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CLINICAL AND IMMUNOLOGICAL EVALUATION OF ASTHMATIC PATIENTS IN A DOUBLE BLIND TREATMENT PROTOCOL WITH TRANSFER FACTOR

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Key words: Extrinsic asthma, Transfer Factor.

SUMMARY

We evaluated clinically and immunologically the therapeutic effect of Transfer Factor (TF) in 17 patients with mild or moderate-severity extrinsic bronchial asthma. TF (1 U) or placebo was administered following a double blind protocol during 6 months (32 doses). The immunological evaluation of the patients and of 21 normal individuals, consisted in immediate hypersensitivity skin tests for common
environmental allergens, delayed hypersensitivity tests (DH) for tuberculin (PPD), C. albicans and T. rubrum, total serum IgE (PRIST), specific serum IgE (RAST), eosinophils count, and CD3+, CD4+ and CD8+ lymphocyte subpopulation counts using the avidin-biotin method. The patients presented 3.05 +/- 1.6 crises per month and used frequently beta-adrenergic drugs and theophylline. Before treatment, there was a higher proportion of positive allergic reactivity skin tests (p < 0.01), higher serum IgE levels (p < 0.001) and eosinophil counts (p < 0.01) among patients than in controls. The CD3+ lymphocyte percentage was less in the patients (p < 0.05) as well as the intensity of the DH tests for C. albicans and T. rubrum (p < 0.05). These data confirm the atopic condition of the selected patients. After treatment, there was clinical improvement, decrease in the frequency of crisis as compared to before treatment (p < 0.001), decrease in the frequency and intensity of cough (p < 0.003) and in the use of conventional drugs (p < 0.002). The DH response to PPD and C. albicans was more intense after treatment (p < 0.02). CD3+, CD4+, and CD8+ subpopulations were not modified, so it will be convenient to study T-cell function further. These results indicate that TF improved the clinical condition but did not modify DH reactivity of the patients. The normalization of the cell immunity tests could be associated to clinical improvement, but the correlation between these immunological and clinical parameters requires a larger number of evaluations.

RESUMEN

Evaluamos clinica e inmunologicamente el efecto terapeutico del Factor de Transferencia (FT) en 17 pacientes con asma bronquial extrinseca de severidad leve o moderada, bajo un protocolo a doble ciegas aplicando 32 dosis (1 U/mL) del FT o de placebo durante 6 meses. La evaluacion inmunologica de los pacientes y de 21 individuos normales, consistio en pruebas de piel para hipersensibilidad inmediata para alergenos ambientales comunes, y retardada (HR) para tuberculina (PPD), C. albicans y T. rubrum, IgE serica total (PRIST), IgE serica especifica (RAST), eosinofilia y cuantificacion de subpoblaciones linfocitarias CD3+, CD4+ y CD8+, con el metodo de la avidina biotina. Los pacientes
presentaron 3.05 +/- 1.6 crisis por mes y utilizaban frecuentemente beta-adrenergicos y teofilina. Antes del tratamiento, el estudio de la reactividad alergica en piel demostro un porcentaje de positividad mas elevados en los pacientes que en los controles (p < 0.01), niveles de IgE serica total y eosinofilia tambien mas elevados en pacientes que en controles (p < 0.001 y p <0.01 respectivamente). El porcentaje de linfocitos CD3+ fue menor en los pacientes (p < 0.05), y asi mismo la intensidad de las pruebas de hipersensibilidad retardada para C. albicans y T. rubrum (p < 0.05). Estos datos confirman la condicion atopica de los pacientes seleccionados. Despues del tratamiento se observo mejoria clinica, se demostro una disminucion en el numero de crisis de asma por mes comparado con el numero de crisis antes del tratamiento (p < 0.001), disminucion de la frecuencia e intensidad de la tos (p < 0.003) y disminucion del uso de medicamentos convencionales (p < 0.002). La respuesta hipersensibilidad retardada al PPD y a C. albicans fue mas intensa despues del tratamiento (p < 0.02). Las subpoblaciones CD3+, CD4+, y CD8+ no se modificaron despues del tratamiento, por lo que seria adecuado estudiar con mas profundidad la funcion de celulas T. Estos resultados indican que el FT mejoro la condicion clinica y no modifico la reactividad de HR de los pacientes. La normalizacion de las pruebas de inmunidad celular podria estar asociada a la mejoria clinica, sin embargo, la asociacion entre estos parametos inmunologicos y clinicos requiere mayor numero de evaluaciones.

