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Delayed-Type Hypersensitivity and Immunoglobulin E in American Cutaneous Leishmaniasis

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Delayed-type hypersensitivity (DTH) and both total and specific immunoglobulin E (IgE) antibody levels were studied during an outbreak of American cutaneous leishmaniasis. Direct correlations were detected between DTH reactivity and either age or the period of evolution of the infection, and a clear association with sex (strongest response in females) was observed. Extremely high, age-dependent, total serum IgE levels were measured in the study group, probably due to concurrent intestinal helminthiasis. A low proportion of the group also had detectable levels of specific anti-*Leishmania* IgE antibody. Total and specific IgE levels were also sex dependent (lowest in females), and an inverse correlation was found between these levels and DTH responsiveness, possibly reflecting the intervention of regulatory influences of T-lymphocyte activity.

The capacity of various helminthic infections to greatly augment total serum immunoglobulin E (IgE) levels is well recognized (9, 23), but in contrast, protozoa have little effect on this immunoglobulin class (5). The existence, however, of anaphylactic reactivity against various protozoa (12, 20, 21, 24), including *Leishmania* (1, 2, 14), has been described. We undertook a study of the total and specific IgE levels in sera from individuals during an outbreak of American cutaneous leishmaniasis (ACL) in Venezuela. In addition, due to the well-recognized association between cellular immune reactivity and IgE synthesis (3), delayed-type hypersensitivity (DTH) skin tests were performed with leishmanin.

MATERIALS AND METHODS

Population characteristics. A total of 126 individuals were examined at random during an outbreak of ACL in Venezuela (Valle Seco, La Laguna). These included 41 apparently healthy subjects, 65 subjects with a single active lesion, and 20 subjects with multiple noncontiguous lesions. The lesions were subjectively assessed by their size and severity, and some were biopsied for both in vitro (liver infusion tryptose medium) and in vivo (hamster) culture and histological examination. The interval between the first appearance of a lesion and the time of study (evolution) was determined when possible (in 80% of the cases). No mucocutaneous lesions were reported in this patient group over the period of 20 months subsequent to the initial examination, and the causal agent was tentatively identified as *Leishmania mexicana*.

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Various aspects of the sex and age structure of the study group are presented in Table 1. The sexes were balanced, but the mean age of the healthy individuals was significantly ($P < 0.01$) higher than that of the ACL patients (27.5 ± 2.4 versus 19.3 ± 2.3 years). In fact, whereas 80.8% of the children examined had lesions, only 53.5% of the adults exhibited lesions ($P < 0.005$). The mean period of evolution of the disease up to the time of the study was significantly ($P < 0.001$) shorter in individuals with single lesions than in those with multiple lesions (Table 1).

Intracutaneous DTH testing. Standard leishmanin (Institute of Tropical Medicine, Caracas, Venezuela) was injected intracutaneously (0.1 ml), and the diameter of induration was measured after 48 h. The leishmanin caused no appreciable reactions when tested in the skin of 20 healthy individuals in a nonendemic area who had no prior history of serious protozoal or bacterial infection.

***Leishmania* extracts.** The AZV strain (19) of *L. mexicana* was cultured in a defined medium (REN) containing no extraneous protein (22) for 10 days. The cultures were subjected to five cycles of freeze-thawing, and the soluble supernatant was collected after centrifugation (16,000 × *g* for 30 min). The pellet was sequentially extracted (24 h; 4°C) with 3 M KCl, then 2 and 4 M urea, and finally 0.1% Triton X-100 plus 0.05% Tween 20, each separate supernatant being immediately dialyzed against three changes of 100 volumes of saline. The protein concentrations of the various fractions were estimated and then equalized, and samples were pooled to provide the complete extract, which was then used in the radioallergosorbent test (RAST).

