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One Year Observation of Immunotherapy for *Artemisia* Hay Fever in China : A Clinical and Immunological Study

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SUMMARY **A controlled trial of one year immunotherapy was conducted in 50 *Artemisia* – sensitive hay fever patients (treatment group). From October 1985 to July 1986, all of the treatment group patients received one year regular injection of *Artemisia* pollen allergen extract totalling 30,000 protein nitrogen units (PNU). For these patients, symptom score indices of the posttreatment 1986 pollination season were compared with those from the pretreatment 1985 season and also with the scores of a similar group of 30 *Artemisia* – sensitive patients treated only with symptomatic medications during the 1986 season (control group). The 1986 symptom scores to the treatment group were significantly improved and the effective rate was 78%. Immunological study with the Human Basophil Degranulation Test (HBDT) showed a significant decrease in degranulation reactions after immunotherapy. Moreover, The decline of the HBDT positive rate in the treatment group was significantly greater in patients with improved symptoms than patients with unchanged symptoms. No difference was observed in basophil degranulation in those patients tested with a pollen – free plant extract, which was not applied in immunotherapy. The results suggested that immunotherapy could induce desensitization of basophils and that the induction might be allergen specific. Basophil desensitization may play an important role in the mechanism of immunotherapy.**

Immuotherapy for pollinosis has been practiced by allergists since 1911.[1,2] It was first introduced in clinical practice in China in the 1950.[3] Although immunotherapy with *Artemisia (Ar)* pollen allergen has been effective for *Ar* hay fever in our clinical trials, observations substantiating the efficacy of the treatment have not yet been reported and the mechanism of its action remains to be clarified. Basophil/mast cell mediator release is the key step in development of allergic reactions.[4] As we have described elsewhere,[5] the modified Human Basophil Degranulation Test (HBDT) is a simple way to assess the degranulation reactions of basophils to pollen allergens. In order to evaluate the efficacy of the treatment and clarify the mechanism of its action at the basophil level, a controlled trial of one-year immunotherapy was undertaken two years ago. Fifty patients suffering typical symptoms of *Ar*-hay fever were selected in 1985 prior to the *Ar*-pollination season. Symptom score diaries were recorded by each patient in the 1985 *Ar*-pollination season, prior to the immunotherapy. After the treatment, symptom score diaries were again recorded by each patient for comparison. Since the severity of *Ar*-allergy symptoms may vary form year to year depending on air pollen concentrations, a second group of 30 untreated patients (control group) similarly completed symptom score diaries in the 1986 *Ar*-pollination season. It provided a comparison of allergic symptoms between immunotherapy-treated and untreated patients during the same season. The HBDTs were performed with *Ar*-pollen and pollen-free plant extracts before and after the treatment to observe the effect of immunotherapy on basophil degranulation reactions.

MATERIALS AND METHODS

Subjects

Before the *Ar*-pollination season in 1985, fifty *Ar*-pollen-sensitive hay fever patients (the treatment group) were selected by definite positive skin testing and typical histories. There were 29 males and 21 females with

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an average age of 39 ± 11, ranging from 14 to 74 years old. They were selected randomly from our out-patients who had not previously received immunotherapy. A similar group of 30 patients (14 males and 16 females, average age 40 ± 9, ranging from 15 to 67 years old) were selected prior to the 1986 pollination season to serve as an untreated control group. The clinical data of the control group matched those of patients in the treated group.

Symptom score diary recording

The best defined *Ar*-pollen season in China ranges from mid-August to the end of September at which time *Ar*-pollen is in flowering.[3] Daily symptom score records were completed by each patient in the treatment group from August to September of 1985. These diaries allowed the patients to quantitate the severity of their symptoms every day: sneezing, stuffy nose, nasal discharge, itchy eyes, cough, and wheezing. In addition, each patient was treated with symptomatic medications, if necessary, and the types and amounts of medications were also recorded. During the hay fever season in 1986, daily symptom scores were again recorded by the patients who had received *Ar*-pollen immunotherapy (treatment group) and by the patients who has not received immunotherapy (control group).

