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

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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 β-adrenergic drugs and teofiline. Before treatment, there was a higher proportion of positive allergic reactivity skin tests ($p < 0.01$), higher serum IgE levels ($p < 0.001$) and eosinophils 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 clínica e inmunológicamente el efecto terapéutico del Factor de Transferencia (FT) en 17 pacientes con asma bronquial extrínseca 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 evaluación inmunológica de los pacientes y de 21 individuos normales, consistió en pruebas de piel para hipersensibilidad inmediata para alergenos ambientales comunes, y retardada (HR) para tuberculina (PPD), *C. albicans* y *T. rubrum*, IgE sérica total (PRIST), IgE sérica específica (RAST), eosinofilia y cuantificación de subpoblaciones linfocitarias CD3+ , CD4+ y CD8+ , con el método de la avidina biotina. Los pacientes presentaron 3.05 ± 1.6 crisis por mes y utilizaban frecuentemente β-adrenérgicos y teofilina. Antes del tratamiento, el estudio de la reactividad alérgica en piel demostró un porcentaje de positividad más elevados en los pacientes que en los controles ($p < 0.01$), niveles de IgE sérica total y eosinofilia tanbién más 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 así mismo la intensidad de las pruebas de hipersensibilidad retardada para *C. albicans* y *T. rubrum* ($p < 0.05$). Estos datos confirman la condición atópica de los pacientes seleccionados. Después del tratamiento se observó mejoría clínica, se demostró una disminución en el número de crisis de asma por mes comparado con el número de crisis antes del tratamiento ($p < 0.001$), disminución de la frecuencia e intensidad de la tos ($p < 0.003$) y disminución del uso de medicamentos convencionales ($p < 0.002$). La respuesta hipersensibilidad retardada al PPD y a *C. albicans* fue más intensa después del tratamiento ($p < 0.02$). Las subpoblaciones CD3+ , CD4+ , y CD8+ no se modificaron después del tratamiento, por lo que sería adecuado estudiar con más profundidad la función de células T. Estos resultados indican que el FT mejoró la condición clínica y no modificó la reactividad de HR de los pacientes. La normalización de las pruebas de inmunidad celular podría estar asociada a la mejoría clínica, sin embargo, la asociación entre estos parámetros inmmunológicos y clínicos requiere mayor número 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 (Ca-

bezas-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 (Fernández and López, 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 5×10^8 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 2×10^6 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 anti-mouse 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.

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.

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 ($69 \pm 10\%$ *vs.* $76 \pm 5\%$; $p < 0.05$). No difference was detected in CD4+ and CD8+ markers.

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 differ-

Table 1
Clinical results. Severity of symptoms of asthmatic patients before and after treatment

	TF group		Placebo group	
	Before	After	Before	After
Cough	1.77 ± 0.9	* 0.55 ± 0.7	1.38 ± 1.1	0.74 ± 1.1
Wheezing	2.11 ± 0.6	1.22 ± 0.9	2.0 ± 0.75	1.25 ± 1.0
Sibilances	1.66 ± 1.1	0.80 ± 1.1	1.5 ± 1.1	0.80 ± 1.1
Use of conventional drugs	1.77 ± 0.8	** 0.66 ± 0.86	1.75 ± 1.0	0.63 ± 0.7
Asthma crises per month	3.12 ± 1.7	*** 1.25 ± 1.1	2.62 ± 1.5	1.75 ± 0.4

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

Table 2
Total serum IgE levels and peripheral blood eosinophils counts before and after treatment

	Before		TF group (9)		Placebo group (8)	
	All patients (17)	Control (21)	Before	After	Before	After
Total Serum IgE	* 1266.4 (2253.5)	124.4 (622.7)	1089 (1630)	** 2180 (4120)	700 (846)	1008 NS (2128)
Eosinophils	* 9.8 ± 5.3	2.2 ± 1.6	9.8 ± 5 NS	11.1 ± 4.0	9.8 ± 5	14.1 ± 11.0 NS

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.

Table 3
Delayed hypersensitivity reaction before and after treatment

	Before		TF group (9)		Placebo group (8)	
	All patients (17)	Control (21)	Before	After	Before	After
PPD (mm^2)	146 ± 187	124 ± 147	146 ± 185	*250 ± 232	181 ± 199	139 ± 147
C. albicans	& 150 ± 244	302 ± 335	61 ± 72	* 681 ± 852	264 ± 399	970 ± 1034
T. rubrum	& 56 ± 146	148 ± 267	101 ± 187	109 ± 220	0 ± 0	1 ± 3

Values are mean ± SD of the induration size (mm^2).
& $p < 0.05$ Patients *vs.* Controls
* $p < 0.05$ TF group after *vs.* before treatment

ences 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).

