assessment was made by comparing the average scores of the first two to those of the last two weeks of treatment.

Both groups of patients used a nasal spray of sodium cromoglycate prior to the administration of either IT or placebo. This drug is usually given in order to prevent and minimize possible side effects of the active DP extract in allergic patients.

METHODS

Blood was obtained by venous puncture and serum separated by centrifugation. Nasal secretions were obtained from a cotton wool plug placed for about 15 min in the nasal cavities.

Sera and secretions were stored at -20°C until analysis. IgE, both total and specific for DP, were measured by fluoro-immunoassay (CAP IgE FEIA and RAST FEIA; Pharmacia Diagnostics AB, Uppsala, Sweden). Sera and nasal secretions were added to a solid phase (cap vials with cellulose) adsorbed with anti-IgE or allergens, and bound IgE measured through the activation of a fluorochrome (4-methylumbelliferyl-D-galactoside) linked to anti-human IgE. The measuring range was 2–2,000 IU/ml for total IgE and 0.35-100 IU/ml for specific IgE, with the IgE standard calibrated against the second International Reference Preparation 75/502 from the WHO.

Tryptase levels were measured by an immunoradiometrical method (Tryptase RIACT; Pharmacia Diagnostics AB, Uppsala, Sweden). Nasal secretions (diluted 1:10) and sera (added to anti-tryptase-coated plastic tubes) were incubated with [^{125}I]anti-tryptase and radioactivity was measured by the emission of gamma radiation. The measuring range was 2–50 U/l. Tryptase standards were calibrated against purified tryptase derived from human lung (Schwartz et al., 1981) and expressed in units (1 U equals 1 µg).

ECP levels were measured by competitive radioimmunoassay (ECP RIA; Pharmacia Diagnostics AB, Uppsala, Sweden). Sera and nasal secretions were incubated in plastic tubes with [^{125}I]-ECP and rabbit anti-ECP, then sepharose-linked anti-rabbit IgG was added and, after centrifugation, radioactivity in the tubes was determined by measuring the emission of gamma radiation. The measuring range was 2-200 µg/l, with the ECP standards calibrated against purified ECP (Peterson et al., 1988).

Statistical analysis was performed by the Student's t-test, the Mann-Whitney test, and linear regression to evaluate the correlation between the parameters studied.

RESULTS

All the patients responded well to the treatment, and no adverse reactions were recorded during or after the administration of intranasal IT or placebo.

The mean value of clinical scores, not different in the two groups at the beginning of treatment, was not significantly modified at the end of the study in both DP-treated and placebo-treated subjects, although a slight decrease was seen in those receiving intranasal IT (mean (±SD) of symptoms scores 18.8±14 *versus* 13.2±12 in DP-treated and 12.5±14.1 *versus* 12.4±13.3 in placebo and treated subjects, respectively).

The threshold value in NPT, measured in AU, was increased in both groups after 10 weeks (mean (±SEM): 34.4±9.6 after *versus* 13.6±4.2 before, in the active group, and 28.7±9.2 *versus* 10.5±3.7 in the placebo group; $p=0.06$ for active treatment, and $p > 0.08$ for placebo). The extent of this change was similar in the two groups.