Pollen counts

Quantitative *Ar*-pollen counts were obtained from a slide sampler at the PUMC Hospital in Beijing.

Allergens

Ar-pollen allergen extract was used both for immunotherapy and HBDT. An *Ar*-pollen-free plant extract was also used for HBDT but not for therapy. The subjects had previously proven to be sensitive to this pollen-free extract.[6] The optimal threshold dilutions for HBDT determined by the preliminary tests were 1 : 100 for the *Ar*-pollen-free plant extract and 1:1,000 for *Ar*-pollen. Allergen standardization through the micro-Kjeldahl technique showed that *Ar*-pollen extract contained 32,830 PNU/ml and that the *Ar*-pollen-free plant extract contained 20,580 PNU/ml.

Immunotherapy

From October 1985 to August 1986, each patient in the treatment group received a series of injections of slowly increasing amounts of allergen as recommended by Patterson *et al.*[7] Injections were given regularly twice a week. The starting dose was carefully adjusted based on the results of skin tests and end point titrations. If a patient developed a significant local reaction to any injection, the dose was readjusted until it reached a total dose of 30,000 PNU.

Human Basophil Degranulation Test (HBDT)

Venous blood (3 ml) was drawn from patients into a heparinized tube and mixed with 3 ml Hepes-ACM buffer (Hepes 25 mM, $CaCl_2$ 1 mM, NaCl 130 mM, KCl 5 mM, adjusted to pH 7.6 using 1 N NaOH). The mixture was gently laid on 3 ml of a Ficoll-Hypaque gradient density solution (density 1.085) and centrifuged at 2,000 rpm for 20 min. The ring containing basophils was collected and washed with 5 ml Hepes-ACM buffer (1,500 rpm for 10 min). The supernatant was discarded, leaving about 0.3 ml of enriched basophil suspension at the bottom of the tube. Four samples of 50 μl of this enriched basophil suspension were deposited into 4 tubes containing 50 μl of control solution (consisting of 25 μl Coca's solution and 25 μl solvent), Hepes-ACM buffer (as a double control), *Ar*-pollen extract, and *Ar*-pollen-free plant extract, respectively. The tubes were incubated in a 37°C water bath for 30 min. Then basophil suspensions were stained by adding 150 μl of Alcian blue dye solution. After 10 min incubation at room temperature, the samples were applied to a haemocytometer and the non-degranulated basophils were counted. The degranulation index (DI) was calculated according to the formula:

$$DI = \frac{X - Y}{X} \times 100$$

X = Basophils (the average value of double control tubes)

Y = Basophils (test tube)

A DI of basophils over 30 % in the presence of allergen was considered as positive.

RESULTS

Symptom score improvement

The daily symptom scores for the treatment and control groups of patients are shown in Figures 1-2. Figure 1 is a comparison of the average daily indices of the treatment group before immunotherapy (during the 1985 *Ar*-pollination season) and after the therapy (during the 1986 season). Figure 2 is a comparison of 1986 indices of the treatment group and of the untreated control group during the 1986 season. The vertical lines represent one standard deviation above the mean. The p values were calculated by Student's *t* test. Figure 1 shows that the 1986 symptom scores of the immunotherapy-treated group were significantly improved when compared with the untreated control group (Fig. 2) or when compared with their scores during 1985 (Fig. 1).

Pollen counts for the 1985 and 1986 *Ar*-pollination seasons are shown in Figure 3 as grains per cubic meter air counted from mid-August to mid-September. They were roughly the same. Figure 4 shows a rough correlation between the pollen count and the total seasonal