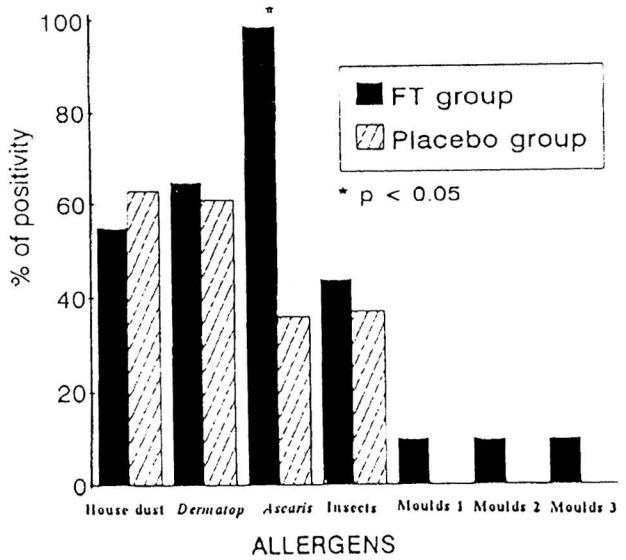

Fig. 1. Percentage of positive skin tests after treatment.

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).

Table 4
Specific serum IgE in asthmatic patients before and after treatment (PRU/mL)

	Before	After	
Allergens	All patients (17)	FT (9)	Placebo (8)
Home dust	3.13 ± 3.5	2.57 ± 2.3	1.68 ± 1.7
Dermatophagoides sp	1.97 ± 1.0	2.0 ± 1.86	0.34 ± 0.4
Ascaris lumbricoides	1.45 ± 0.7	2.30 ± 1.7	ND
Insects	1.29 ± 1.6	1.25 ± 1.7	ND
Moulds	1.02 ± 0.7	0.67 ± 0.7	0.45 ± 0.4

ND: not determined.
Values greater than 0.35 PRU/mL were considered positive.

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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REFERENCES

BUSSE, W. W. (1991). Respiratory infections: Their role in airway responsiveness and the pathogenesis of asthma. *J. Allergy Clin. Immunol.* **85:** 671-683.

CABEZAS-QUIROGA, R.; S. ESTRADA-PARRA; L. PADIERNA; J. PADIERNA; C. FERNÁNDEZ y P. LÓPEZ (1990). Inmunoterapia con el Factor de Transferencia en pacientes con herpes zoster. *Biotecnología Aplicada.* **7**:52-57.

CAREY, J.T.; M.M. LEDERMAN; Z. TOOSI; K. EDMONDS; S. HODDER; L.H. CALABRESE; M.R. PROFFITT; C.E. JOHNSON and J.J. ELLNER. (1987). Augmentation of skin test reactivity and lymphocyte blastogenesis in patients with AIDS treated with transfer factor. *JAMA* **257**:651-655.

CESKA, M. and U. LUNDKVIST (1972). A new and simple radioimmunoassay method for the determination of IgE. *Immunochemistry* **9**:1021.

CORBIEL, L.; J. L. CEUPPENS; G. VAN DER BERGHE; H. CLAEYS and M. CASTEELS-VAN DAELE (1984). Immunological observations before and after successful treatment of Chronic mucocutaneous Candidiasis with Ketoconazole and transfer factor. *Eur. J. Pediatrics.* **143**: 45-48.

CORRIGAN, C. J. and A.B. KAY (1990). CD4 T-lymphocyte activation in acute severe asthma. *Am. Rev. Respir. Dis.* **141**:970-977.

DELGADO, O; L. E. ROMANO; E. BELFORT; F. PIFANO; J. V. SCORZA and Z. ROJAS. (1981). Dialyzable leukocyte extract therapy in immunodepressed patients with cutaneous leishmaniasis. *Clin. Immunol. Immunopathol.* **19**:351-359.

DWYER, J. M. (1990). The rise and fall and rise again of transfer factor. *In: Recent advances in transfer factor and dialyzable leukocyte extracts.* Section I. Introduction of transfer factor or dialyzable leukocyte extracts. Fujisawa, Sasakawa, Iikura, Komatsu, and Yamaguchi eds. Maruzen Co. Ltd, Tokyo, pp. 23-29.

FAN, Z.; F. KONG; G. CHAI; and J.GUO (1990). Comparison of the efectiveness between porcine and human spleen transfer factor in the treatment of bronchial asthma. In: *Recent Advances in Transfer Factor and Dialyzable Leukocyte Extracts.* Section V. Clinical Studies of Immunological Anormalities. Fujisawa, Sasakawa, Iikura, Komatsu, and Yamaguchi eds. Maruzen Co. Ltd, Tokyo, pp. 287-293.

FENG-YIZHEN, M. D.; Z CUI; X SUN; H. HUANG; Z CHe; and L. KANG, (1990). Evaluation of the clinical and immunological effects of transfer factor (TF), Thymosin (Th) and Placenta Factor (PF) on asthma in children. In: *Recent Advances in Transfer Factor and Dialyzable Leukosyte Extracts.* Section V. Clinical Studies on Immunological Anormalities. Fujisawa, Sasakawa, Iikura, Komatsu, and Yamaguchi eds. Maruzen Co. Ltd, Tokyo, pp. 278-281.

FERNÁNDEZ, C. y P. LÓPEZ (1986) Obtención y caracterización del factor de transferencia extraído de leucocitos que produjeron interferón. *II Seminario Cubano sobre Interferon y I Seminario Cubano sobre Biotecnología*, La Habana, pp. 549-559.