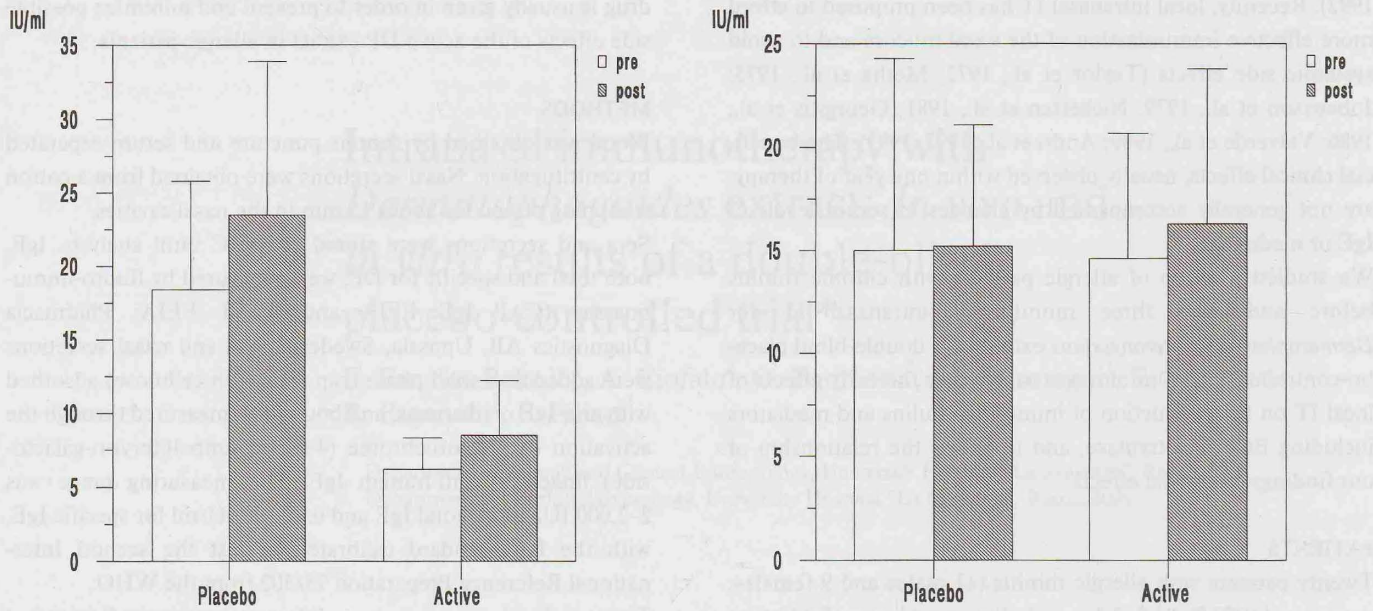

Figure 1. Right panel: Specific IgE to DP in nasal secretion prior and after the treatment in patients with allergic rhinitis. Values expressed as IU/ml, according to the Pharmacia CAP System. Left panel: The same in sera from the patients. Mean±SEM indicated.

Table 1. Geometric mean (and range) of total IgE values in sera and secretions of patients treated by DP intranasal IT and placebo, at the beginning and at the end of the treatment.

	placebo		active	
	pre	post	pre	post
IgE in nasal secretions (IU/ml)	30.3 (4.7–343.7)	54.6 (4.6–379.5)	35.6 (3.3–337.8)	35.7 (8.1–468.2)
IgE in sera (IU/ml)	117.3 (20.3–717.3)	109.1 (12.9–762.3)	79.4 (31.5–402.3)	67.6 (23.6–456.1)

Levels of total IgE in secretions were similar in the two groups before the treatment, but they showed opposite changes after the 10-week treatment. In placebo-treated patients IgE levels increased, whereas they persisted at the same level in actively-treated cases (Table 1). Serum IgE levels also remained stable in both groups. The changes in total IgE in secretions and sera from either group did not correlate with changes in clinical score or nasal reactivity. Specific IgE to DP in nasal secretions from actively-treated subjects were similar in subjects of the two groups before starting the therapy. No change was recorded in either group after treatment (Figure 1). A positive correlation was found between total and specific IgE changes in secretions, both in actively-treated ($p=0.03$) and placebo-treated patients ($p<0.01$). No correlation was found, however, with changes in clinical scores or nasal reactivity in both groups. Serum-specific IgE levels were higher in the placebo group at the beginning of treatment, and increased slightly, but not significantly, in both groups of patients (Figure 1).