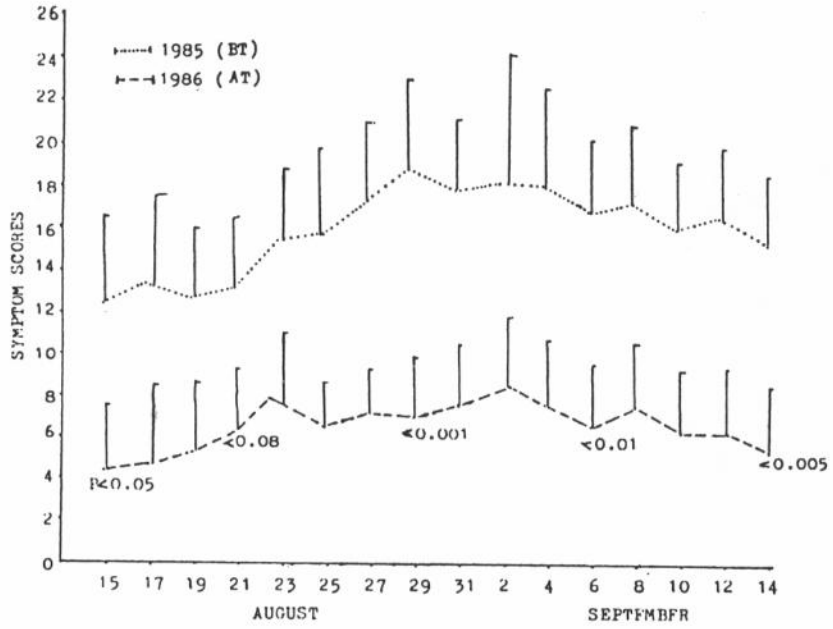

Fig. 1 Daily symptom scores of the treatment group are compared for 1985 (before therapy) (BT) and for 1986 (after therapy) (AT). The p value and 1 SD are shown for each point.

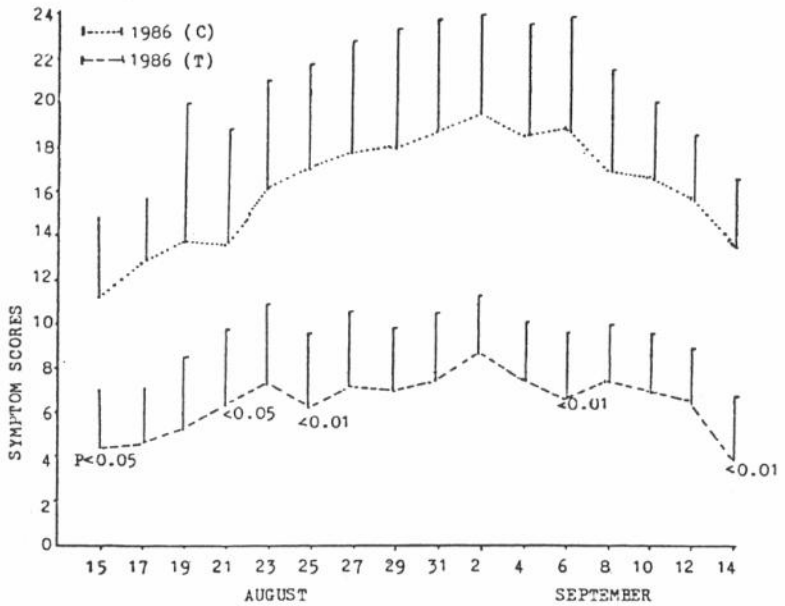

Fig. 2 Daily symptom scores of the treatment group (T) are compared with scores of the untreated control group (C) in 1986. The p value and 1 SD are shown for each point.