INTRODUCTION

Different studies have shown that patients with extrinsic asthma have high allergic reactivity to common environmental allergens (Rackemann, 1947), high levels of total serum IgE (Ishizaka, 1981), and increased eosinophils count in peripheral blood and sputum (Fukuda et al., 1985). They often show low cell-mediated immune response toward specific antigens (Leung and Geha, 1986), and a high susceptibility to viral and bacterial infections (Busse, 1991). Many therapeutic methods have been applied to these patients, but the results are seldom fully satisfactory. Some of these procedures represent a high risk to the...
patient for adverse side effects during or after the treatment (Spitzer et al., 1992). Therefore, the evaluation of new, non-conventional treatment protocols in these patients is still important.

A dialyzable extract obtained from peripheral blood leukocytes, called Transfer Factor (TF), has been used in the treatment of a variety of viral (Cabezas- Quiroga et al., 1990) bacterial, fungal (Corbiel et al., 1984) and parasitic infections (Delgado et al., 1981), that are often associated to a depressed cell-mediated immunity (Carey et al., 1987). TF has also been used in patients with malignant diseases who have similar immunological alterations (Miller et al., 1988).

TF is a non immunogenic preparation containing low molecular weight molecules, capable of transferring immunological information to non responder individuals, mainly for delayed hypersensitivity reactions (Lawrence, 1955). It is well known that atopic patients frequently show severe viral or fungal infections and asthmatic patients may specifically suffer respiratory infections that worsen their clinical picture (Lemanske et al., 1989). Therefore, asthmatic patients with a possible depression of cell-mediated immunity are candidates for TF treatment.

The aims of this study were to evaluate the therapeutic effect of TF on the clinical symptoms of extrinsic asthma patients and its possible modulation of their immunological response and allergic reactivity.

MATERIALS AND METHODS

Study population

We evaluated 17 patients (mean age 29.5 +/- 14.0 years) with extrinsic asthma, of low (5 crises per year) to moderate (5 to 12 crises per year) severity. None of the patients had been treated with specific hyposensitization or systemic steroids. No respiratory infection was detected in any of the patients. A control group consisting in 21 healthy subjects (29.2 +/-8.0 years), with no family or
personal atopic history was used for comparison of baseline data. The patients' written informed consent was obtained, and the study was approved by the Ethical Committee of the Institute of Biomedicine, Caracas.

Transfer Factor

We used a dialyzable extract from normal human blood donor leukocytes, previously induced by Sendai virus to stimulate interferon alpha production (Fernandez and Lopez, 1986). The TF was prepared at the Center for Biological Research, La Habana, Cuba.

Treatment

We performed a randomized double-blind, 6 month protocol. Nine patients were treated with TF and 8 patients received placebo (saline solution pH 7.2). One unit of TF (equivalent to the extract obtained from $5 \times 10^8$ total leukocytes) or the placebo was administered subcutaneously, twice weekly for 8 weeks and then once weekly up to 6 months. Each patient received 34 units. Patients were clinically evaluated monthly and were allowed to use conventional treatment when necessary: 2 or 3 daily doses of b-agonists.

Symptom severity was evaluated according to the Institute of Biomedicine, Caracas, Allergy Clinic scale. Treatment administration, as well as all clinical and laboratory evaluations were done blindly. The code was broken only for the analysis of the results.

Immediate hypersensitivity skin testing

Cutaneous prick tests were performed with partially purified extracts of common environmental allergens. These were: house dust, *Dermatophagoides pteronyssinus*, *Aspergillus fumigatus*, niger and flavus, *Rhizopus sp*, *Hormodendrum sp*, *Alternaria sp*, *Fusarium sp*, *Candida sp*, *Penicillium sp*, house mosquito, fly, butterfly, honey bees, cockroach, dog and cat epithelia, *Melinis minutiflora* pollen, *Ascaris lumbricoides* antigens, negative control,
histamine (Linch et al., 1984). Positive reactions were taken as immediate wheal diameters of equal to or larger than 3 mm.

**Delayed hypersensitivity skin testing**

Delayed hypersensitivity tests were performed before and after the 6 month treatment, with tuberculin (PPD; 2 IU/0.1 mL), *Candida albicans* antigens (300 mg/mL) and *Tricophytum rubrum* antigens (1:100). Positive reactions were recorded when a 10 mm or larger induration was observed after 48 hours.

**Serum IgE levels**

The PRIST (Phadebas, Pharmacia, Sweden) technique for the measurement of total serum IgE level was used. The results were expressed in international units (IU/mL).

A paper disk RAST technique (Ceska and Lundkvist, 1972) was used for the measurement of specific IgE against common environmental allergens (Wide et al., 1967). The positivity of the tests for specific IgE was taken as 0.35 PRU/mL (level 1), according to the *Phadebas* (Pharmacia, Sweden) RAST scale.

