

SEROLOGICAL BEHAVIOUR OF LEPROMATOUS SERA IN RELATION TO COMPLEMENT FIXATION TESTS FOR SYPHILIS, CHAGAS' DISEASE AND BRUCELLOSIS*

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It is well known that the serological diagnosis of syphilis, among lepromatous lepers, can present difficulties due to cross-reactivity with lipoidal antigens, of anti-lipoidal antibodies present in leprous sera (Schmidt, 1961). The rate of non-specific reactions varies with the technic and with the antigen used (Almeida, 1962).

However in a study of 467 lepromatous lepers, Almeida, Lima & Carvalho (1955), found the quantitative complement fixation test of Wadsworth, Maltaner & Maltaner (1938), highly specific for syphilis, when cardiolipin was used as antigen. It was pointed out that the evaluation of the specificity of the reactions, in many cases, was plagued by uncertainties, since reliable information of clinical antecedents could not be obtained. In this study was found 6-per-cent of reactive sera in 467 lepers, a value that could be considered higher than that found among non-lepers (Almeida, Pedreira de Freitas & Brandão, 1954).

On the other hand, lepromatous sera could react with *Trypanosoma cruzi* antigen, in complement fixation tests. Pedreira de Freitas (1956) found two sera among 33 lepromatous sera, which gave positive complement fixation tests with *T. cruzi* antigen, suggesting non-specific reactions since, by clinical data, Chagas' disease could be excluded.

Information could not be found, in previous publications, on the reactivity of leprous sera with antigens prepared from *Brucella abortus* cultures, in complement fixation tests for the detection of brucellosis.

It seems desirable to test lepromatous sera by the "quantitative complement fixation technic" with antigens standardized for the serodiagnosis of syphilis, Chagas' disease and brucellosis, and verify the influence of the contents in leprous antibodies, titrated by complement fixation test, with antigen prepared from tubercle bacilli, on the rate of positive reactions.

MATERIAL AND METHODS

HUMAN SERA

Lepromatous lepers, from the leprosariums of Aymorés and Pirapitingui (São Paulo, Brazil) were bled for this study. Blood was kept at room temperature for two hours and the collected serum transferred to ampoules, numbered correspondingly to the accession number of the specimen. Volumes of 2.5 ml were found convenient for lyophilization in the Edwards Centrifugal freeze-dryer, model 30P.

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Final drying was achieved under high vacuum in the presence of phosphoric pentoxide for 48 hours. Empoules were sealed at reduced pressure and kept in the ice-box. At the same time the serum was processed, one aliquot part was taken apart for serological investigation.

When necessary, the dried serum was reconstituted by adding 2.5 ml of double distilled water and then filtered through a wet pad in a micro-Seitz filter. The reconstituted serum is kept iced and then, for inactivation, is heated at 56°C for 30 minutes, just before the test is to be performed.

COMPLEMENT-FIXATION TECHNIC

The reagents, as borate-buffered saline, anti-sheep amboceptor, complement, sheep erythrocytes were prepared according the directions given else where (Almeida, 1958).

The antigens used in this study were: *Cardiolipin 72*, prepared by Sylvana Chemical Co., New Jersey, and standardized by the method of Maltaner & Maltaner (1945); *Trypanosoma cruzi* antigen prepared and standardized by the method of Pedreira de Freitas & Almeida (1949); *Brucella abortus* antigen, prepared from the strain B-99-2252 according the technic described by Almeida (1961).

The antigen employed for titration of leprosy antibody was prepared from cultures of *Mycobacterium tuberculosis*, in Long's medium, by the method of Faure (1940). The antigen was then standardized by the technic of iso-hemolytic curves, as described by Almeida (1956, 1958).

The complement-fixation technic varied in some details, with the system. So, the preliminary incubation for leprosy, Chagas' disease and brucellosis, was carried out in water-bath, at 37°C, for 90 minutes, but for syphilis, it was in the ice-box, at 3°-6°C, for four hours.

The period of incubation at 37°C that is allowed for hemolysis was the same for all systems: 30 minutes in the water-bath.

The volume of reaction was adjusted to 1.0 ml, by adding 0.5 ml of cold salt solution, after the incubation for hemolysis, in order to have the necessary volume required for the use of EEL photo-colorimeter. Hemolysis is evaluated by the optical density found, when the filter 545 was used and the apparatus zeroed with distilled water.