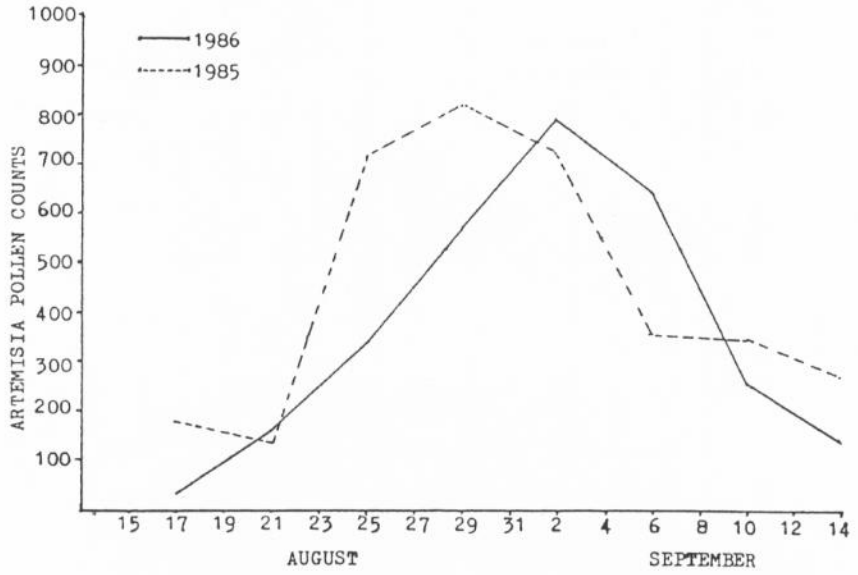

Fig. 3 Pollen counts in the 1985 and 1986 *Artemisia* pollination seasons.

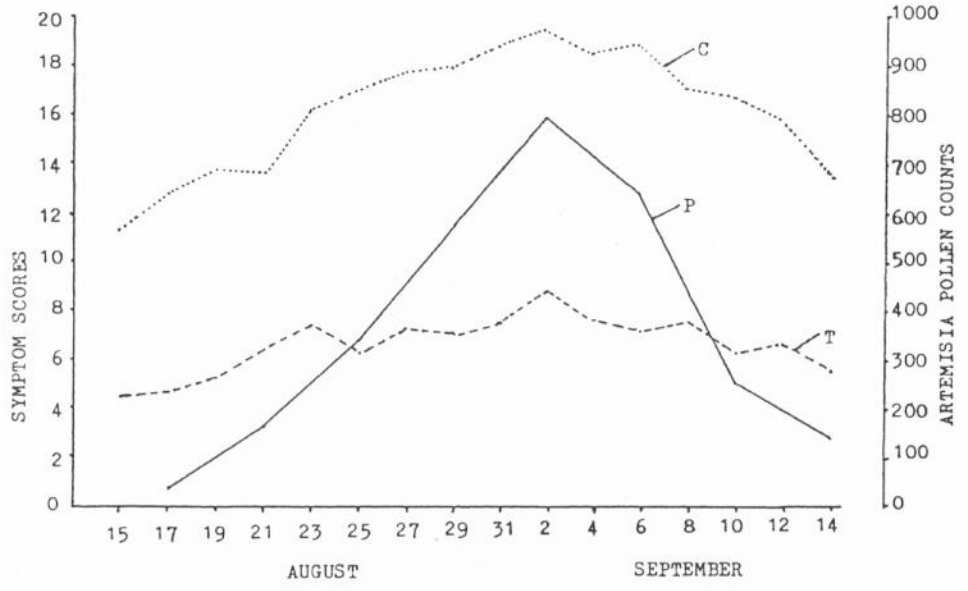

Fig. 4 The daily relationship between pollen count (P) and patient symptom scores for the control (C) and treatment group(T) in 1986.

symptom scores of the patients in 1986.

For the treatment group, the degree of improvement in symptoms varied. Thirty-nine of the patients had a reduction in their total seasonal symptom scores for the 1986 pollination season when compared with their scores for the 1985 season. The remaining 11 patients had no definite amelioration of their symptoms. The effective rate was therefore 78 %.

HBDT

HBDT tests were performed with *Ar*-pollen allergen (*Ar*-HBDT) and *Ar*-pollen-free plant allergen (*An*-HBDT) before and after immunotherapy. In the treatment group, the percentage of patients with a positive *Ar*-HBDT was 30 % after immunotherapy. This value was significantly lower than that prior to therapy (72 %). With *An*-HBDT tests performed at the same time, the positive percentage was 62 % and this compared with a pre-therapy value of 64 %; there was no significant change after immunotherapy. These results are shown graphically in Figure 5. In addition, Table 1 shows that the percentage of positive reactions for the *Ar*-HBDT test was significantly lowered in the group of patients for whom therapy was effective. It was not significantly changed in the group for whom therapy was ineffective.