**Blood eosinophils counts**

Differential eosinophils counts were performed on blood smears stained with Wright solution.

**T cell subpopulations assay**

The monoclonal antibodies ior-T3, ior-T4, and ior-T8 were prepared at the Center of Molecular Immunology, La Habana, Cuba. These antibodies were used at the following dilutions: 1:20, 1:5 and 1:200 respectively.

Twenty milliliters of heparinized blood was obtained by venepuncture and lymphocytes were separated using a
Histopaque (Sigma) density gradient. The cells were resuspended at 2 x 10^6 cells/mL and smears were prepared. The immunostaining was performed using the avidin-biotin immunoperoxidase technique (Hsu et al., 1981), as modified by Hoffman et al. (1982). The slides were sequentially incubated for 30 min. at 25 C with normal horse serum diluted 1:20 in PBS, then primary mouse monoclonal antibody, biotinylated horse antimouse antibody (50 mg/mL) (Vector, Burlingame, Calif.), and the avidin-peroxidase complex (Vectastain kit, Vector). Five-minute washes with PBS were performed between the incubation steps.

The slides were then incubated with aminoethyl carbazole in the presence of hydrogen peroxide for 10 min. After a 5 min. washing they were counterstained with methyl green, washed again for 5 min. and mounted in glycerol-gelatin.

A total of 200 cells was counted under standard light microscopy, and the percentage of positive cells for each surface marker was calculated.

**Statistical analysis**

The results were expressed as group means and were compared by the Student's t-test for unpaired and paired data.

The total and specific IgE levels were logarithmically transformed and the means +1 standard deviation were calculated and compared by the Student's "t" test.

**RESULTS**

**Baseline evaluations**

**Clinical data**

The group of 17 asthmatic patients who participated in the protocol showed symptoms of low or moderate severity according to the scale used (table 1). Fourteen were classified as mild asthmatics and 3 suffered of moderate asthma. The non asthmatic control individuals were all free of symptoms and family or personal history of atopy. The TF
and placebo treated groups were equivalent for age, sex and severity of symptoms before treatment.

**Table 1**

Clinical results. Severity of symptoms of asthmatic patients before and after treatment

<table>
<thead>
<tr>
<th></th>
<th><strong>Tf group</strong></th>
<th></th>
<th><strong>Placebo group</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Before</strong></td>
<td><strong>After</strong></td>
<td><strong>Before</strong></td>
<td><strong>After</strong></td>
</tr>
<tr>
<td>Cough</td>
<td>1.77 +/- 0.9</td>
<td>* 0.55 +/- 0.70</td>
<td>1.38 +/- 1.10</td>
<td>0.74 +/- 1.1</td>
</tr>
<tr>
<td>Wheezing</td>
<td>2.11 +/- 0.6</td>
<td>1.22 +/- 0.90</td>
<td>2.00 +/- 0.75</td>
<td>1.25 +/- 1.0</td>
</tr>
<tr>
<td>Sibilances</td>
<td>1.66 +/- 1.1</td>
<td>0.80 +/- 1.10</td>
<td>1.50 +/- 1.10</td>
<td>0.80 +/- 1.1</td>
</tr>
<tr>
<td>Use of conventional drugs</td>
<td>1.77 +/- 0.8</td>
<td><strong>0.66 +/- 0.86</strong></td>
<td>1.75 +/- 1.00</td>
<td>0.63 +/- 0.7</td>
</tr>
<tr>
<td>Asthma crises per month</td>
<td>3.12 +/- 1.7</td>
<td>***1.25 +/- 1.10</td>
<td>2.62 +/- 1.50</td>
<td>1.75 +/- 0.4</td>
</tr>
</tbody>
</table>

^ *p < 0.001 TF before vs. TF after  
^ **p < 0.002 TF before vs. TF after 
^ ***p < 0.003 TF before vs. TF after

During TF treatment patients did not show any secondary adverse reaction. Controls were not treated.

**Allergic reactivity**

Before TF therapy, skin testing showed a higher percentage of positivity towards house dust (p < 0.001), *Dermatophagoides sp.* (p < 0.01), insects (p < 0.01) and *A. lumbricoides* antigen (p < 0.05) in patients, as compared to normal controls. Patients also had higher levels of total serum IgE (p < 0.001) and eosinophils counts (p < 0.001)
than controls (table 2). These results confirmed the atopic background of the asthmatic patients.

