

# Lymphocyte proliferative responses to the purified *Dermatophagoides farinae* major allergen in untreated and hyposensitized atopic patients

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We investigated the proliferative response of lymphocytes from mite-sensitive patients (RAST D.far > 3.5 PRU/ml) in the presence of the major allergen Der.f.I purified from *Dermatophagoides farinae*. Comparative studies were carried out with peripheral blood mononuclear cells from non-atopic donors (RAST = 0), and from patients undergoing hyposensitization treatment (5 to 24 months). According to Student's t-test, there was no significant difference in the Der.f.I-induced proliferation of peripheral blood mononuclear cells from normal donors, untreated atopic patients and hyposensitized patients. In conclusion, it was impossible to discriminate between normal donors, atopic patients and hyposensitized patients with regard to their circulating lymphocyte responses to the purified major allergen Der.f.I.

**Key words:** allergen; mite; *Dermatophagoides farinae*; hyposensitization; lymphocyte transformation.

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The allergen-induced blast transformation observed with human peripheral blood mononuclear cells (PBMC), was first described in 1963 (16). Several authors have reported higher proliferative responses of cells isolated from atopic patients than the responses obtained with cells from normal donors (8-10, 13, 17, 20, 21, 24, 26); others observed no such difference (4, 11, 19). Conflicting results have been reported concerning the effect of hyposensitization treatments, including a decrease in the blast response (1, 3, 7, 8, 13, 24, 25), an increase or absence of any modification (3, 13, 26). The differences between many experimental parameters may be responsible for such discrepancies (e.g. nature of the cells, origin of the added serum, nature of the allergen, length of culture period, etc.). Most studies have been performed with pollen extract or purified ragweed antigen E, whereas fewer studies have used mite extracts or purified allergens. In the present study, we have investigated the blast transformation of

PBMC induced by a purified major allergen from *Dermatophagoides farinae* mites (Der.f.I), the biochemical and immunochemical properties of which have been previously characterized (5, 14, 15). To avoid a possible interference of natural IgG anti-Der.f.I antibodies occurring in normal human serum, IgE antibodies present in serum of atopic patients, or suppressive factors described in serum of hyposensitized patients, the cultures were performed with fetal calf serum instead of human serum.

## MATERIAL AND METHODS

### *Study subjects*

Blood was obtained from 53 subjects by venipuncture into a heparinized syringe. Eighteen were normal donors (Centre National de Transfusion, Paris); 35 were atopic patients (outpatients from the allergology clinic of the Pasteur

Table 1  
Characteristics of the study subjects

| Donors | Age  |       | Sex |    | Total IgE (IU/ml) |         | RAST <i>D. farinae</i> (PRU/ml) |          |        | Clinical symptoms |         |          |
|--------|------|-------|-----|----|-------------------|---------|---------------------------------|----------|--------|-------------------|---------|----------|
|        | mean | range | M   | F  | mean              | range   | 0                               | 3.5-17.5 | > 17.5 | asthma            | rinitis | conjunct |
| Normal | 42±3 | 24-57 | 14  | 4  | 125±45            | <10-565 | 18                              | -        | -      | -                 | -       | -        |
| Atopic | 34±3 | 16-71 | 15  | 20 | 730±160           | 45-5100 | -                               | 15       | 20     | 17                | 23      | 8        |

Institute Hospital). Serum IgE concentrations were determined by the paper radioimmunosorbent test (PRIST, Pharmacia Diagnostic, Uppsala, Sweden); results were expressed as international unit per ml (1 IU/ml = 2 ng/ml). *Dermatophagoides farinae* specific IgE antibodies were evaluated by the Phadebas radioallergosorbent test (RAST, Pharmacia Diagnostic); results were expressed as Phadebas RAST Units/ml (PRU/ml). All selected atopic patients had > 3.5 PRU/ml (class 3 or 4). Donor characteristics are summarized in Table 1.

Sixty-five percent of the atopic patients were undergoing or had begun hyposensitization treatment during the period of experimentation; 68% were desensitized with a purified *D. farinae* extract combined with calcium phosphate (Allergens Pasteur), and 32% with the purified *D. farinae* extract in association with another allergen (*Dactylis*, cat, *D. pteronyssinus*, or ambrosia). Patients were injected subcutaneously once a week with an increasing amount of allergen, up to a total of 1.5 µg *D. farinae* extract at the end of the 1st month, 15 µg the 2nd month, and 150 µg the 3rd month; then 75 µg were injected once a month, starting at the 14th week of treatment. The main medication used by the patients during the study period consisted of antihistaminics, but none used corticosteroids or aspirin during the time of the experiments.