RAST. The method employed for the RAST was basically comparable to that previously described (10). Each test serum was comparatively evaluated by using either *Leishmania* extract or human serum albumin

TABLE 1. Characteristics of study population (126 subjects)

Patient characteristics	% Group	% Male	Age (yr)[a]	Evolution (days)[a,b]
Sex				
Male	52.4	NR[c]	19.3 ± 1.7	61.8 ± 8.1
Female	47.6	NR	24.4 ± 2.0	69.3 ± 8.3
Age group[d]				
6 to 16 yr	41.9	59.6	NR	55.0 ± 8.0
16 to 50 yr	47.6	45.8	NR	75.1 ± 9.0
Lesions				
None	32.5	53.7	27.5 ± 2.3	0
Single	51.6	51.6	17.8 ± 1.7	55.8 ± 5.8
Multiple	15.8	55.0	22.2 ± 3.6	101.4 ± 13.3

[a] Mean ± standard error of the mean.
[b] Period between first appearance of lesion and time of study.
[c] NR, Not relevant.
[d] Only the largest groups are presented.

controls, and selected sera were tested for their specificity by RAST inhibition (11) with soluble extract; net inhibitions of 63 to 77% were obtained. Although the RAST employed was developed in our laboratory, it was standardized against the commercially available Phadebas (Pharmacia Diagnostics, Sweden) kit. To avoid the necessity of establishing arbitrary reference units, RAST results were expressed as the ratio of the mean counts per minute of the *Leishmania* test over that of the albumin control for each serum. "Positivity" was defined, where necessary, as a ratio of ≥6.5, a figure that has been tentatively established in the course of our comparison of allergic history, immediate skin test reactivity, and RAST results in 630 individuals tested with common environmental allergens.

Total serum IgE. Serum IgE levels were measured by the application of a paper radioimmunosorbent test (4), which, while being developed in our laboratory, was standardized against the Phadebas assay.

Statistical analysis. All data were processed by using programs developed by the Health Sciences Computing Facility, University of California. Continuous data were tested for the normality of their distribution and then grouped according to the discrete variables defined; means ± standard error of the mean were determined. Where appropriate, one- or two-way analysis of variance was applied, correlation coefficients were determined, and the distributions between the discrete variables were analyzed by the χ^2 test; the Yates correction factor was applied where necessary.

RESULTS

DTH reaction diameters to leishmanin. The skin test reaction diameters were significantly greater ($P < 0.02$) in females than in males and in adults than in children (Table 2). As might be anticipated, the reaction diameters were greater in the patients than in the apparently healthy controls ($P < 0.005$); the individuals with multiple lesions exhibited the strongest reactivity (Table 2). In fact, in males a significant correlation was detected between the period of evolution of the infection and the DTH reaction size ($r = 0.54$; $P < 0.01$). That the higher skin test reactivity in females was actually due to the influence of the leishmanial infection is demonstrated by the fact that no significant difference was detected between the apparently healthy males and females (10.1 ± 2.3 and 8.7 ± 2.2 mm, respectively); the sex difference was contributed largely by the group with single lesions (11.2 ± 1.4 mm for males and 19.8 ± 2.2 for females; $P < 0.002$). It is of interest to note that in the healthy group, there was also a clear correlation between age and skin test diameter ($r = 0.47$; $P < 0.01$). For example, healthy children demonstrated skin reaction diameters of only 2.8 ± 1.3 mm, compared with 11.1 ± 2.1 mm in healthy adults ($P < 0.025$). Children with single lesions had reaction diameters of 12.0 ± 1.7 mm ($P < 0.02$ compared to controls), and those with multiple lesions had reaction diameters of 18.3 ± 3.8 mm ($P < 0.01$).

A very high proportion of apparently healthy subjects exhibited DTH reaction diameters of ≥5 mm, as did a significantly ($P < 0.01$) greater fraction of the patients (Table 2). No sex difference was observed in this respect, although adults were more reactive ($P < 0.01$) than children.

