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A Comparative Study of the Complementary Activity of Serum in the Polar Forms of Leprosy and in the Leprosy Reaction[1]

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The importance of immunologic processes in the outcome and evolution of infection caused by *Mycobacterium leprae* has led to much research. Nevertheless, the problem remains obscure to a large extent, in view of the difficulties inherent in this type of investigation. It is presumed that the defense mechanism is predominantly cellular, but the presence of antibodies in patients' serum, and the variations in complementary activity, as demonstrated in this paper, justify interest in a more nearly complete study of the serology of leprosy.

In determining the complement in sera from lepromatous patients, we frequently found some titers deviated excessively from the usual level, although the possibility of technical failure was totally excluded. As a matter of fact, Almeida (1), in determining the titers of antibodies of lepromatous patients' sera, had already warned us of inexplicable variations that he found in successive determinations at short intervals in the serum of the same patient. According to Eliasberg (4) lepromatous serum does not contain any free complement. According to Bonatti and Olmos Castro (3), on the other hand, free complement does exist, and is found at normal or slightly diminished levels in the serum of lepromatous patients. Since a conspicuous and frequent clinical occurrence in the L form of leprosy is the lepra reaction, the intensity of which has been attributed to fluctuation in the immunologic state, we consider it reasonable to repeat our studies, separating the lepromatous sera into two groups: viz., sera from patients in lepra reaction (erythema nodosum and multiform types), and sera from nonreacting patients.

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MATERIALS AND METHODS

Our material consisted of 88 serum samples, distributed as follows: Group L (lepromatous), 37 samples; Group T (tuberculoid), 33 samples; Group R (lepra reaction), 18 samples.

A vein was punctured to obtain the blood, which was then left to coagulate at room temperature; the serum was separated on the same day and kept in the refrigerator at between 4° and 6°C overnight.

The determinations of K (complement unit) and 1/n (angular inclination) were made by the technic of Maltaner and Maltaner and their colleagues (8), described by Almeida (2) who gives an extended bibliography. Much of the terminology for the calculations involved was established by von Krogh (6) in 1916. The average K and 1/n values of each group (lepromatous, tuberculoid and lepra reaction) were compared and analyzed statistically to determine the significance of differences observed.

RESULTS AND DISCUSSION

The K and 1/n values in each one of the 88 samples are presented in Table 1, on the bottom line of which the arithmetic average is stated.

It is observed that the arithmetic average of K in the L group ($\bar{x}_1 = 0.00350$) is only slightly lower than that of the T group ($\bar{x}_2 = 0.00361$), and, further, that both are noticeably lower than the average in group R ($\bar{x}_3 = 0.00740$). At the same time, the averages of the angular inclinations in groups T and L are practically identical (0.2573 and 0.2578), but they are much lower than the average value of 1/n in group R (0.4764).

The statistical analysis of these results aims to ascertain whether or not there is sig-

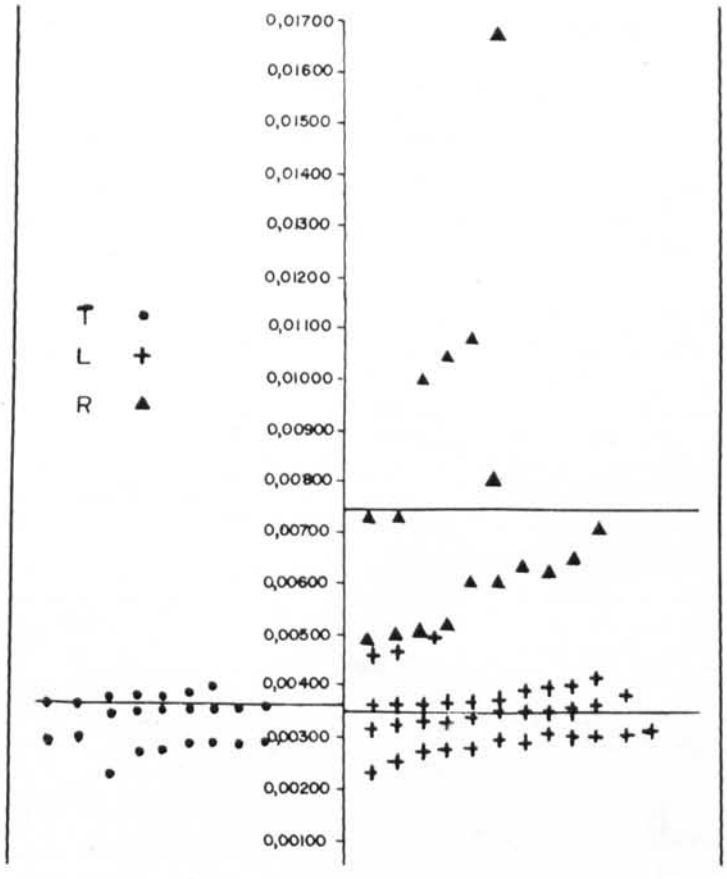

FIG. 1. Distribution of the K values in the three groups (dispersion graph). The horizontal lines are the media of K values in each group.