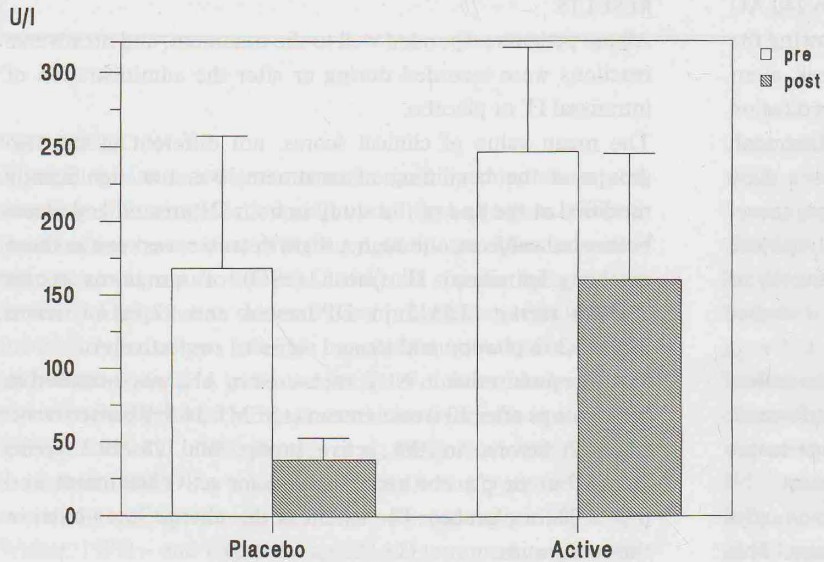

Figure 2. Levels of mast cell tryptase found in nasal secretions from patients with chronic rhinitis allergic to DP, before and at the end of the intranasal treatment with either placebo or active DP extract. Mean±SEM is indicated.

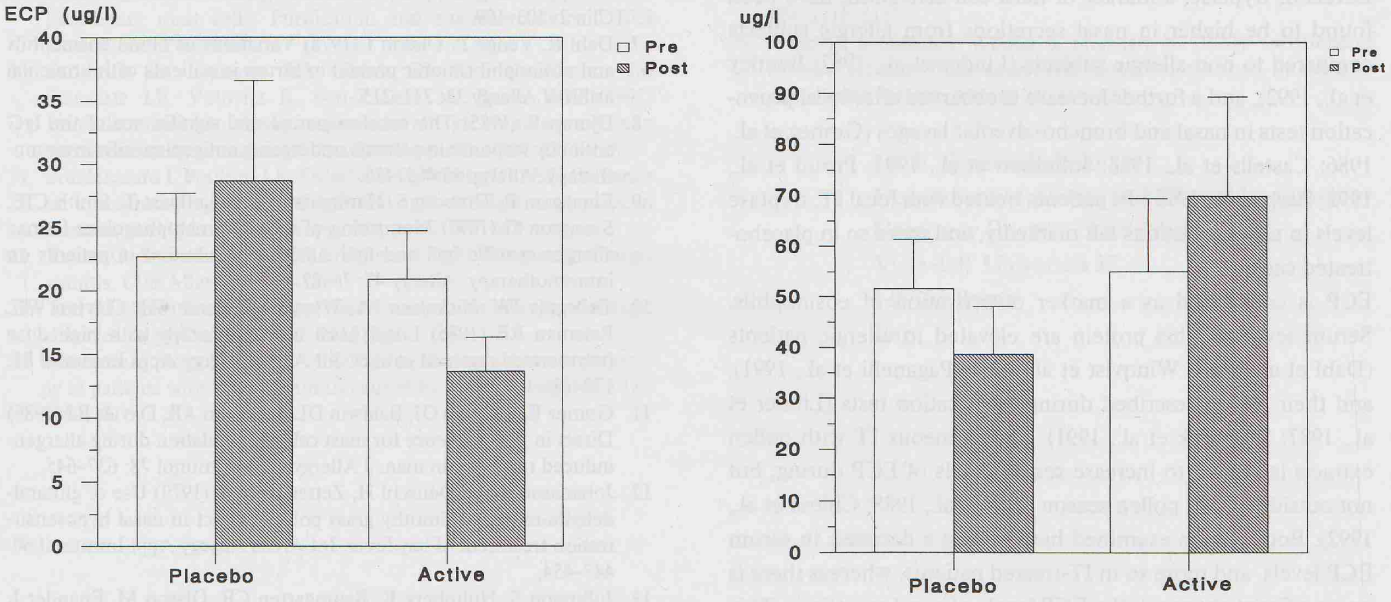

Figure 3. Levels of ECP found in nasal secretions (right panel) and sera (left panel) of patients with allergic rhinitis treated by either DP extract or placebo, before and at the end of the study. Mean±SEM is indicated.

Tryptase levels in sera were undetectable. Levels of this mast-cell-derived protein in nasal secretions from actively-treated patients before the therapy were greater, but not significantly, compared to placebo-treated patients. In both groups a similar decrease was observed. At the end of treatment tryptase levels in actively-treated patients were higher compared to placebo-treated patients (Figure 2). Levels of ECP in nasal secretions before the therapy were similar in the two groups. After three months a small decrease was observed in the placebo group, and a very slight increase in the active group. These changes were not statistically significant.

Serum levels of ECP were lower in actively-treated patients before therapy, and decreased by one-third in this group, whereas they slightly increased in placebo-treated patients (Figure 3). Variations of ECP levels in nasal secretions from both groups correlated positively with changes in nasal reactivity ($p < 0.05$). No correlation was found with changes in clinical score, total and specific IgE, and nasal fluid tryptase levels. Serum ECP levels were not related to any of the other parameters studied.

