Complement and the Second Component of Complement in Leprosy[1]

E. W. Saitz, R. E. Dierks and C. C. Shepard[2]

In lepromatous leprosy the antigen of *Mycobacterium leprae* and antibodies to at least some of its components are apparently present in the body simultaneously. The antigen is characteristically present as large numbers of bacilli, which are located in well vascularized tissue. Antibody can be demonstrated with the Middlebrook-Dubos hemagglutination test, which involves carbohydrate antigen of the tubercle bacillus, and with complement fixation tests, which involve an undefined mixture of antigens of tubercle bacilli (reviewed in (12)). The role of serologic reactions in producing the manifestations of leprosy is not yet clear. Many have suggested that erythema nodosum leprosum (ENL) is the result of serologic reactions (14).

Evidence that antigen-antibody reactions may be occurring in the patient can be gained from the amount of complement (C') present in the sera. Thus in serum sickness, acute nephritis, and systemic lupus erythematosis, diseases in which antigen-antibody reactions presumably occur *in vivo*, C' titers are depressed (reviewed in (3, 6)). However, C' titers are raised in a number of acute inflammatory conditions, such as acute infectious diseases, rheumatic fever, rheumatoid arthritis, and myocardial infarction (6, 11). In lepromatous leprosy C' has been reported to be absent (5), normal or slightly diminished (4), and more recently to be normal in patients without reaction but depressed in patients with reaction (2). The disparity in results may have arisen because the technics used to measure C' were not adequate, especially as regards concentration of Mg^{++} and Ca^{++} or perhaps because the sera were not handled properly to preserve complement. A more recent abstract (13) mentions that C' was found elevated in lepromatous disease with ENL and amyloidosis.

In the detection of antigen-antibody reactions *in vivo* the measurement of the second component of complement (C'2) is potentially more sensitive than the measurement of C' (1). Antigen-antibody aggregates (AgAb) react with whole C' to form AgAbC' 1, 4, 2. This complex may then decay back to AgAbC' 1, 4 in which state it may again react with more C' 2; this cyclic reaction can lead to selective reduction of C' 2 titers. During renal homograft rejection in man there may be a small reduction in C', but a pronounced reduction in C' 2, presumably reflecting an *in vivo* complement fixation (7).

The relationship between C' H_{50} and the titers of individual components has been discussed by Mayer (9). Usually the supply of C'2 is the determining factor in C'H_{50} titers, but when C'2 is depressed C'H_{50} titers reflect the concentration of C'3 and other factors also.

MATERIALS AND METHODS

Sera. These were obtained from leprosy patients at the U.S. Public Health Service Hospital, Carville, Louisiana, and from healthy persons at the National Communicable Disease Center. As soon as clotting began, the blood was centrifuged for 10-15 minutes, and the serum withdrawn and immediately frozen at -70°C or on dry ice. Later all sera were rapidly thawed and redistributed in 0.5 ml. portions and then refrozen and held at -70°C. They were not thawed again until just before the tests.

C' determinations. The LBCF procedure

[1] Received for publication 13 May 1968.

[2] E. W. Saitz, M.D., R. E. Dierks, D.V.M., M.P.H., Ph.D., C. C. Shepard, M.S., M.D., National Communicable Disease Center, Bureau of Disease Prevention and Environmental Control, Public Health Service, U.S. Department of Health, Education and Welfare, Atlanta, Georgia 30333. *Present address:* E. W. Saitz, Children's Hospital of Pittsburgh, Pittsburgh, Pennsylvania 15213; R. E. Dierks, Veterinary Medical Research Institute, Iowa State University, Ames, Iowa 50010.

(8) for $C'H_{50}$ was employed, with volumes totaling 3.0 ml. so that the amount of hemolysis could be read in the Beckman DU spectrophotometer (541 mμ). The cells were sensitized at 37°C for 30 minutes, and incubation with complement was at 37°C for 60 minutes. In this procedure, a slight modification of that described by Mayer (10), the diluent contains Ca^{++}, Mg^{++}, and gelatin, and the 50% hemolytic titer is estimated from the Van Krogh plot of the corrected spectrophotometer readings.

C'2 determinations. These were performed by the two-step procedure described by Austen and Beer (1), except that all volumes were one-half those specified. An aliquot of one control serum was included in each test. Since its titers did not vary more than ±10% from the mean, the determined titers for the test sera are reported without correction.