nificance in the differences between arithmetic averages found in the material of a reduced number of cases. This was done by applying the "t" test for small samples, according to the formula:

$$t = \frac{\bar{x}_1 - \bar{x}_2}{s} \cdot \sqrt{\frac{n_1 \cdot n_2}{n_1 + n_2}}$$

For the probability levels "P" for the different "t" values we used the values published by Fisher and Yates ([5]).

Thus, for example, by obtaining the "t" value for the K in Group L and in Group T, it was found that "t" = 0.73, which, for 68 degrees of liberty, corresponds to a "P" value between 0.40 and 0.50, a fact indicating that there is no significant difference between the value of the complement unit (K) in Group L and that in Group T, within the dimensions of the samples examined. At the same time, we found no significance in the difference between the average angular inclination value (1/n) in Group L and in Group T. These two groups are therefore similar insofar as complementary activity is concerned. The contrary occurred with Group R, whose arithmetic averages of K and 1/n proved in the statistical analysis to be significantly different from the average of the other group (Fig. 1). It can be stated, therefore, that the complement titers in serum from patients in lepra reaction are indeed lower than in the nonreacting polar forms. In one of our cases of lepra reaction it was quite impossible to obtain complementary activity even with 0.30 ml. of serum, a fact practically meaning absence of complement (Table 1).

It seems to us that this decrease in complementary activity is due to fixation of the complement by antigen-antibody complexes formed in the lepra reaction. As to the nature of the antigen in question, two hypotheses are to be considered.

TABLE 1. *Values for complement K and angular inclination 1/n in the sera of 37 lepromatous patients (L), 33 tuberculoid patients (T) and 38 patients with lepra reactions (R).*

K			1/n		
L	T	R*	L	T	R*
0.00340	0.00424	0.00730	0.30	0.35	0.24
0.00280	0.00368	0.00730	0.25	0.27	0.37
0.00276	0.00292	0.01664	0.26	0.21	0.40
0.00320	0.00350	0.00704	0.29	0.24	0.55
0.00300	0.00286	0.00800	0.22	0.25	0.60
0.00312	0.00336	0.00640	0.28	0.24	0.82
0.00360	0.00424	0.00600	0.35	0.25	0.49
0.00368	0.00368	0.01040	0.27	0.22	0.49
0.00376	0.00440	0.01000	0.25	0.19	0.63
0.00350	0.00356	0.00800	0.23	0.24	0.74
0.00468	0.00408	0.00600	0.22	0.23	0.34
0.00236	0.00432	0.00504	0.21	0.24	0.42
0.00270	0.00300	0.00520	0.22	0.17	0.40
0.00312	0.00416	0.00496	0.29	0.25	0.36
0.00320	0.00350	0.00624	0.19	0.19	0.46
0.00332	0.00368	0.00504	0.30	0.21	0.33
0.00336	0.00350	0.00624	0.22	0.42	0.46
0.00376	0.00268	....	0.20	0.46	...
0.00372	0.00500	....	0.27	0.22	...
0.00500	0.00220	....	0.21	0.26	...
0.00360	0.00284	....	0.35	0.23	...
0.00408	0.00408	....	0.22	0.23	...
0.00352	0.00356	....	0.31	0.22	...
0.00256	0.00464	....	0.26	0.28	...
0.00360	0.00266	....	0.35	0.34	...
0.00360	0.00348	....	0.28	0.30	...
0.00350	0.00372	....	0.24	0.21	...
0.00360	0.00500	....	0.25	0.27	...
0.00300	0.00350	....	0.23	0.25	...
0.00404	0.00356	....	0.23	0.31	...
0.00322	0.00284	....	0.25	0.24	...
0.00364	0.00384	....	0.18	0.26	...
0.00348	0.00280	....	0.25	0.26	...
0.00310	....	....	0.27	...	...
0.00464	....	....	0.28	...	...
0.00424	....	....	0.32	...	...
0.00400	....	....	0.22	...	...
0.00350	0.00361	0.00740	0.2578	0.2573	0.4764

* One serum had so little effect that a K could not be assigned (see text).