The method for complement-fixation reactions and titrations was based on the isofixation method (Almeida, 1956) and described for the standardization of antigens employed in tests with leprosy sera (Almeida, 1958), and can be summarized as follows:

Complement was titrated daily against 5% suspension of sheep erythrocytes, maximally sensitized by anti-sheep amboceptor, and dilutions of complement to contain in 0.1 ml 6 units (50% hemolytic unit) were prepared and kept in ice. Complement for the quantitative complement fixation test should fulfill all the requirements of normal guinea-pig serum (Almeida, 1958).

Borate-saline (Wadsworth, 1947) was used as diluent for complement, antigen and amboceptor. Diluent containing magnesium salts should not be used, since the presence of magnesium enhances the hemolytic activity of complement, which is inhibited by human serum (Maltaner & Almeida, 1949).

The total volume of the reaction mixture is 0.5 ml of which 0.05 ml is serum (or dilution of serum), 0.1 ml the dilution of antigen, 0.1 ml complement dilution containing multiple 50% hemolytic units. Volume of 0.3 is completed with saline and 0.2 of maximally sensitized sheep cells are added after preliminary incubation.

The degree of hemolysis is recorded and the number of units of complement required for 50% hemolysis is calculated by the aid of conversion factors for each of the systems.

Specimens that fail to give a significant degree of reaction as shown by inhibition of hemolysis, were reported as negative.

All specimens giving 90-per-cent or less of hemolysis were submitted to the quantitative test, with serial dilutions of serum, and 3 and 6 units of complement. The amount of antigen used is the dose of maximal reactivity.

The serum titer is given in terms of the maximal reaction obtained with 0.05 ml of undiluted serum. Titers below 10 are determined with undiluted serum, but in order to have the endpoint titer of a highly reactive serum, smaller amounts of serum are tested. When two dilutions of complement are used, a linear relation can be drawn, between complement required for 50% hemolysis and the amounts of serum tested with optimum doses of antigen.

From this linear relationship the serum titer is computed as the slope of the line (Almeida, 1956, 1958) or as the amount of complement required for 50% hemolysis, when 0.05 ml of undiluted serum is used (Wadsworth, Maltaner & Maltaner, 1938).

The end-point titration were carried out with a single dose of antigen, in order to have the increment ratio of complement in relation to the serum, not dependent of the amount of antigen used. This technic is in accordance with the method described by Almeida (1956) for various complement-fixation systems.

RESULTS AND COMMENTS

Titration of sera from 2433 lepromatous lepers, with tubercle antigen, permitted their classification in four major groups. The non reacting sera or those with titers up to 20 belong to the first group. The second group includes titers from 21 to 200; a third group is for titers from 201 to 2000. The last one includes all sera of titer higher than 2000.

When the rate of reactions obtained with cardiolipin was computed in each group, it was apparent some cross-reactivity between leprous antibody levels and the incidence of syphilitic reactors, as it is shown in table I.

A similar observation could be done with the results obtained with *Trypanosoma antigen*. The rate of reactions for Chagas' disease increases as the level of leprous antibodies is higher, as it is shown in table II.

When these data were plotted, regression lines could be drawn (Fig. 1) showing the correlation between the level of leprous antibody and the serum cross-reactivity with cardiolipin and with *T. cruzi* antigen. The steep slope of the reactions with cardiolipin shows that the correlation can be missed when the total incidence of positive reactions for syphilis is computed in a group of lepromatous lepers and compared with that found among non-leprous population.

The distribution of reactors for syphilis and for Chagas' disease among the groups of lepromatous lepers was not casual, as it is shown by χ^2 test, no significant at the level of 0.05.

From the results obtained and presented in table III, it can be said that the incidence of "chagasic" among "syphilitic" was not significantly different of that observed in the whole group of 2433 lepers.

No significant effect of the leprous antibody on the reactivity for *Brucella abortus* antigen could be found; 11 sera reacted, 6 from the group of 1421 leprous sera whose titers were less than 200 and 5 in the remaining 1012 sera of titers higher than 200; these eleven sera did not react with cardiolipin. Reactions with *B. abortus* were not observed among the 109

lepromatous sera that reacted with cardiolipin. The findings confirm the previous observations of Almeida (1961) who detected 5 reactions with cardiolipin among 123 "brucellic sera"; this incidence could be in accordance with 815 reactions with cardiolipin among 18,826 blood-donors (Almeida, 1961).