RESULTS

The results are given in Figure 1 and Table 1. The results for the sera from normal persons agree with those reported in the literature (11). There were four lepromatous patients who never had ENL, and their values fell in the same range as the normals. There were 17 lepromatous patients who had ENL at the time of bleeding and all of these had distinctly elevated titers. There were four lepromatous patients who did not have ENL at the time of bleeding but did at other times, One developed it eight months later, another six months later, and one had had ENL two months previously and developed it again three months later. The fourth was apparently free of ENL at the time of bleeding, but had "a little" ENL nine days earlier and developed a more serious crop two days later. However, this patient was receiving vigorous treatment for ENL, including ACTH (40 units every other day) and azathioprine (50 mgm. three times a day), and had recently received prednisolone and indomethacin, as well as desipramine, phenazocine, glutethimide, and aspirin, and some of these drugs may have affected the complement titers. All of these four patients had normal values.

Three of the four borderline patients had elevated $C'H_{50}$ and C'2 levels. The values for the single tuberculoid patient were normal.

In some of the sera from patients with ENL there was possibly a slight reduction of C'2 titers in relation to $C'H_{50}$ titers. However, the reduction was so small that it could not be considered significant.

DISCUSSION

Lepromatous patients without ENL had normal $C'H_{50}$ and C'2 levels. In clear distinction, nearly all lepromatous patients with ENL had elevated levels of $C'H_{50}$ and C'2. Three of four patients with borderline leprosy had similarly raised values.

TABLE 1. *Complement levels in leprosy patients and in normal persons.*

Subjects	$C'H_{50}$			C'2		
	Av.	Range	Number	Av.	Range	Number
Normal	52.1	41–61	7[a]	598	471–686	5
Lepromatous without ENL	47.0	43–49	4	536	510–571	4
Lepromatous with ENL, but not at time of bleeding	52.5	49–55	4	483	425–588	4
Lepromatous with ENL at time of bleeding	73.7	59–90	17[b]	852	715–1020	16
Borderline	65.5	58–72	4	798	658–893	4
Tuberculoid	44		1	446		1

[a] Includes 2 patients for whom C'2 values were not available.
[b] Includes 1 patient for whom C'2 values were not available.