1. Bacillary antigen (hetero-antigen), resulting from the destruction of bacillary bodies, which would stimulate the formation of hetero-antibodies.

2. Cellular antigen (autoantigen), resulting from the destruction of lepromatous granulomata, which stimulates the formation of autoantibodies.

The first of these hypotheses seems to us less probable, because the destruction of bacilli, a frequent occurrence during the regressing stage of the illness, is not always accompanied by decrease in the complementary activity of the serum from lepromatous patients. This occurs only during the course of lepra reactions.

On the other hand, the presence of autoantigen/autoantibody complexes has already been admitted by Olmos Castro and Arcuri ([7]) to occur, usually among lepromatous

patients. Furthermore, according to these authors, the lepra reactions would appear as a consequence of unbalance in these complexes, with a predominance of autoantigen. It would seem to us, however, that, if this were so, the complementary activity should be lessened, even in the absence of the reaction. But this does not correspond to what we were able to observe. It seems more probable that the formation of these complexes occurs only during the reaction. During this stage, because of the patient's hypersensitivity to the liberated cellular materials (autoantigens), autoantibodies would be formed, with the constitution of complement-fixing complexes. We think also that the mechanism of hypersensitivity might be the explanation for an evidently individual factor in the lepra reaction.

SUMMARY

The authors studied complementary serum activity in 88 leprosy patients, divided into three groups: Group T, 33 tuberculoid cases; Group L, 37 lepromatous cases; and Group R, 18 cases in lepra reaction (erythema nodosum and multiform types). The titration technic was that of Maltaner and Maltaner as described by Almeida, and the designations used for component and angular inclination were those of von Krogh. The average of complement unit (K) and of angular inclination (1/n) were compared statistically, with the following results:

1. The average values of Groups T and L did not present significant differences.

2. The average values of Group R presented differences that were significant in relation to the other two groups studied.

3. The complementary activity in Group R was clearly decreased.

4. It is probable that this decreased complementary activity is related to the autoantigen/autoantibody complement-fixing complexes found in circulating blood. Their appearance may be explained by an immunologic phenomenon peculiar to lepromatous patients in a reactional stage.

RESUMEN

Los autores estudiaron la actividad del complemento en suero de 88 pacientes leprosos, divididos en tres grupos: Grupo T, 33 casos tuberculoides; Grupo L, 37 casos lepromatosos; y Grupo R, 18 casos de lepra en reacción (tipos eritema nudoso y multiforme). La técnica de titración fué la de Maltaner y Maltaner descripta por Almeida, y las designaciones usadas para las inclinaciones componentes y angulares fueron las de von Krogh. Los términos medios de la unidad de complemento (K) y de la inclinación angular (1/n) fueron comparadas estadísticamente, con los siguientes resultados:

1. Los valores promedios de los grupos T y L no presentaron diferencias significativas.

2. Los valores promedios del Grupo R presentaron diferencias que fueron significativas en relación a los otros dos grupos estudiados.

3. La actividad complementaria en el grupo R estaba claramente disminuida.

4. Es probable que esta disminución de la actividad complementaria, esté relacionada a los complejos auto-antigeno/anticuerpo complemento-fijador encontrados en la sangre circulante. Su aparición puede ser explicada por un fenómeno inmunológico peculiar a los pacientes lepromatosos en el estadio reaccional.

RÉSUMÉ

Les auteurs ont étudié l'activité complémentaire du sérum chez 88 malades de lèpre, divisés en trois groupes: un groupe T, avec 33 cas tuberculoïdes; un groupe L, avec 37 lépromateux, et un groupe R, comprenant 18 cas de réaction lépreuse (érythème noueux et types multiformes). La technique de titration était celle de Maltaner et Maltaner telle qu'elle a été décrite par Almeida, et les désignations utilisées pour le constituant et pour l'inclinaison angulaire étaient celles de von Krogh. Les moyennes obtenues pour l'unité de complément (K) et pour l'inclinaison angulaire (1/n) ont été comparées statistiquement, ce qui a fourni les résultats suivants:

1. Les valeurs moyennes n'ont pas présenté de différences significatives entre les groupes T et L.

2. La moyenne du groupe R a présenté des differences qui étaient significatives par rapport celles des deux autres groupes étudiés.

3. L'activité du complément était nettement abaissée dans le groupe R.

4. Il est probable que cette activité abaissée du complément est en relation avec les complexes de fixation du complément autoantigène/auto-anticorps trouvés dans le sang circulant. Leur apparition peut être expliquée par un phénomène immunologique propre aux malades lépromateux en état de réaction.

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