# Serum Complement Profile in Human Leprosy and its Comparison with Immune Complex Diseases[1]

Kunal Saha and Asit K. Chakraborty[2]

Patients with leprosy suffer from a number of immunologic abnormalities. Borderline patients develop reversal reactions initiated by cell-mediated immune mechanisms to *M. leprae* ([11]). Also lepromatous patients having a high bacillary load suffer from *erythema nodosum leprosum* (ENL) lesions in which deposits of complement and immunoglobulins have been detected ([33]). The ENL reactions, which are very similar to the Arthus phenomenon, are thought to result from the sudden release of the bacilli from macrophages into the tissue of patients with high antibody titers ([11, 32]). Activation of the complement cascade brought about by the immune complexes ([9]) thus formed, should obviously be an important feature of the disease. However, evidence to show that ENL reactions are the phlogistic effects of complement activation are far from clear ([18]). Several authors have studied the role of serum complement in different immunologic reactions; its involvement in allergic reactions leading to tissue damage is well known ([5, 14]). The main aim of the present communication is to report the complement profile in patients suffering from leprosy and to delineate the pathway of complement activation in this disease.

## MATERIALS AND METHODS

**Human materials.** Sixty-six biopsy-proven leprosy patients from the Leprosy Home, Delhi, formed the basis of the present study. Skin biopsies were examined histopathologically ([22]) and borderline cases were grouped with the respective polar forms. Borderline borderline (BB) forms were not included in the study. These patients were on usual dapsone therapy and were not receiving steroids or thalidomide. Twenty normal adults belonging to the lower socio-economic strata were included in the control group. Twelve patients with acute glomerulonephritis, seven cases of lupus nephritis, and six patients suffering from biopsy-proved membranoproliferative glomerulonephritis were taken as another type of control.

**Collection of sera.** Blood samples were collected from all patients as well as controls. Sera were separated and stored at -20°C. Fresh samples of sera were used for the estimation of total hemolytic complement. The sera drawn from leprosy patients were adequately protected from heat prior to assay for total hemolytic complement. All complement components were quantitated in the stored samples which were aged under identical conditions.

**Immunologic technics.** Total hemolytic complement (CH50) was estimated in fresh serum samples according to the method described by Campbell *et al* ([6]). The hemolysin was obtained commercially from the Central Research Institute, Kasuali, India. The components Clq, C3, C4, C5, C8, C9 and Cl-inactivator were quantitated in the stored serum samples by the single radial immunodiffusion method ([17]), using monospecific human antisera and reference standards. Anti-Clq antiserum was obtained from Behring Institute, West Germany; anti-C3, anti-C4 and anti-C5 antisera were supplied by Meloy Laboratories, U.S.A.; and anti-C8 and anti-C9 antisera (IgG fraction) were obtained from Cordis Corporation, U.S.A. The levels of Clq, Cl-inactivator, C8 and C9 in the patients and normal controls were compared with a WHO reference standard serum 67/97 and were expressed in units per 100 ml, taking the WHO standard as 100 units per 100 ml. The levels of serum C3 and C4 were expressed in mg per 100 ml. In the patients with nephritis only CH50 and early components were estimated.

**Identification of cryoglobulins.** In a separate study, sera collected from 16 leprosy pa-

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[2] K. Saha, M.Sc., M.B.B.S., Ph.D., F.A.A.A., Associate Professor, Department of Bacteriology, Govind Ballabh Pant Hospital, New Delhi 110001; and A. K. Chakraborty, M.Sc., Ph.D., Lecturer, Department of Biochemistry, University College of Medical Sciences, New Delhi 11016. Correspondence should be directed to Dr. Saha.

tients, including 14 cases with lepromatous leprosy and 2 cases with tuberculoid leprosy, were studied for the composition of their cryoglobulins. The cryoprecipitates, obtained by keeping 10 ml serum samples at 4°C for ten days, were washed thoroughly with ice cold buffer saline till they were free from any protein, as monitored by a spectrophotometer. These precipitates were then dissolved in a minimum volume of barbitone buffer and subjected to analysis by immunoelectrophoresis and immunodiffusion in agarose gel against several monospecific antisera, e.g., anti-human IgG, IgA, IgM, IgD, C3, fibrinogen, factor VIII, alpha-2-macroglobulin and ceruloplasmin. These antisera were obtained from Meloy Laboratories, U.S.A. and Nordic Laboratory, the Netherlands.