#### Cell cultures

Peripheral blood mononuclear cells (PBMC) were obtained after centrifugation of blood diluted 1:1 in RPMI-1640 medium (Flow Lab., USA) on Ficoll-Hypaque (MSL, Eurobio, France). After washings, the cells were resuspended in RPMI-1640 medium supplemented

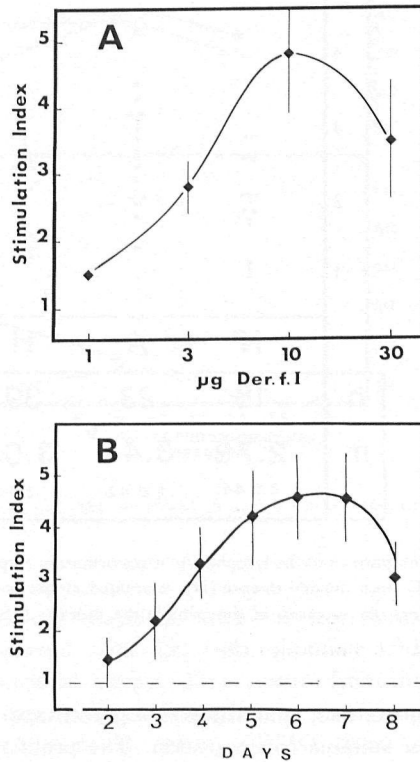


Fig. 1. A: Lymphocyte transformation induced by increasing amounts of the purified allergen Der.f.I. The data, expressed as stimulation index (SI = cpm in the presence of the allergen/cpm in the control culture), are the means ± SE of the results on day 5 obtained with the peripheral blood mononuclear cells from 6 different atopic patients. B: Kinetics of the lymphocyte response to Der.f.I (10 µg/2 × 10<sup>6</sup> cells/well). The results are the mean ± SE of experiments performed with PBMC from 6 different atopic patients.

with antibiotics (Streptomycin, 100 µg/ml and penicillin, 100 IU/ml) and fetal calf serum (FCS) (IBF, France). The final concentration of FCS was adjusted to 2% once preliminary

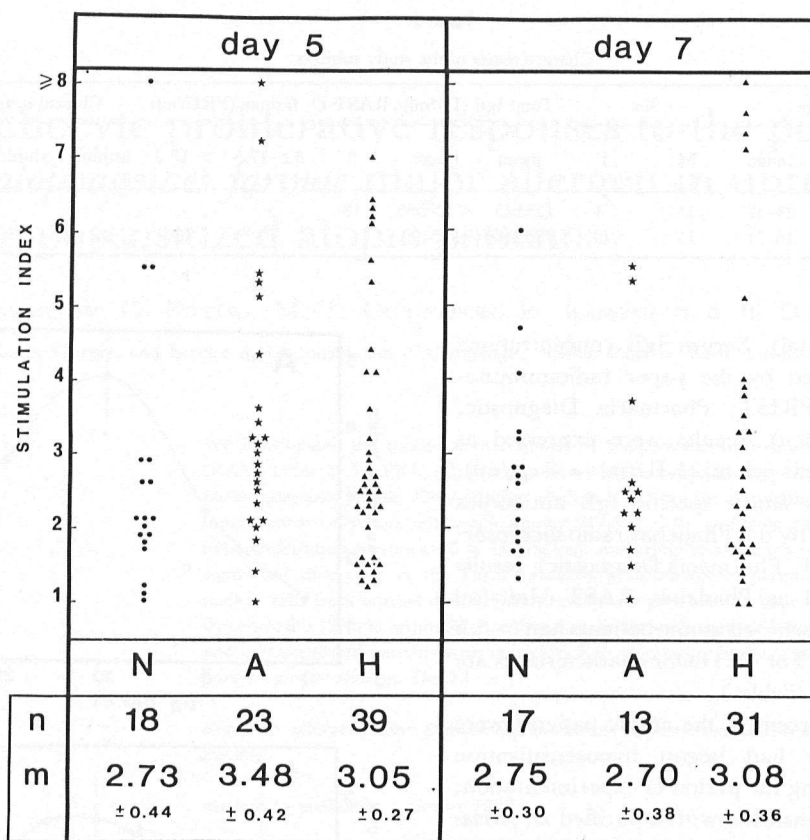


Fig. 2. Comparison of the lymphocyte transformation responses on days 5 and 7 to the purified major allergen Der.f.I (10  $\mu$ g) by PBMC from normal donors (N), untreated atopic patients (A), or hyposensitized atopic patients (H). (n = number of experiments; m = mean of the stimulation indexes  $\pm$  SE)

