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Circulating Immune Complexes Detected by Clq Solid Phase Assay in Leprosy[1]

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The presence of circulating immune complexes (CIC) has been described in leprosy both in the lepromatous (LL) and in the tuberculoid (TT) forms of the disease ([6]). CIC have been particularly implicated in the pathogenesis of some leprosy complications such as erythema nodosum leprosum and Lucio's phenomenon ([1, 2]).

In the lepromatous polar form the CIC levels appear to be very high in comparison to those found in the tuberculoid form ([1, 14]); however, not all authors found such a discrimination ([12]).

The aim of this paper is to study the presence of CIC by Clq solid phase assay (ClqSPA) in the sera of 63 leprosy patients and to correlate our results with the presence of autoantibodies which have already been examined in the same series of patients ([8]).

MATERIALS AND METHODS

Patients. Sixty-three Somalian patients from the Leprosy Hospital of Alexandra Island of Jilib, south of Mogadishu, were studied. All the patients were classified according to the clinical and histological criteria of the Ridley-Jopling classification ([11]). Only the polar TT forms (28 cases) and polar LL forms (35 cases) were included in the protocol. No patients presenting "immunologic complications" (leprosy reactions) were considered in this study.

The age of the patients ranged from 16 to 65 years with an average age of 38 years. The duration of the disease at the time of the study was from 2 to 10 years. All the patients were under treatment with dapsone (DDS) and/or rifampin; patients receiving corticosteroids were excluded from the protocol.

In addition, these patients and healthy native controls had been previously studied by one of the authors of this paper ([8]) for the presence of autoantibodies directed against thyroglobulin, thyroid-microsome, parietal cells, nuclei, smooth muscle, and mitochondria.

Fifty-five native and 97 healthy European subjects, age and sex-matched, were used as controls. All sera were stored at −70°C until tested.

CIC determination. CIC were checked by ClqSPA ([4]) with some modifications ([10]). Briefly, 50 μl of Clq, isolated according to Yonemasu and Stroud ([15]), in a phosphate-buffered saline (PBS) 0.15 M pH 7.2, at a concentration of 30 mg/l, was incubated in polyvinyl flexible culture plates (Linbro Flow Laboratories) for 3 days at 4°C. After three washes with cold PBS, the plates were filled with 0.01% gelatin solution in PBS and incubated at room temperature for 2 hr. After three more washes with cold PBS, the plates were used in the immune complex assay. Fifty μl of serum sample was incubated with 100 μl of disodium ethylenediamine-tetraacetate (EDTA) 0.2 M pH 7.5 for 30 min at 37°C to block any complement activation. We then added 350 μl of PBS, containing 0.05% Tween-20; 50 μl of this final solution was poured into the wells of the plate and incubated for 1 hr at 37°C and for 30 min at 4°C. Unbound proteins were then removed by washing three times with cold PBS. Immune complexes bound to the Clq-coated wells were detected by incubating each well with 25 μg of

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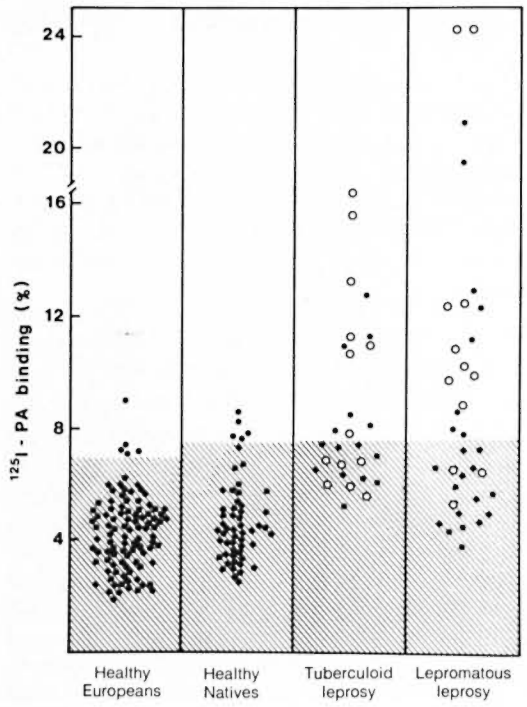

THE FIGURE. Circulating immune complexes in 28 tuberculoid and in 35 lepromatous leprosy patients compared to 55 healthy native and to 97 healthy European subjects. Shaded areas refer to the Mean ± 2 S.D. values for healthy Europeans and healthy natives. ○ = with one or more autoantibodies; ● = without autoantibodies.

Staphylococcus aureus protein A (Pharmacia Fine Chemicals) radiolabeled with ^{125}I by the chloramine T method ([5]). The plates were incubated for 2 hr at 4°C, and after three more washes with cold PBS they were dried with warm air and fixed with Hibitane Spray; the wells were cut and placed in single tubes and the radioactivity measured in a gamma-counter. As positive controls we used various dilutions of heat-aggregated gamma-globulins. The assay was performed in duplicate. The blank, which was less than 2% of the total radioactivity, was determined in wells similarly processed but filled with PBS-Tween instead of serum. In each plate at least ten fresh sera from healthy controls were used. Positivity was defined as two standard deviations (S.D.) above the mean of the controls. Results were expressed as percentage of the maximum binding, corresponding to that of a preparation of aggregated IgG (8 mg/ml). Statistical analysis was performed by the Student's *t* test.