Table 2

Total serum IgE levels and peripheral blood eosinophils counts before and after treatment

<table>
<thead>
<tr>
<th></th>
<th>Before</th>
<th>TF group (9)</th>
<th>Placebo group (8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All patients</td>
<td>Control</td>
<td>Before</td>
</tr>
<tr>
<td></td>
<td>(17)</td>
<td>(21)</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>1266.4</td>
<td>124.4</td>
<td>1089</td>
</tr>
<tr>
<td><strong>NS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>serum IgE</strong></td>
<td>(2253.5)</td>
<td>(622.7)</td>
<td>(1630)</td>
</tr>
<tr>
<td><strong>Eosinophils</strong></td>
<td>9.8+/-5.3</td>
<td>2.2+/-1.6</td>
<td>9.8+/-5.0</td>
</tr>
<tr>
<td><strong>NS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Total serum IgE levels are expressed as geometric mean + 1SD in IU/mL
Eosinophils are expressed in percentages of the whole leukocyte population
^ *p < 0.001 patients vs. control before treatment
^**p^ < 0.05 TF group before and after treatment
NS: non-significative

Delayed hypersensitivity reactions and T cell subpopulations

Before treatment, the skin test responses to *C. albicans* and *T. rubrum* antigens were smaller (p < 0.05) in patients than in controls (table 3). Similarly, the number of CD3+ positive T cells was less in the patients than in the controls (69 +/-10% vs. 76 +/- 5%; p < 0.05). No difference was detected in CD4+ and CD8+ markers.
Table 3

Delayed hypersensitivity reaction before and after treatment

**Before**

<table>
<thead>
<tr>
<th>TF group (9)</th>
<th>Placebo group (8)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>All patients Control</strong></td>
<td><strong>Before</strong></td>
</tr>
<tr>
<td><strong>After</strong></td>
<td><strong>(17)</strong></td>
</tr>
<tr>
<td>PPD (mm^2)</td>
<td>146 +/- 187</td>
</tr>
<tr>
<td></td>
<td>181 +/- 199</td>
</tr>
<tr>
<td><em>C. albicans</em></td>
<td>&amp;150 +/- 244</td>
</tr>
<tr>
<td></td>
<td>264 +/- 399</td>
</tr>
<tr>
<td><em>T. rubrum</em></td>
<td>&amp;56 +/- 146</td>
</tr>
<tr>
<td></td>
<td>0 +/- 0</td>
</tr>
</tbody>
</table>

Values are mean +/- SD of the induration size (mm^2)

* p < 0.05 Patients vs. Controls

**Evaluation after treatment**

**Clinical data**

Evaluation of the TF and placebo groups was performed immediately after the six months of treatment (table 1). TF patients showed a statistically significant decrease in the number of asthma crises per month (p < 0.001), cough episodes (p < 0.003) and utilization of conventional treatment (p < 0.002). No differences were detected in wheezing. No differences were found when symptom severities were compared between the TF and placebo groups.

**Allergic reactivity**

The positivity percentage in immediate hypersensitivity skin tests did not show substantial changes after
treatment. Nevertheless, an increased allergic reactivity towards *A. lumbricoides* antigen (p < 0.05) was detected in the TF group (figure 1). The levels of specific IgE antibodies toward house dust, *Dermatophagoides sp*, and *Ascaris lumbricoides* antigens, insects and molds, are shown in table 4. The total serum IgE levels and peripheral blood eosinophils counts increased after treatment in the TF patients (table 2).

**Table 4**

Specific serum IgE in asthmatic patients before and after treatment (PRU/mL)

<table>
<thead>
<tr>
<th>Allergens</th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All patients (17)</td>
<td>FT (9)</td>
</tr>
<tr>
<td>Home dust</td>
<td>3.13 +/- 3.5</td>
<td>2.57 +/- 2.3</td>
</tr>
<tr>
<td><em>Dermatophagoides sp</em></td>
<td>1.97 +/- 1.0</td>
<td>2.0 +/- 1.86</td>
</tr>
<tr>
<td><em>Ascaris lumbricoides</em></td>
<td>1.45 +/- 0.7</td>
<td>2.30 +/- 1.7</td>
</tr>
<tr>
<td>Insects</td>
<td>1.29 +/- 1.6</td>
<td>1.25 +/- 1.7</td>
</tr>
<tr>
<td>Moulds</td>
<td>1.02 +/- 0.7</td>
<td>0.67 +/- 0.7</td>
</tr>
</tbody>
</table>

ND: not determined

Values greater than 0.35 PRU/mL were considered positive.

**Delayed hypersensitivity reactions and T cell subpopulations**

TF treated patients showed an increase response towards PPD and *C. albicans* antigens (p < 0.05) when compared with their previous responses. However, when the TF group was compared with the placebo group no significant differences were observed (table 3).