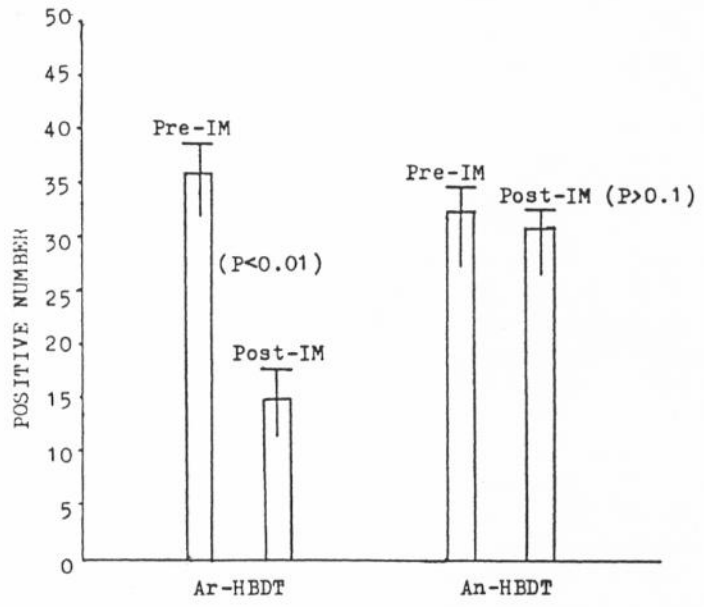

Fig. 5 Comparison of the results of *Ar*-HBDT and *An*-HBDT tests for pre-immunotherapy and postimmunotherapy patients.

DISCUSSION

The number of hay fever patients receiving immunotherapy is expanding rapidly in China. However, the lack of objective quantitative observations on immunotherapy has led many people, both inside and outside the specialty, to look at the technique with a questioning eye and to doubt its usefulness. The first controlled study was carried out in 1949.[8] Since then there has been a lot of work on the evaluation of immunotherapy for ragweed hay fever in North America.[9-14] These reports have shown that 60-80 % of the treated patients improved. The results of our controlled study confirmed the effectiveness of immunotherapy for *Artemisia* hay fever in China. The effective rate was 78 %, similar to that given in the reports cited above.

At present, the viewpoints concerning the mechanisms of

Table 1 Comparison of *Ar*-HBDT and *An*-HBDT results for effectively and ineffectively treated patients*

Subjects	Effective group		Ineffective group		p value
	Total number	Positive reactions	Total number	Positive reactions	
Ar-HBDT	39	8 (20.0%)	11	7 (63.6%)	$p<0.01$
An-HBDT	39	24 (61.5%)	11	7 (63.6%)	$p>0.1$

* After one-year of specific *Ar*-pollen immunotherapy.

immunotherapy can be summarized as follows:[15]

(1) Decrease in IgE antibodies;

(2) Suppression of the seasonal rise in IgE antibodies in pollinosis;

(3) Increase in IgG blocking antibodies;

(4) Reduction of histamine release from basophils challenged with allergens.

Against viewpoint (1) is the fact that IgE antibody levels vary widely in the great majority of patients.[16-17] Also, in another study IgG antibody or blocking antibody rose significantly after immunotherapy but the relationship between the serum level of blocking antibody and symptom relief was neither precise nor constant.[18]

Reduced sensitivity of basophils/mast cells is another reasonable explanation for the mechanism of immunotherapy. The present investigation demonstrated that the basophil degranulation reaction to *Ar*-pollen allergen was significantly decreased after immunotherapy. Moreover, the decrease in reaction to *Ar*-HBDT was greater in patients who responded favorably to treatment than in patients who did not respond. We did not observe any significant difference in *An*-HBDT reactions before and after immunotherapy. This may suggest that immunotherapy can induce desensitization of basophils and that the induction is allergen specific. A previous *in vitro* study showed that basophil desensitization was antigen specific,[19] and may play an important role in the mechanism of immunotherapy.

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