experiments had established this percentage as the most suitable concentration. The proliferative response was expressed as stimulation index (SI = cpm in the presence of the allergen/cpm in the control culture);  $2 \times 10^6$  PBMC were seeded in multidish 96 microwells (Nunc, Denmark) in a final volume of 200  $\mu$ l and left at 37°C in an air-CO<sub>2</sub> incubator (Heraeus, Germany) (7.5% CO<sub>2</sub>) for 5 to 7 days, unless otherwise specified. Cells were stimulated with the major allergen Der.f.I purified from *Dermatophagoides farinae* prepared as previously described (5, 14) (10  $\mu$ g/well, unless otherwise specified) or with pokeweed mitogen (PWM, Gibco, USA) (5  $\mu$ g/ml). (Der.f.I is the new international nomenclature for what was previously termed the Ag.11 allergen, which pos-

sesses cross-reactivity with the major allergen from *Dermatophagoides pteronyssinus*, Der.p.I (12)). Twenty-four hours before the end of the culture, 20  $\mu$ l of 3H-thymidine (0.25  $\mu$ Ci/well; 5 Ci/mmol, Amersham, UK) were added. Cultures were made in triplicate. Cells were harvested on a glass fiber filter with a Skatron apparatus (Norway), and filters were placed in vials with scintillation liquid (Econofluor, N.E.N., USA) and radioactivity was measured in a  $\beta$ -counter (Kontron, Switzerland).

## RESULTS

The purified major allergen Der.f.I induced lymphocyte transformation as assessed by its ability to increase 3H-thymidine uptake by

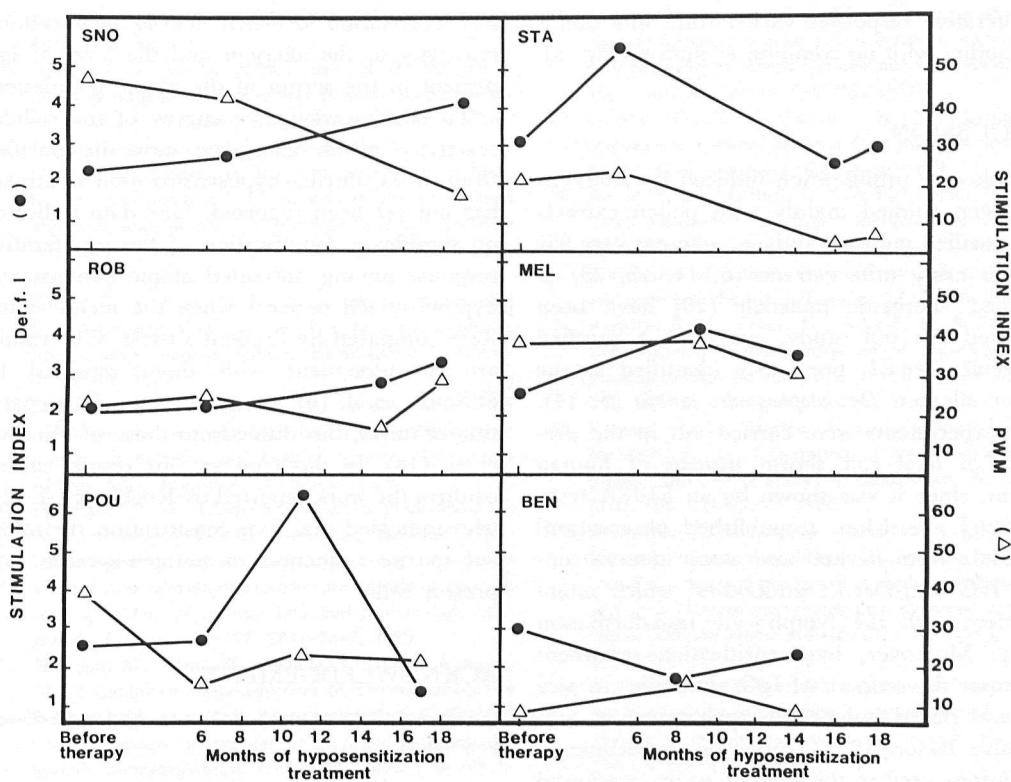


Fig. 3. Lymphocyte transformation on day 5, induced by Der.f.I (10 µg) (●), or PWM (5 µg) (Δ) in 6 different patients during hyposensitization treatment.