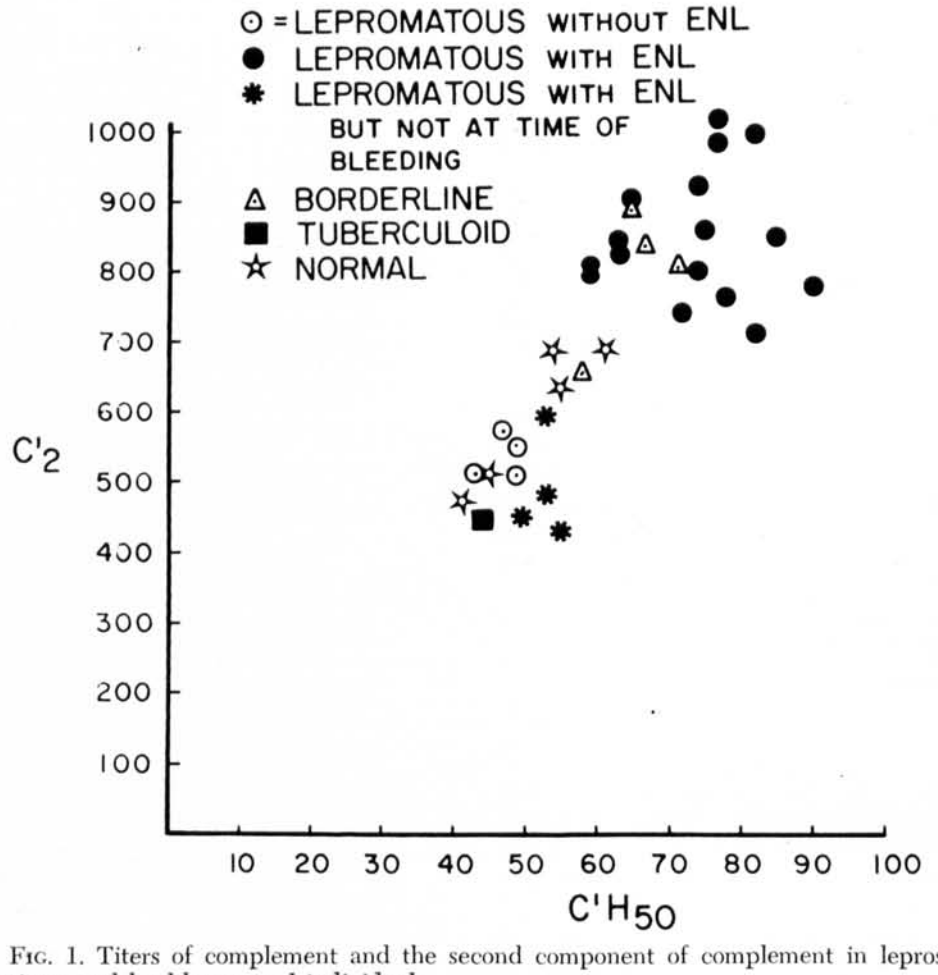

FIG. 1. Titers of complement and the second component of complement in leprosy patients and healthy normal individuals.

These elevations are characteristic of the "acute phase" reaction seen in a variety of infectious and noninfectious conditions in which there is distinct tissue reaction. Our results show that this increase in whole complement titers is accompanied by an increase in C'2. A distinct depression in C'2, such as that described during graft rejection ([7]), was not observed, even in lepromatous patients with heavy loads of bacilli present in their tissues. In the patients with elevated complement titers, there remains a possibility that antigen-antibody reactions were occurring *in vivo*, but any accompanying complement fixation was more than compensated for by the increased amounts of C'2, which were perhaps even triggered by tissue damage resulting from the immunologic reaction. However, our results by their nature offer no evidence on this possibility.

The disagreement of our results with most of the older results may be ascribed to the inadequacy of the earlier technic. Our measurements of $C'H_{50}$ agree with those of Sheagren *et al* ([13]), who probably used a very similar technic for measuring $C'H_{50}$ titers. Our measurements of $C'H_{50}$ titers were supported by the measurements of C'2. As mentioned, the latter measurement may be a more sensitive method for observing a depression due to reactions *in vivo* between antigen and antibody.

SUMMARY

Titers of whole complement ($C'H_{50}$) and the second component of complement (C'2) were measured in the sera of

healthy, normal persons and patients with leprosy.

In normals the $C'H_{50}$ titers ranged from 41-61 units and the C'2 titers from 449-686 units, in agreement with values reported in the literature. In lepromatous patients without ENL the titers were in the normal range. In lepromatous patients with ENL the titers were distinctly elevated (59-90 $C'H_{50}$ units and 715-1,020 C'2 units.). Lepromatous patients who were between attacks of ENL at the time of bleeding had normal titers. Three of four patients with borderline leprosy had elevated titers. One tuberculoid patient had normal values.

Elevations, such as those seen in lepromatous patients with ENL and in most borderline patients, are characteristic of a number of other diseases marked by acute tissue reaction. Distinct depressions, such as those observed in conditions presumably associated with antigen-antibody reaction *in vivo*, were not seen in leprosy patients.

RESUMEN

Títulos de complemento total ($C'H_{50}$) y del segundo componente del complemento (C'2) fueron medidos en el suero de personas sanas y normales y en enfermos con lepra.

En personas normales los títulos del $C'H_{50}$ variaron de 41-61 unidades y títulos de C'2 fueron de 449-686 unidades, en acuerdo con los valores informados en la literatura. En enfermos lepromatosos sin ENL los títulos tuvieron un margen normal. En enfermos lepromatosos con ENL los valores estuvieron diferentemente elevados (59-90 $C'H_{50}$ unidades y 715-1,020 C'2 unidades). Enfermos lepromatosos que estaban en medio de ataques con ENL al tiempo de tomar muestras de sangre, tuvieron títulos normales. Tres de cuatro pacientes con lepra borderline tuvieron títulos elevados. Un enfermo tuberculoide tuvo valores normales.

Alzas, tales como aquellas observadas en enfermos lepromatosos con ENL y en la mayoría de los enfermos borderline, son características de un número de otras enfermedades que se identifican por reacciones agudas de los tejidos. Diferentes descensos como acquellas que se observan en condiciones presumiblemente asociades con la reacción antígeno-anticuerpo *in vivo*, no se observaron en enfermos de lepra.