When diameters of ≥10 mm were considered, significantly fewer ($P < 0.01$) of the controls were positive compared with the number of positive controls when 5 mm was taken as the threshold. This proportion was also lower ($P < 0.005$) in controls than in patients, and once more, no sex difference was found in the proportion positive (Table 2). Adults were again more responsive ($P < 0.005$) than children. It is of relevance to note that the mean period of evolution of infection was considerably higher ($P < 0.01$) in patients who were skin test positive

TABLE 2. DTH reactivity and total and specific IgE in sex, age, and clinical groups

Patient characteristics	DTH			Total IgE		Specific IgE	
	Mean diam (mm) ± SEM	% >5 mm	% >10 mm	log	Geometric mean (IU/ml)	Mean	% Positive
Sex							
Male	12.0 ± 1.1	82.5	57.9	3.447 ± 0.060	2,799	3.71 ± 0.29	20.0
Female	16.3 ± 1.6	86.0	66.0	3.202 ± 0.071	1,592	2.15 ± 0.22	3.4
Age group[a]							
6 to 15 yr	11.0 ± 1.4	69.6	45.6	3.364 ± 0.074	2,312	3.55 ± 0.33	17.3
16 to 50 yr	16.0 ± 1.5	94.0	70.0	3.309 ± 0.066	2,037	2.79 ± 0.23	8.5
Lesion							
None	9.5 ± 1.6	72.2	41.7	3.461 ± 0.077	2,891	3.34 ± 0.38	9.8
Single	15.5 ± 1.4	88.5	65.4	3.303 ± 0.061	2,009	3.00 ± 0.24	9.7
Multiple	18.3 ± 1.7	94.7	89.5	3.148 ± 0.153	1,406	3.25 ± 0.50	25.0
Single + multiple[b]	17.0 ± 1.5	90.1	71.8	3.254 ± 0.091	1,788	3.10 ± 0.30	13.4

[a] Only the largest age groups are considered.
[b] Total infected group.

compared with those who were negative (76.0 ± 7.4 and 35.3 ± 8.3 days, respectively).

Total serum IgE. For statistical analysis, the IgE concentrations were normalized by logarithmic transformation, but for descriptive purposes the geometric means are also presented (Table 2). Male total IgE levels were significantly ($P < 0.01$) higher than those of females, but no statistically significant difference was detected between child and adult means when the group was considered as a whole (Table 2). However, significant correlations between age and log IgE levels were detected in certain subgroups of the population, such as healthy adults ($r = 0.49$; $P < 0.01$).

When the clinical status of the group was considered, total IgE levels were somewhat ($P < 0.05$) higher in the healthy individuals than in the patients, particularly those with multiple lesions (Table 2). This difference was even more evident in children: the level was 4,645 IU/ml in healthy individuals and 783 IU/ml in those with multiple lesions ($P < 0.02$). In females, who generally had the highest skin reaction diameters and lowest IgE levels, a weak inverse correlation was found ($r = -0.29$; $P < 0.05$).

Specific IgE. The levels of IgE antibody against *Leishmania*, estimated by RAST, correlated significantly with total IgE ($r = 0.77$; $P < 0.001$) and were higher ($P < 0.001$) in males than in females. A weakly significant increase ($P < 0.05$) was also detected in children over adults (Table 2). No significant differences were, however, observed when the individuals were grouped according to their clinical status. A significant inverse correlation existed between skin reaction diameters and specific IgE levels in various subgroups within the population, such as the children with positive (≥5 mm) skin tests ($r = -0.36$; $P < 0.05$).

Applying the criterion previously described, 11.9% of the whole group were positive by RAST, and no significant difference was demonstrated between apparently healthy controls and patients (Table 2). A strong sex difference did, however, exist in that males were more frequently ($P < 0.02$) positive. In fact, 86.7% of the positive RAST values occurred in males ($P < 0.001$). There was also a weak bias ($P < 0.05$) toward children, but no difference in the period of evolution of the infection was observed. When the patients were considered, the mean DTH skin reaction size in those with a negative RAST was significantly greater than in those with a positive RAST (16.4 ± 1.9 versus 4.5 ± 1.7 mm; $P < 0.02$).

It is relevant to note here that none of the 23 sera from individuals from a nonendemic area, but infected with various intestinal helminths, had significant anti-*Leishmania* IgE levels (mean RAST level of 1.7 ± 0.4).