Although a tendency toward an increase in the peripheral blood lymphocyte subpopulations was observed, no statistically significant differences were detected after treatment (result not shown).
DISCUSSION

The immunological response of asthmatic patients is a key aspect of the etiopathology of bronchial asthma. The most important immunological alterations found in this disease are a high allergic reactivity toward common environmental allergens (Zimmerman et al., 1988), activation of CD4+ lymphocytes associated with previous sensitization to environmental allergens, which induce a high IgE production (Corrigan and Kay, 1990) and high peripheral blood and bronchial eosinophilia (Gleich, 1990).

The etiopathology of asthma is complicated by multiple immunological and non immunological factors like a specific genetic pattern and abnormal biochemical responses, such as a low sensitivity threshold to histamine and beta adrenergic blockade.

We developed a treatment protocol using TF, which is capable of modulating the immunological response.

The characterization of the asthmatic patients before treatment revealed their atopic background, compared with the non asthmatic, control group.

The clinical evaluation revealed a statistically significant improvement of the symptoms in the TF group after treatment. Similar results have been reported by Feng-Yizhen et al. (1990), after the application of 10 to 24 doses of TF in a 6 month period. These authors found a decreased frequency and severity of asthmatic crises and reduced use of conventional anti-asthmatic drugs. Our results also agree with Khan et al. (1978), who demonstrated clinical improvement in a double blind cross-over study of 15 asthmatic patients. In the present study no difference was found when we compared the severity of the symptoms between the TF and the placebo group, probably due to the small number of patients evaluated.

No changes were found in the immediate hypersensitivity skin tests toward common environmental allergens after
treatment. However, we detected a significant increase in the skin test response to *A. lumbricoides* in the TF group. There is no published information available on the influence of TF on immediate hypersensitivity reactions, particularly on skin tests. Therefore, the change in the allergic response to *A. lumbricoides* might be associated with an increased prevalence of intestinal parasitism in this group of individuals. We could not confirm this possibility as we did not perform feces examination.

The specific IgE levels to common environmental allergens did not show changes after treatment. Nevertheless, increased anti-*A. lumbricoides* IgE levels were detected in the treated patients.

It is possible that a longer evaluation of the patients is necessary to detect clearer variations in IgE levels. Indeed, data obtained from patients under specific hyposensitization treatments show that changes in IgE levels occur only slowly during treatment (Peng et al., 1992).

Reports in the literature are variable in this respect. Some authors report no changes (Khan et al., 1978; Lu, 1983) while others have found reductions (Feng-Yizhen et al., 1990; Zhao et al., 1990) on IgE levels after TF treatment.

TF treatment produced a significant increase of the delayed hypersensitivity reactions when these were compared with the placebo group. This confirms the previous results of Khan et al., (1976; 1978) and Fan et al., (1990). These authors demonstrated the capacity of TF to transfer these reactions to non-responder individuals.

The immunological mechanism of this transferred response could be related to the action of TF on naive T cells, inducing their capacity for a specific response. The other possibility is that TF could act on memory T-cells and be integrated as a part of the T-cell receptor, producing a stronger secondary response (Dwyer, 1990). These ideas could support the future possibility of therapeutic trials
based on the binding capacity of peptides, which may compete with MHC class I and II to modulate the immunological response in allergic diseases (O'Heir et al., 1991). Moreover, TF could link to MHC class II molecules and thus prevent specific T cell activation toward allergens and subsequent IgE synthesis.

The quantification of CD3+, CD4+ and CD8+ peripheral blood T lymphocyte subpopulations did not show significant changes after TF treatment. Other studies have shown that patients with extrinsic asthma treated with TF increased their number of CD3+ and CD4+ peripheral blood populations (Zhao et al., 1990). Nevertheless, the possibility that other aspects of cell mediated immunity, more related to the function of these cells in allergy, and not only to their number, has to be explored. For example, serum IL-4 levels, soluble CD23 receptors or T-cell activation markers may provide relevant data on T cell regulation and function in TF-treated asthma patients.

Different results have been reported in various treatment protocols of TF therapy in asthma, probably due to the non-standardized potency of the TF lots. It would be very important to develop an in vitro analysis to standarize the actual potency of the different fractions used and determine the optimal dose according to its biological activity, and not only the number of cells used.

The present work demonstrates the importance of performing new and more detailed clinical trials on the use of TF as a non conventional treatment in extrinsic asthma patients.

Future studies must be more precise on dose, period of treatment and the possible mechanisms by which TF modulates the allergic responses in extrinsic asthma.

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Fig. 1. Percentage of positive skin tests after treatment.