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SPECIFIC IgE ANTIBODIES TO *LEISHMANIA BRAZILIENSIS* IN PATIENTS WITH MUCOCUTANEOUS LEISHMANIASIS

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SUMMARY

A total of 116 sera of patients suffering from South American mucocutaneous leishmaniasis were analysed using the RAST (*L. braziliensis*-specific) and RIST techniques. Results of total and specific IgE levels were correlated with different clinical stages of the disease. The level of total and specific IgE appeared to be higher among patients with cutaneous ulcers than among those presenting mucocutaneous lesions, *i. e.* espundia. Furthermore, non-treated patients presented higher concentrations of total and specific IgE than did treated ones.

KEY-WORDS: IgE, Mucocutaneous leishmaniasis, *Leishmania braziliensis;* Espundia, Human.

INTRODUCTION

One form of mucocutaneous leishmaniasis in the New World is produced by *Leishmania braziliensis braziliensis:* it is called « espundia », and presents typical clinical aspects with a high proportion of secondary mucosal localizations. Mucocutaneous involvement represents the final self-destructive stage which may occur several years after disappearance of the initial sore, producing severe mutilation of the face. These disfiguring effects are evidence of the gravity of the disease. The immune response of infected patients

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follows different patterns, reflecting the clinical form of the infection. Previous data showed that most patients with mucocutaneous leishmaniasis had delayed hypersensitivity reactions and specific antibodies against leishmanial antigens [18, 7]. On the other hand, a recent study [8] suggests a link between the development of severe forms and the outbreak of immune complexes and anti-IgG antibodies in espundia.

Immunoglobulin E is massively produced during helminthic infection in man and animals [5, 12]. Bacterial and viral infections are of minor importance in raising IgE levels [1]. Several recent papers show that protozoal parasitic infections do not increase serum IgE levels [9]. Some studies have already been published concerning the production of total IgE in South-American leishmaniasis [4] and of specific IgE in acute human Chagas disease [15].

Recently, a radioimmunoadsorption method [6] and a radioallergosorbent test [13] have been adapted to the quantification of specific IgE antibodies. The obtaining of a soluble lyophilized antigenic extract of *L. braziliensis* has enabled these techniques to be used for detection of specific IgE antibodies in sera from patients with mucocutaneous leishmaniasis.

MATERIALS AND METHODS

Parasite, culture and preparation of antigenic extracts.

L. b. braziliensis M 1670 (LV 65, ITMAP 1810) used in the present work was isolated from a human nasal *para state* lesion, in Brazil, by Drs R. L. Lainson and J. J. Shaw. Infected hamsters were obtained from Dr M. Chance, Liverpool, and the parasites were transferred *in vitro*. Large-scale culture was carried out in GLSH monophasic liquid medium containing lactalbumin hydrolysate, calf serum and haemoglobin in Hank's physiological solution [11, 14]. An approximate yield of 1 g (wet weight) of organisms per litre of culture medium was obtained. The organisms were pelleted by continuous flow centrifugation (JCF-Z, Beckman) at 5,000 rpm at 2° C, then washed 4 times in cold Hanks-Wallace solution in order to eliminate serum contaminants from culture medium. Culture forms were then resuspended in 1 % NaCl at a ratio of 1 g wet weight per 4 ml solution. This suspension was frozen at — 25° C in a precooled « Bio-X-press » (LKB) for disintegration of cells and tissues. The suspension was disrupted at — 25° C by 5 pressings at a pressure of 2,000 kg/cm² (28,000 psi). The whole homogenate was then centrifuged at 30,000 *g* for 1 h at 2° C in order to separate insoluble from soluble antigen. The supernatant fluid was dialyzed at 100 times its volume in demineralized water for 21 h at 2° C and then lyophilized.

Sera.

The 116 sera were collected on the eastern slopes of the Andean Cordillera (La Paz Department, Bolivia). The leishmanial infection was checked by the intra-

B/T = bound/total (radioactivity).
d. f. = degree of freedom.
IU = international unit.
MC = more than one cutaneous ulcer.
MMC = more than one mucocutaneous lesion (espundia).
OC = one cutaneous ulcer.
OMC = one mucocutaneous lesion (espundia)
RAST = radioallergosorbent test.
RIST = radioimmunosorbent test.

dermal test with *L. braziliensis* antigen (Gorgas Memorial Laboratory, Canal Zone, Panama) and by serological tests (precipitation in gel and indirect immunofluorescence).