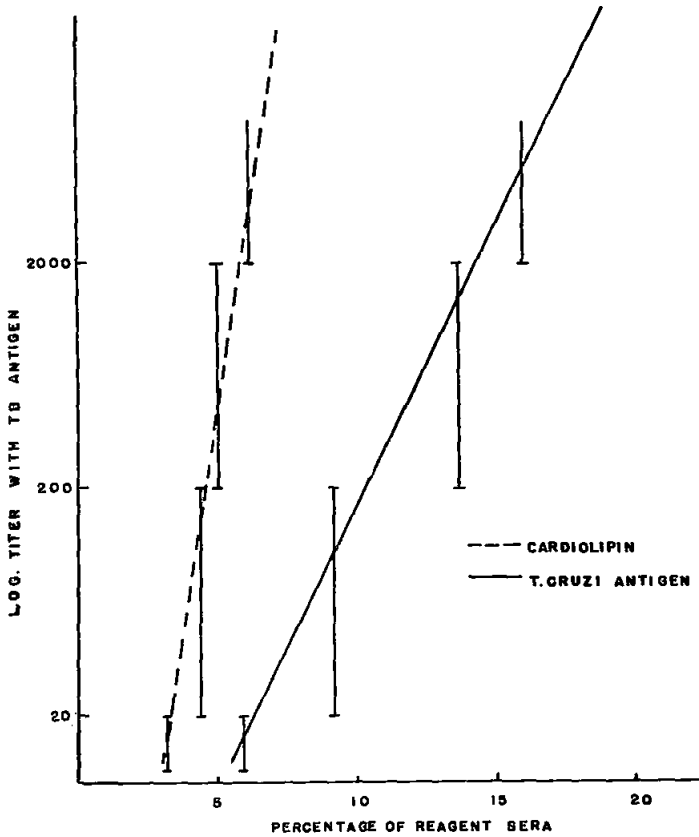
TABLE I — COMPLEMENT-FIXATION REACTIONS WITH CARDIOLIPIN AMONG 2,433 LEPROMATOUS LEPERS CLASSIFIED ACCORDING TO THE LEVELS OF LEPROUS ANTIBODIES.

Leprous antibody titer	Results with cardiolipin		Total
	Reactions	Non-reacting	
Up to 20	17 (3.34%)	492	509
21 — 200	39 (4.28%)	873	912
201 — 2000	30 (4.81%)	593	623
higher 2000	23 (5.91%)	366	389
	109 (4.48%)	2324	2433
$\chi^2 = 10.04$ (3)		P less than 0.02	

TABLE II — COMPLEMENT-FIXATION REACTIONS WITH *TRYPANOSOMA CRUZI* ANTIGEN, AMONG 2,433 LEPROMATOUS LEPERS OF DIFFERENT LEVELS OF LEPROUS ANTIBODY.

Leprous antibody titer	Results with <i>T. cruzi</i> antigen		Total
	Reactions	Non-reacting	
Up to 20	30 (5.89%)	479	509
21 — 200	89 (9.75%)	823	912
201 — 2000	84 (13.48%)	539	623
higher 2000	62 (15.93%)	327	389
	265 (10.89%)	2168	2433
$\chi^2 = 30.56$ (3)		P less than 0.001	

EFFECT OF LEPROMATOUS ANTIBODIES ON THE QUANTITATIVE C.F. TESTS WITH CARDIOLÍPIN AND T. CRUZI ANTIGEN IN 2433 SERA.



These data suggest that there is no relationship of brucella infection to biologic false poitive tests for syphilis, as pointed out by Carpenter, Miller, Boak & Heiskell (1961).

On the other hand, reactions with *B. abortus* were more often found among patients with positive complement fixation test with *T. cruzi* antigen. We do not have data to exclude a possible serological cross-reactivity, but professional activities and living conditions prevailing in farms may favor the infections by *T. cruzi* and by brucella, in those lepers coming from rural zones where the two infections are endemic. Table IV presents the results obtained by complement-fixation test for brucellosis and Chagas' disease in a total of 2433 lepromatous lepers.

TABLE III — COMPLEMENT-FIXATION REACTIONS WITH *T. CRUZI* ANTIGEN AND *CARDIOLIPIN* IN SERA OF 2433 LEPROMATOUS LEPERS.