peripheral blood mononuclear cells from atopic patients; the optimal amount of allergen was 10 µg per assay (50 µg/ml) (Fig. 1A), reaching a plateau between the 5th and the 7th day of culture (Fig. 1B). Similar parameters were obtained with PBMC from non-atopic donors (data not shown). Pokeweed mitogen (PWM) was used as a positive control of the proliferation since, in contrast to blast transformation induced by Con.A or PHA, both of which peak at the 3rd day of culture and then decrease rapidly, PWM-induced transformation peaks at the 3rd day of culture and remains very high up to the 7th day of culture. The cell reactivity assessed by PWM was independent of the transformation induced by Der.f.I, and there was no correlation between the amplitude of the cell response to Der.f.I and the amount of total IgE present in the serum of untreated atopic patients (data not

shown). Such a lack of correlation was observed with the cells obtained both from untreated atopic donors and from hyposensitized patients. Lymphocyte proliferation was also detected when PBMC from normal donors and PBMC from hyposensitized patients were cultured in the presence of Der.f.I (Fig. 2). The mean value of the stimulation indexes obtained with the cells of untreated atopic patients was slightly higher than that obtained with cells from normal donors on day 5; similarly, the mean value of the SI obtained with the PBMC from hyposensitized patients was slightly higher than that of cells obtained from untreated atopic patients on day 7. However, these differences were not significant according to Student's *t*-test ( $P > 0.1$ ). When patients were tested at different periods after the beginning of their hyposensitization treatment, the lymphocyte

proliferative responses varied from one donor to another with no common evolution (Fig. 3).

## DISCUSSION

Lymphocyte proliferation induced by allergens has been studied mainly with pollen extracts and purified ragweed antigen, whereas very few studies using mite extracts (6, 11, 13, 23) or purified allergenic molecule (20) have been reported. In our study, we used a purified molecule Der.f.I, previously identified as the major allergen *Dermatophagoides farinae* (5, 14). Our experiments were carried out in the presence of fetal calf serum instead of human serum, since it was shown by an ELISA technique (J. Le Mao, unpublished observation) that sera from normal and atopic donors contain IgG anti-Der.f.I antibodies, which might interfere with the lymphocyte transformation assay. Moreover, hyposensitization treatment increases the amount of IgG antibodies in sera (7) and might induce the appearance of suppressive factors (25). Under the experimental conditions used in the present study, we found no significant differences in the responses of cells isolated from normal donors and atopic patients; both groups had similar stimulation indexes when PBMC were cultured in the presence of the purified allergen. Results obtained from patients suffering from rhinitis, asthma or both, and those obtained from hyposensitized patients did not correlate with clinical scores. The similarity in lymphocyte transformation induced by mite allergens observed in cells from healthy controls and atopics is in agreement with the recent report of Halvorsen et al. (11) who studied the T-cell responses to a *D. farinae* preparation, but differs from other reports (13, 20, 25) which indicated a higher stimulation of the cells from an atopic population than from normal controls. Interestingly, Rawle et al. (20), using the purified major allergen from *D. pteronyssinus* (Der.p.I) which cross-reacts with Der.f.I, reported that cells from patients suffering from atopic dermatitis responded better to allergen stimulation than those isolated from patients with rhinitis or asthma. In contrast to two other reports (2, 21), we did not observe

any correlation between the *in vitro* cellular reactivity to the allergen and the level of IgE present in the serum of the atopic population.

To our knowledge, a survey of the cellular reactivity to an allergenic molecule purified from mites, during hyposensitization treatment has not yet been reported. Our data indicated no significant modification of the proliferative response among untreated atopic patients and hyposensitized patients when the mean values were compared by Student's t-test. Our results are in agreement with those reported by D'Souza et al. (6), who used a crude preparation of mites, but differ from those of Hiratani et al. (18). In this context, our results cannot confirm the work reported by Rocklin et al. (21) who indicated that hyposensitization treatment led to the induction of antigen-specific suppressor cells.

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