Sixty-nine patients were classified into four groups based on clinical criteria according to the localization and number of lesions: 1) five patients with only one cutaneous ulcer (OC); 2) three patients with several cutaneous ulcers (MC); 3) eighteen patients with only one mucocutaneous lesion (OMC); and 4) forty-three patients with multiple mucocutaneous lesions (MMC).

The criterion of treatment with N-methylglucamine (Glucantime) allowed classification of patients into 3 groups: 1) twenty patients who were never treated (non-treated); 2) thirty-three patients in the process of treatment (treated); and 3) sixteen patients treated but presenting evolutive lesions (intermediary group; for statistical tests, they were alternatively included in groups 1 or 2).

Quantification of total IgE.

Total IgE levels were determined using the radioimmunosorbent test (RIST) « Phadebas » (Pharmacia, Uppsala, Sweden). In order to standardize the test, WHO serum 78/341 was used as a reference. IgE levels were estimated using the standard curve and the confidence limits of the assay ($p \leqslant 0.05$) established by repeated measurement of a single serum. Results are expressed in international units (IU) per millilitre of serum.

Quantification of specific IgE antibodies against L. braziliensis.

All sera were tested by the radioallergosorbent test (RAST) used to detect IgE antibodies to *L. braziliensis*. Ten mg of antigens were mixed with 100 mg CNBr-activated microcrystalline cellulose particles [**13**] during the coupling procedure. The suspension of *L. braziliensis* conjugates was added, in 0.5 ml aliquots, to 0.05 ml serum for each test in triplicate. After washing the non-specific IgE, the suspension was incubated with 0.05 ml radioactive I^{125}-labelled anti-IgE ($\simeq 0.8$ µg; 185 KBq, Pharmacia AB, Uppsala, Sweden). Both steps of incubation were carried out under vertical rotation. The specific IgE antibody titre was proportional to the uptake in radioactivity (bound = B) compared to total radioactivity (total = T) added per tube, and was classically expressed in per cent B/T.

The different B/T ratios were directly compared with those obtained using 15 control sera from Europeans presenting high IgE levels in relation to *Graminae* antigens with a range of 1,000-8,000 IU/ml total IgE (mean 2334 IU, SD 1918 IU). In order to investigate cross-reactivity, 5 Algerian hydatid diseases *(Echinococcus granulosus)* with a range of 684-6,050 IU/ml total IgE (mean 3,765 IU, SD 2,670 IU) and 4 African schistosomiasis *(Schistosoma mansoni)* with a range of 1,200-6,160 IU/ml total IgE (mean 3,313 IU, SD 1769 IU) were studied in patients with high levels of IgE antibodies.

Statistical analysis.

The non-parametrical statistical tests [**3, 17**] of Kruskall and Wallis were used, as well as the regression coefficients of Spearman and Kendall.

RESULTS

Total and specific IgE concentration.

Results of the RIST technique demonstrated a significant increase in total IgE level in the sera of 116 Bolivian patients with mucocutaneous

leishmaniasis. Although exhaustive parasitological studies were not carried out in these patients, the high prevalence of intestinal helminthiasis in the Bolivian population demonstrates the need for further consideration of these factors as possible inducers of the total IgE level. As we had no serum from healthy Bolivian controls, it was not possible to compare probable poly-parasited leishmanian patients with European controls allergic to *Graminea*.