TABLE 1. *Circulating immune complexes in 28 tuberculoid and in 35 lepromatous leprosy patients compared to controls.*

Subjects	No. of cases	No. (%) positive	Mean (±S.D.)
Healthy Europeans	97	5 (5.2)	4.19 (±1.34)
Healthy natives	55	5 (9.1)	4.69 (±1.46)[a]
Tuberculoid leprosy	28	13 (46.4)	8.64 (±3.00)[b]
Lepromatous leprosy	35	17 (48.6)	9.24 (±5.32)[b]

[a] $p < 0.05$, Student's *t* test, compared to healthy Europeans.
[b] $p < 0.001$, Student's *t* test, compared to healthy natives.

RESULTS

The CIC levels in normal European and native subjects and in LL and TT leprosy are reported in The Figure. The mean values of CIC levels in leprosy patients are very high in comparison with those of the healthy natives and even higher in comparison with those of the healthy Europeans (Table 1). The differences between the means of healthy natives and tuberculoid patients and of healthy natives and lepromatous patients are very significant ($p < 0.001$ in each instance). The difference between the means of healthy Somalians and healthy Europeans is also significant ($p < 0.05$).

In Table 2, mean CIC levels in leprosy patients are presented in relation to the presence or absence of autoantibodies in the sera. It can be seen that CIC levels are higher in subjects with autoantibodies; in LL patients the difference between the means of subjects positive and negative for

TABLE 2. *CIC levels (Mean ± S.D.) in lepromatous (LL) and tuberculoid (TT) patients with (±) and without (−) autoantibodies.*

Patients	No.	Mean (±S.D.)
LL (+)	12	11.76 (±6.26)[a]
LL (−)	23	7.93 (±4.35)
TT (+)	13	9.45 (±3.80)[b]
TT (−)	15	7.94 (±2.14)

[a] $p < 0.05$, Student's *t* test, compared to LL (−).
[b] Not significant, Student's *t* test, compared to TT (−).

the presence of autoantibodies is significant ($p < 0.05$), but the difference in TT patients is not statistically significant.

DISCUSSION

CIC in leprosy patients have been detected by many authors with different methods ([1,3,9,12]), but only a few tests ([6,14]) have been carried out to discriminate between the lepromatous and tuberculoid subgroups of the leprosy spectrum ([11]).

The differences between the tuberculoid and lepromatous polar forms of the Ridley-Jopling classification are not only histological and clinical but also and especially immunological. In fact, the TT form has been traditionally considered as a pathological condition characterized by a good cell-mediated immunity, a poor antibody response, and a small number of mycobacteria, as opposed to the lepromatous polar form in which the cell-mediated immunity is depressed and a large number of autoantibodies, CIC, and mycobacteria are present ([13]). In our cases, we have not been able to confirm these findings in the literature since there is no difference in the incidence of autoantibodies in the two polar forms of the disease ([8]). In addition, there is no difference in the percentage of cases positive for CIC in the two polar forms of the leprosy spectrum. On the other hand, we have found a higher incidence of CIC in those sera which contain autoantibodies.

Our results agree with the data from a WHO collaborative study. In this study ClqSPA was sensitive, precise, and accurate in the detection of various preparations of IgG aggregates and of CIC present in systemic lupus erythematosis (SLE) and in vasculitis but was unable to discriminate between the TT and LL polar forms of leprosy ([6]). The use of protein A instead of an antiserum against the Fc portion of IgG was also a cause of its failure to discriminate between the two polar forms. This may suggest that the CIC population(s) responsible for the higher levels found by some authors in the lepromatous form probably involve mycobacterial antigens and is (are) perhaps formed by either IgG_4 which does not bind complement or by a small quantity of IgM, which is poorly revealed by protein A but which would cause positive results in other tests such as those described in the WHO collaborative study ([6]).

In conclusion, our paper confirms a high incidence of CIC in leprosy; CIC levels are higher in patients with autoantibodies; finally, there is no difference between tuberculoid and lepromatous patients, perhaps because ClqSPA is unable to detect those CIC which involve mycobacterial antigens.

SUMMARY

Sixty-three sera of patients with leprosy were tested for the presence of circulating immune complexes (CIC) by Clq solid phase assay (ClqSPA).

The mean values of CIC levels in leprosy patients were very high in comparison to native and European controls. No difference was found in the tuberculoid and lepromatous forms, but there was a good correlation between the presence of CIC and autoantibodies.

RESUMEN

Se buscaron complejos inmunes circulantes (CIC) por un ensayo con Clq en fase sólida en 63 sueros de pacientes con lepra.

Los valores promedio de los niveles de CIC en los pacientes con lepra fueron muy altos en comparación con los valores de los controles nativos y europeos. No se encontraron diferencias entre las formas lepromatosa y tuberculoide pero hubo una buena correlación entre la presencia de CIC y la presencia de autoanticuerpos.

RÉSUMÉ

On a recherché la présence de complexes immuns circulants (CIC), par l'essai en phase solide Clq (ClqSPA) dans 63 échantillons de sérum recueillis chez des malades atteints de lèpre.

Les valeurs moyennes des taux de CIC chez des malades de la lèpre étaient fort élevées lorsqu'on les compare à des témoins autochtones ou européens. Aucune différence n'a été observée entre les formes tuberculoïde et lépromateuse, mais on a constaté une corrélation satisfaisante entre la présence de CIC et les auto-anticorps.

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