DISCUSSION

Individuals in a zone of ACL were tested for their DTH reactivity by the intracutaneous injection of a leishmanin preparation that provoked negligible reactions in individuals from a nonendemic area. When skin reaction diameters greater than a commonly accepted positive threshold (5 mm) were considered, almost all the patients responded, but so did a high proportion of the apparently healthy controls. When a diameter of 10 mm was taken as the threshold, significantly fewer controls reacted, although patients remained highly positive. The small DTH reactions are possibly due to either prior or cross-reactive infections, whereas larger responses (e.g., >15 mm) may result from the specific current infection. A clear relation was

found between age and skin test diameters (and percent positivity), and this might indicate the accumulation of past specific and cross-reactive immune responses with age, rather than differences in immune reactivity per se. If no selective bias influenced our study, this possibly also provides a partial explanation (6) for our observation of a significant preponderance of adults in the apparently healthy sector of the population examined. The dependence of intense DTH reactions on the infectious process was demonstrated by their particularly large diameters in patients with multiple lesions and their direct relation to the period of evolution of the infection. These results also revealed the existence of a significant sex-associated responsiveness. Thus, whereas the DTH reaction sizes were comparable between healthy males and females, the diameter in female patients was clearly greater than in infected males, although the frequency of positive reactivity was actually equal between the sexes.

Helminthic infections are clearly associated with striking elevations in the levels of total serum IgE (9, 23), but this is not the case for protozoa (5). In the group that we examined, the mean IgE level was very high (2,138 IU/ml), but this is almost certainly due to the frequent intestinal helminthic infections experienced by these individuals (9, 15, 23). It is of interest that in "normal" populations, males have significantly higher IgE levels than females (13, 18, 23), and in our study group this sex difference was maintained despite intense stimulation of synthesis by helminthic infection. Another observation in this respect is that, in contrast to a number of other studies (13, 18), we found a direct relation between age and total IgE levels, particularly in young adults. This unusual result might be explained by a cumulative effect of repetitive helminthic infections on the basal IgE levels, which therefore increase with age. Significantly lower levels of IgE were measured in patients with multiple lesions than in the apparently healthy controls. This observation might parallel that of low IgE levels in bovine trypanosomiasis (16) that are associated with elevated catabolic rates in this disease. There may, however, exist an alternative or complementary explanation for this phenomenon. Thus, elevated total IgE levels have been associated with deficiencies in T-cell function, possibly as a result of inadequate suppressive activity (3). We detected an inverse correlation between total IgE levels and DTH reaction diameters, the latter variable possibly providing a global indication of T-cell activity. It is perhaps for this reason that the highest DTH reactivity and lowest IgE levels coexisted, both in the same clinical group (multiple lesions) and in the same sex (females).

There have appeared in the literature some reports of the existence of anaphylactic-type reactivity against various protozoa (12, 20, 21, 24), including *Leishmania* (1, 2, 14). We found that a low proportion of the study population possessed detectable serum levels of IgE antibody against *Leishmania*. This antibody showed a strong direct correlation with total serum IgE levels, which might be due, as is also possibly the case in amoebiasis (20), to the nonspecific adjuvant effect (17, 23) of concurrent helminthiasis on IgE synthesis. By using appropriate background compensation, and RAST inhibition, we established that we were actually measuring low-level specific IgE antibody and not simply observing an artifact caused by the effect of high total IgE levels on the RAST procedure (7). Both children and males showed significantly higher levels of anti-*Leishmania* IgE antibody than adults or females, respectively. Indeed, 86.7% of the positive RAST reactions were found to occur in males, thus reinforcing the concept of the operation of a sex-linked influence in the synthesis of this antibody. As described for total IgE, an inverse correlation was also found between the specific antibody and DTH skin reactions, possibly reflecting a regulatory role of T cells.

It is perhaps somewhat surprising that anti-*Leishmania* IgE antibody occurred at a comparable frequency in the apparently healthy controls and in patients. This observation has, however, been made in other parasitic infections (8) and might be related to the existence of subclinical or incipient infections in the controls. It might also be postulated that the IgE antibody detected was in fact directed against cross-reactive (20) helminth antigens. This cannot, however, explain the totality of the response, as significant antibody levels were not detected in individuals who, although unexposed to the protozoan, were infected with a number of intestinal helminths.

In conclusion, therefore, we detected low titers of specific IgE antibody against *Leishmania* in a proportion of the study population, and these were apparently more dependent on the cellular immune reactivity than on the actual clinical status of the individuals.

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