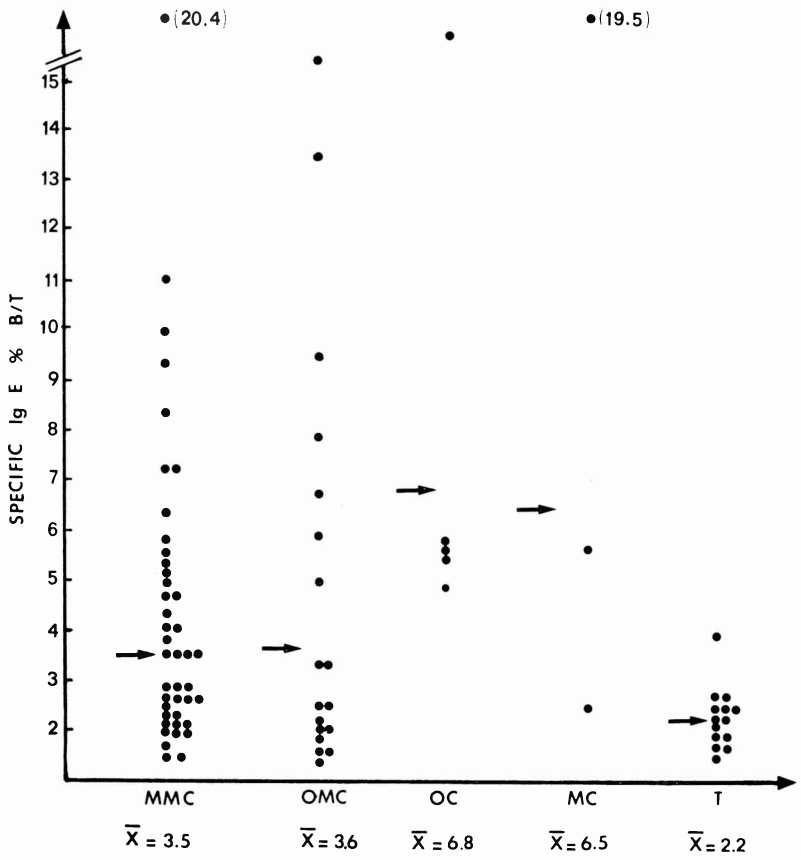

FIG. 1. — *Specific IgE levels (with geometrical mean concentration) related to clinical criteria.*

T = controls.
For the other symbols, see list of abbreviations.

Figure 1 shows the distribution of specific IgE levels related to clinical criteria in 69 patients. The concentration of specific IgE against *L. braziliensis* expressed by the B/T ratio in per cent varied from the basic (1.1) to the maximum level (20.4). Patients showed a specific IgE rate different from that of control Europeans (T) presenting anti-*Graminea* specific IgE ($0.01 > p > 0.001$).

A very slight non-specific absorption was observed in the RAST test for Algerian hydatid disease (geometrical $\bar{x} = 3.2$ % B/T) and African schistosomiasis (geometrical $\bar{x} = 3.1$ % B/T) with high levels of IgE antibodies.

Human infection by *L. braziliensis* thus appeared to provoke a production of specific IgE.

The Kendall test showed a significant correlation ($r = 0.714$; $2p < 0.001$) between specific and total IgE levels.

Role of the stage of the disease in levels of total and specific IgE.

The Kruskall and Wallis test showed that total and specific IgE levels varied according to the different stages of the disease; these results are expressed in table I.

TABLE I. — **Total (IgE) and specific (B/T) IgE levels related to clinical criteria.**

	MMC	OMC	MC	OC	d. f. = 3 χ^2 (0.05) = 7.81	
B/T	44.39	42.30	61.00	67.30	35.00	H = 29.81
N	43	18	3	5	69	
IgE	35.49	33.20	56.33	60.00	38.00	H = 10.12
N	45	20	3	7	75	

d. f. = degree of freedom.
N = number of sera.

A simple variance analysis (table II) reveals that patients presenting a single or multiple cutaneous lesions (OC and MC) showed an increase in total and specific IgE compared with patients presenting one or several mucocutaneous sores (OMC and MMC), as expressed in table II, contrasts 1 and 4.

Within the same type of sore (whether cutaneous or mucocutaneous), the number of lesions did not have any effect upon the IgE levels.

Effect of treatment on total and specific IgE rates.

A difference existed at the level of total and specific IgE rates according to treatment (except in one case: that of non-treated + intermediary group/treated). In the case of non-treated/treated + intermediary group, the correlations were significant (0.03 for specific IgE; 0.01 for total IgE).

The Kruskall and Wallis test proved that the differences previously noted were not due to the intermediary group. For the specific IgE, W was 56.97 for the non-treated, 40.21 for the intermediary and 43.24 for the treated group (d. f. = 3/H = 13.70/χ^2 (0.05) = 7.815). For total IgE,

TABLE II. — **Simple analysis of the variance of total and specific IgE levels related to clinical criteria.**

— *Specific IgE*

Source of variation	Sum of squares	d. f.	Variant ratio	Contrast	Sum of squares	Test	Conclusion
Factorial	109.48	3		1. [MC + OC] ↔ [MMC + OMC]	101.13	6.376	significant
Residual	1046.80	66	15.86	2. [MC] ←——→ [OC]	4.1	0.259	not significant
Total	1156.30	69		3. [MMC] ←——→ [OMC]	4.2	0.266	not significant
				3.98 < F1; 66; 0.95 < 4 7.01 < F1; 66; 0.99 < 7,08			