FUKUDA, T.; C.E. DUNNETE; S.J. REED; M.S. ACKERMAN and G.J Gleich (1985). Increased number of hypodense eosinophilia in the blood of patients with bronchial asthma. *Amer. Rev. Respir. Dis.* **132:** 981.

GLEICH, G.(1990). The eosinophils and bronchial asthma: current understanding. *J. Allergy Clin. Immunol.* **85**:423.

HOFFMAN, F. M.; R. J. BILLING; J. W. PARKER and C. R. TAYLOR (1982). Cytoplasmic as opposed to surface Ia antigens expressed on human peripheral blood lymphocyte and monocytes. *Clin. Exp. Immunol.* **49**:355-363.

HSU, S.M.; L. RAINE and H. FAUGER (1981). Use of avidin-biotin peroxidase complex(ABC) in immunoperoxidase techniques: a comparation between ABC and unlabelled antibody (PAP) procedures. *J. Histochem. Cytochem.* **92**:577-580.

ISHIZAKA, T. (1981). Analysis of triggering events in mast cells for immunoglobulin E mediated histamine release. *J. Allergy Clin. Immunol.* **67**:90.

KHAN, A.; W. SELLARS; J. PFLANZER; J.M. HILL; D. THOMETZ y J. HAENKE. (1976). Asthma and T cell inmunodeficiency improvement with transfer factor and inmunopeptide 1. *Ann. Allergy.* **37**:267-274.

KHAN, A.; W. SELLARS, W. GRATER; M.F. GRAHAM; J. PFLANZER; A. ANTONETTI; J. BAILEY, Y N.O. HILL. (1978). The usefulness of transfer factor associated with frequent infections. *Ann. Allergy* **40**:229-232.

LAWRENCE, H.S. (1955). The transfer factor in humans of delayed skin sensitivity to streptococcal M substance and to tuberculin with disrupted leukocyte. *J. Clin. Invest.* **34**:219-232.

LEMANSKE, R.F. JR.; E.C., DICK; C.A.SWENSON (1989). Rhinovirus upper respiratory infection increases airway reactivity in late asthmatic reactions. *J. Clin. Invest.* **83:** 1-10.

LEUNG, D. Y. AND R.S. GEHA (1986). Immunoregulatory abnormalities in atopic dermatitis. *Clin. Rev. Allergy.* **4**: 67.

LU, W. (1983). Serum IgE, skin test and asthma. *Chinese J. Tuberc. Respir.* **6**:171.

LYNCH, N.R.; L. MEDOUZE; M.C. DI PRISCO-FUENMAYOR; O. VERDE; R.I. LÓPEZ and C. MALAVÉ (1984). Incidence of atopic disease in a tropical environment: Partial independence from intestinal helminthiasis. *J. Clin. Allergy and Immunol.* **73**:229.

MILLER, L.L.; L.E. SPITLE; R.E. ALLEN and D.R.MINOR (1988). A randomized, double-blind, placebo-controlled trial of transfer factor as adyuvant therapy for maligmant melanoma. *Cancer.* **61**:1543-1548.

O'HEIR, R.E; R. BUCH; J.B. ROTHBARD and R.L. JONOTHAN (1991). An *in vitro* model of peptide-mediated immunomodulation of the human T cell response to *Dermatophagoides sp*, (house dust mite). *J. Allergy Clin. Immunol.* **87:** 1120-1127.

PENG, Z.; R.M. NACLEIRO; P.S. NORMAN and N.F. ADKINSON (1992). Quantitative IgE and IgE-subclases responses during and after long term ragweed immunotherapy. *J. Allergy Clin. Immunol.* **89**:519-529.

RACKEMANN, F.M.(1947). A working classification of asthma. *Am. J. Med.* **3**: 6.

SPITZER, W.O.; M.P.H. SAMMY SUISSA; M.P.D. ERNST; R.I. HORWITZ; B.HABBICK; D. COCKCROFT; J.F. BOIVIN; M.MC NUTT; A.S. BUIST and A.S. REBUCK (1992). The use of b-agonist and the risk of death and near death from asthma. *N. Engl. J. Med.* **326**:501-506.

WIDE, L.; H.H. BENNICH and S.G.O. JOHANSSON (1967).Diagnosis of allergy by *in vitro* test for allergen antibodies. *Lancet* **ii**:1105-1107.

ZHAO, J.; Z. ZHANG; F. LU; J. LIU; S. WANG; Y. LIU; S. HOU; H. ZHENG; B. HUO and E. WU (1990). *99 Section V Clinical Studies on Immunological Abnormalities.* Fujisawa, Sasakawa, Iikura, Komatsu, and Yamaguchi eds. Maruzen Co. Ltd, Tokyo, pp:294-298.

ZIMMERMAN B; S. FEANNY and J. REISMAN (1988). Allergy in asthma. I. The dose relationship of allergy to severety of childhood asthma. *J.Allergy Clin. Immunol.* **81**:63-70.