DISCUSSION

We studied the early effects of local intranasal IT in a double-blind placebo-controlled short-term trial with DP extracts, on a selected group of patients with allergic rhinitis and single sensitization to DP. We looked for changes in clinical symptoms and levels of IgE and inflammatory mediators in both serum and nasal secretions.

Despite the evidence of good tolerability of the intranasal therapy, we could not obtain unequivocal proof of the effectiveness of local IT, since no significant changes in our multi-parametric analysis were detected by comparing the active *versus* the placebo treatment.

The two groups did not show major clinical differences, since their mean clinical scores at the end of treatment were not significantly affected, as compared to the initial scores. However, nasal reactivity decreased in both groups, and nearly to the same extent.

Sodium cromoglycate represents an anti-allergic drug acting on mast cells, the primary effector of allergic reactions (Kay et al., 1987). In this respect, as both groups of patients used a nasal spray of this drug prior to the administration of either IT or placebo, even a low-dose treatment with this drug may be responsible for the improvement of nasal reactivity, as shown by the patients receiving placebo.

Traditional subcutaneous immunotherapy is known to reduce serum IgE levels, usually after several months of treatment (Terr, 1969; Djurup, 1985; Sondergaard et al., 1992). Serum IgG – and particularly IgG_4 – increased at the same time, suggesting a possible mechanism of down-regulation (Peng et al., 1992; Einarsson et al., 1992).

Very little is actually known on the mucosal production of IgG_4 during IT. Since most studies were performed on serum levels, this potentially-protective antibody isotype might be involved in the local effects of IT. In this study total IgE was unchanged in nasal secretions and sera from patients treated with IT, but increased in nasal secretions from patients who received placebo. This suggests that intranasal IT may exert locally a protective effect and may control IgE-increased synthesis by other mechanisms. We have evidence that local amounts of DP-specific IgG_4 do increase both in our actively- and placebo-treated cases (data not shown), perhaps due to natural or therapeutical exposure to the allergen.

Specific IgE to DP tended to increase in serum, but not in nasal secretions of either group. A significant correlation between total and allergen-specific IgE was observed in the secretions, indicating that patients with higher secretory levels of IgE also produced more IgE antibodies to DP. No correlation was however found with clinical parameters.

Levels of tryptase, a marker of mast cell activation, have been found to be higher in nasal secretions from allergic subjects compared to non-allergic subjects (Linder et al., 1987; Bentley et al., 1992), and a further increase is observed after local provocation tests in nasal and broncho-alveolar lavages (Gomez et al., 1986; Castells et al., 1988; Juliusson et al., 1991; Proud et al., 1992; Rasp et al., 1993). In patients treated with local IT, tryptase levels in nasal secretions fall markedly, and more so in placebo-treated cases.

ECP is considered as a marker of activation of eosinophils. Serum levels of this protein are elevated in allergic patients (Dahl et al., 1978; Winqvist et al., 1981; Paganelli et al., 1991), and their rise is described during provocation tests (Linder et al., 1987; Sedgwick et al., 1991). Subcutaneous IT with pollen extracts is known to increase serum levels of ECP during, but not outside of, the pollen season (Rak et al., 1988; Chiesa et al., 1992). Both groups examined have shown a decrease in serum ECP levels, and more so in IT-treated patients, whereas there is no significant change in the ECP levels of nasal secretions. This may indicate that a short course of intranasal IT cannot induce differential changes in the local level of this mediator. The change in serum levels of ECP was the only value that correlated with the decrease in nasal reactivity. This is particularly important, because it may constitute the basis for an indirect assessment of this clinical parameter.

Recent studies (Andri et al., 1992, 1993) have shown the appearance of significant clinical benefit after 3–6 months' treatment, suggesting that mucosal modifications of responsiveness to allergen may occur quite early.

The decrease of nasal reactivity, possibly associated with a decrease of local IgE production and with changes in the synthesis of mediators from eosinophils and mast cells, was however not significantly different in the groups studied. The lack of increased IgE levels in nasal secretions from actively-treated subjects, as compared to the increased levels found in the placebo group, suggests that intranasal IT exerted an inhibitory effect on the synthesis of this antibody class, without significantly reducing the local production of inflammatory mediators.

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