— *Total IgE*

Source of variation	Sum of squares	d. f.	Variant ratio	Contrast	Sum of squares	Test	Conclusion
Factorial	291,710,252	3		4. [MC + OC] ↔ [MMC + OMC]	272,306,595	14.4	significant
Residual	1,361,164,654	72	18,905,064	5. [MC] ←——→ [OC]	12,530,880	0.66	not significant
Total	1,652,874,906	75		6. [MMC] ←——→ [OMC]	6,872,776	0.36	not significant
				6.96 < F1; 72; 0.99 < 7.01			

d. f. = degree of freedom.

the W was 53.35 for the non-treated, 31.75 for the intermediary and 32.76 for the treated group (d. f. = 2/H = 13.55/χ^2 (0.05) = 5.991).

A simple variance analysis of specific IgE and total IgE demonstrated that the non-treated group was responsible for differences observed in variance [IgE (intermediary group) = IgE (treated); IgE (intermediary group) ≠ IgE (non treated); IgE (non-treated) ≠ IgE (treated)]. Past or current treatment seemed to provoke a decrease in specific IgE level.

DISCUSSION

The RIST technique demonstrated a significant increase in the total IgE level in the sera of 116 Bolivian patients with mucocutaneous leishmaniasis. The high prevalence of intestinal helminthiasis in this Bolivian population plus the absence of an analysis of « normal » populations from the same geographical zone prevent any interpretation. In the RAST test, a negligible non-specific absorption with *L. braziliensis* antigen was provoked against sera of individuals from a non-endemic area with high levels of IgE antibodies against *Graminae* antigens, *E. granulosus* and *S. mansoni*. Moreover, the Kendall test shows a significant correlation between specific and total IgE levels in sera with mucocutaneous leishmaniasis. Thus, during the cutaneous stage (one or multiple sores) of the infection by *L. braziliensis*, an increase in total and specific IgE is observed. On the other hand, treatment provokes a decrease in total and specific IgE. The fact that total and specific IgE levels are lower in patients with mucocutaneous leishmaniasis than in those with cutaneous lesions is not due to human genetic problems; indeed, the two developmental stages of the disease are carried out by the same genetically homogeneous population.

The developmental of IgE responses during the cutaneous stage could protect against further development of mucocutaneous disease; conversely, the development of the mucocutaneous disease might have as its consequence a reduction in IgE levels. Finally, an immunosuppressive phenomenon might frequently involve a stimulation of IgE. In cases of severe leishmaniasis (human kala-azar) [16, 10], recent data would seem to show a T-cell defect with significant anomalies in cell-mediated immunity. Spontaneous or post-treatment cure could renew the T-cell functions. The production of IgE is also a T-regulated response [2], but thus far, no work on either experimental or human leishmaniasis supports the hypothesis of an interaction between IgE molecules and different cell populations.

Our recent unpublished work shows that patients with Old World-type leishmaniasis, that is, kala-azar (60 sera), « oriental sore » (45 sera) and diffuse cutaneous leishmaniasis in Ethiopia (10 sera), do not develop significant levels of total and specific IgE; thus, mucocutaneous South American leishmaniasis appears to be a unique entity.

Hence, the observation of hyperimmunoglobulinaemia E only in patients with mucocutaneous ulcers suggests the existence of two opposite poles

in the immune response between cutaneous and mucocutaneous localization, and provides the basis for new approaches in the exploration and evaluation of the immune response during the course of this disease. Longitudinal observations of patients in the cutaneous stage are required in order to shed further light upon this distinction.

RÉSUMÉ

IgE SPÉCIFIQUES DE « LEISHMANIA BRAZILIENSIS » DANS LA LEISHMANIOSE CUTANÉO-MUQUEUSE

Un total de 116 sérums de patients atteints de leishmaniose cutanéo-muqueuse Sud-Américaine a été analysé par des techniques de RAST spécifique de *Leishmania braziliensis* et de RIST. Les résultats des IgE totales et spécifiques sont comparés avec les différents stades cliniques de la maladie. Le niveau des IgE totales et spécifiques est plus élevé chez les patients OC et MC (ulcères cutanés) que chez les patients avec des lésions muco-cutanées OMC et MMC (espundia). Les patients non traités présentent des concentrations d'IgE totales et spécifiques plus élevées que chez les patients traités.

MOTS-CLÉS : IgE, Leishmaniose cutanéo-muqueuse, *Leishmania braziliensis;* Espundia, Homme.

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