RÉSUMÉ

Dans le sérum de personnes normales et en bonne santé, et de malades atteints de lépre, on a mesuré les titres du complément entier ($C'H_{50}$) et du deuxiéme constituant du complément (C'2).

Chez les sujets normaux, les titres de $C'H_{50}$ ont varié de 41 à 61 unités, et les titres de C'2 de 449 à 686 unités, ce qui est en accord avec les valeurs rapportées dans la littérature. Chez les malades lépromateux sans ENL, les titres étaient situés dan les valeurs normales. Chez les malades lépromateux souffrant d'ENL, les titres étaient trés nettement élevés (de 59 à 90 unités pour $C'H_{50}$ et de 715 à 1,020 unités pour C'2). Les malades lépromateux, soumis aux prélèvements de sang entre deux attaques d'ENL, présentaient des titres normaux. Trois des quatre malades atteints de lépre borderline témoignaient de titres élevés. Un malade tuberculoïde présentait des valeurs normales.

De telles élévations, de l'ordre de celles observées chez les malades lépromateux souffrant d'ENL et chez les plupart des malades atteints de lépre border-line, sont caractéristiques de plusieurs autres maladies au cours desquelles une réaction tissulaire aigüe est notée. On n'a pas observé chez les malades de la lépre des diminutions nettes, telles que celles qui sont observées dans des conditions que l'on suppose associées à des réactions antigènes-anticorps *in vivo*.

REFERENCES

1. Austen, K. F. and Beer, F. The measurement of second component of human complement ($C'2^{hu}$) by its interaction with $EAC'1a^{gp},4^{gp}$ cells. J. Immunol. **92** (1964) 946-957.
2. de Azevedo, M. P. and de Melo, P. H. A comparative study of the complementary activity of serum in the polar forms of leprosy and in leprosy reaction. Internat. J. Leprosy **34** (1966) 34-38.
3. Boltax, A. J. and Fischel, E. E. Serologic tests for inflammation. Serum complement, C-reactive protein and erythrocyte sedimentation rate in myocardial infarction. American J. Med. **20** (1956) 418-427.
4. Bonatti, A. A. and Olmos Castro, N. Dosaje del complemento en sueros leprosos. Rev. Argentina Dermatosif. **29** (1945) 301-302.
5. Eliasberg, J. Über das Fehlen freien Komplementes im Blute Leпröser. Deutsche Med. Wchnschr. **37** (1911) 302-304.

6. Gewurz, H., Pickering, R. J., Muschel, L. H., Mergenhagen, S. E. and Good, R. A. Complement-dependent biological functions in complement deficiency in man. Lancet **2** (1966) 356-360.
7. Guiney, E. J. Austen, K. F. and Russell, P. S. Measurement of serum complement during homograft rejection in man and rat. Proc. Soc. Exper Biol. & Med. **115** (1964) 1113-1117.
8. [Laboratory Branch Task Force.] Standardized diagnostic complement fixation method and adaption to micro test. Public Health Monograph No. 74, 1965.
9. Mayer, M. M. On the destruction of erythrocytes and other cells by antibody and complement. Cancer Res. **21** (1961) 1262-1269.
10. Mayer, M. M. Complement and Complement Fixation. *In* Experimental Immunochemistry. Kabat, E. A. and Mayer, M. M., Eds. Charles C. Thomas, Springfield, Illinois, 2nd Ed., 1961, pp. 133-240.
11. Rapp, H. G. and Borsos, T. Complement research. Fundamental and applied. J. American Med. Assoc. **198** (1966) 1347-1354.
12. Ross, H. The blood in leprosy: Morphology, chemistry, immunology. A rereview. Leprosy Briefs **6** (1955) 21-23; 26-47.
13. Sheagren, J. N., Block, J. B., Trautman, J. R. and Wolff, S. M. Immunologic reactivity in leprosy. Clin. Res. **15** (1967) 300.
14. Skinsnes, O. K. Immunology in leprosy. II. The immunological spectrum of leprosy. *In* Leprosy in Theory and Practice. Cochrane, R. G., and Davey, T. F., Eds. Bristol, John Wright & Sons, Ltd.; Baltimore, Williams and Wilkins Co., 2nd ed., 1964, pp. 156-182.