C. F. reactions with cardiolipin antigen	C. F. reactions with <i>T. cruzi</i> antigen		Total
	Reaction	Non-reacting	
Reaction	12	97	109
Non-reacting	253	2071	2324
	265	2168	2433
$\chi^2 = 0.00161$ P higher than 0.99 (1)			

TABLE IV — COMPLEMENT-FIXATION REACTIONS WITH *T. CRUZI* ANTIGEN AND WITH *B. ABORTUS* ANTIGEN IN SERA OF 2433 LEPROMATOUS LEPERS.

C. F. reactions with <i>B. abortus</i> antigen	C. F. reactions with <i>T. cruzi</i> antigen		Total
	Reaction	Non-reacting	
Reaction	4	7	11
Non-reacting	261	2161	2422
	265	2168	2433
$\chi^2 = 7.38$ P less than 0.01 (1)			

Our data show that the amount of leprosy antibodies, determined by the quantitative complement-fixation method, have a significant influence on the increase of reactors with cardiolipin and with trypanosoma antigen. This cross-reactivity is more pronounced with *T. cruzi* antigen than with *cardiolipin*. Sera reacting with *B. abortus* antigen reacted more frequently with *T. cruzi* antigen, but the possibility of the presence of the two infections could not be excluded.

Cross-reactivity between brucellosis and syphilis, could not be found, as far as serological tests are concerned.

SUMMARY

Leprous antibodies were titrated in 2,433 lepromatous lepers and the sera were grouped according their titers and then tested with *cardiolipin*, *T. cruzi* antigen and *B. abortus* suspension. The quantitative complement-fixation method was employed for all serological investigations. Results were as follows: in 509 sera of titers less than 20, 17 (3.34%) reacted with *cardiolipin* and 30 (5.98%) with *T. cruzi* antigen; in 912 sera of the second group (titers from 21 to 200), 39 (4.28%) reacted for syphilis and 89 (9.75%) for Chagas' disease; in the third group of 623 sera (titers from 201 to 2000), 30 sera (4.81%) reacted with *cardiolipin* and 84 (13.48%) with *T. cruzi* antigen; in the last group of 389 sera (titers higher than 2000), 23 (5.91%) reacted with *cardiolipin* and 62 (15.93%) with *T. cruzi* antigen. It was observed a correlation between the level of anti-leprosy antibodies and the rate of positive complement fixation tests with *cardiolipin* and with *T. cruzi* antigen, but not between reactions for syphilis and for Chagas' disease. However 4 sera reacted with *B. abortus* in the group of 265 "chagasic sera"; this incidence was significantly higher than that observed among non reactors with *T. cruzi* antigen (7 reactions among 2,168 sera).

RESUMO

Anticorpos anti-lepra foram titulados por reação quantitativa de fixação de complemento, com antígeno lipopolissacarídeo de bacilo da tuberculose, em soros de 2433 doentes de lepra lepromatosa. Os soros, classificados de acôrdo com seus títulos, foram examinados por reação de fixação de complemento, para sífilis, moléstia de Chagas e brucelose, empregando-se respectivamente os antígenos de *cardiolipina*, antígeno benzeno-cloroformado de *Trypanosoma cruzi* e suspensão de *Brucella abortus*. Os resultados foram os seguintes: em 509 soros de títulos menores que 20, 17 (3,34%) reagiram com *cardiolipina* e 30 (5,98%) com antígeno de *T. cruzi*; em 912 soros de títulos entre 21 e 200, 39 (4,28%) reagiram para sífilis e 89 (9,75%) para moléstia de Chagas; entre os 623 soros de títulos entre 201 e 2000, 30 (4,81%) reagiram com *cardiolipina* e 84 (13,48%) com *T. cruzi*; no último grupo, de 389 soros, de títulos maiores que 2000, 23 (5,91%) reagiram com *cardiolipina* e 62 (15,93%) com antígeno de *T. cruzi*.

De acôrdo com êsses dados, há nítida influência do teor de anticorpos anti-lepra sôbre a incidência de reações de fixação de complemento positivas para sífilis e para moléstia de Chagas. Quanto às reações com *B. abortus* tal influência não foi observada, mas quatro soros reagiram com êsse antígeno entre 265 soros reagentes para moléstia de Chagas. Tal incidência era significativamente maior que a observada no grupo "não chagásico", onde foram encontradas apenas 7 reações positivas de fixação de complemento para brucelose, entre 2168 soros não reagentes com antígeno de *T. cruzi*. Embora não se possa excluir uma reatividade cruzada entre moléstia de Chagas e brucelose, é de se notar que os pacientes com tais reações positivas provinham de zona rural, onde as condições de vida favorecem o contágio de ambas as infecções.

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