**Study of alternate pathway of complement activation.** In another experiment, the levels of factor B (C3 proactivator) and its breakdown product (Ba) were quantitated in the sera of 18 lepromatous patients and 10 normal subjects by single radial immunodiffusion using anti-human-C3-activator antiserum (Behring) ([19]). The serum factor B levels in the patients and the normal subjects were compared with a WHO reference standard serum 67/97 and they were expressed in units per 100 ml, taking the WHO standard as 100 units per 100 ml.

For quantitation of serum Ba fragment level, similar procedure was used ([19]). In brief, 0.2 ml serum was mixed with 0.2 ml 36% polyethylene glycol 6000 (BDH), the mixture was incubated at 4°C for three hours and then it was centrifuged. The concentration of Ba fragment in the supernatant was compared with that in a similar supernatant obtained from a WHO reference standard serum 67/97, using immunodiffusion plates containing anti-human-C3-activator antiserum (Behring).

## RESULTS

**Patient materials.** Skin biopsies of 39 patients with lepromatous leprosy, including 9 females, showed borderline lepromatous leprosy (BL) in six instances. Among them, 14 patients were suffering from ENL during the time of serum sample collection. Similar histologic examination of skin biopsies of 27 patients with tuberculoid leprosy, including 7 females, showed borderline tuberculoid leprosy (BT) in only 6 patients, and polar tuberculoid leprosy in the remaining subjects.

In the control group of patients with nephritis, twelve subjects including seven females had acute glomerulonephritis (AGN). Their ages varied from 8 to 25 years. Among them, nine had edema, two anasarca, seven oliguria, ten hematuria, eleven hypertension, and upper respiratory tract infection was seen in six cases. Nine patients showed high blood urea levels ( $>$ 40 mg/100 ml), and seven patients had raised antistreptolysin 0 titers ( $>$ 500 Todd units per 100 ml). The latter finding indicated streptococcal infection in these seven cases. Only two patients had heavy albuminuria (3.5 gm daily), and in the remaining ten patients the daily albumin excretion varied from 2.6 gm to 3.5 gm. Histologic examination of renal biopsies was possible only in eight cases; the results were consistent with exudative proliferative glomerulonephritis. The duration of illness was short and ranged from 2 to 18 days.

All seven patients with lupus nephritis (LN) were females; their ages ranged from 10 to 40 years. The onset of their clinical symptoms was gradual. Four had edema, one had anasarca and two had oliguria; another two had hematuria and five had hypertension. The daily excretion of albumin was moderate and varied from 1.6 gm to 3.6 gm. Only two had blood urea above 40 mg per 100 ml. LE cells were seen in all seven cases. Renal biopsy showed focal and diffuse proliferative lesions but the wireloop pattern was not found in any of the cases.

Six cases of membrano-proliferative glomerulonephritis (MPGN), based on clinical presentation and assisted by biopsy findings, were also included in our control group of patients with nephritis. All had edema and albuminuria, which varied from 2.6 gm to 13.1 gm in 24 hours. Their serum albumins ranged from 1.8 gm to 4.2 gm per 100 ml. Blood urea levels varied from 16 mg to 282 mg per 100 ml, and the range of serum cholesterol was from 118 mg to 560 mg per 100 ml.

**Serum complement levels.** Total hemolytic complement was estimated in the sera collected from 20 normal adults, and from 10 patients with lepromatous leprosy (LL) including 7 cases with ENL and 3 cases without ENL, and from 10 patients with tuberculoid leprosy. The results are shown in Table 1. In comparison to the normal control group, the mean CH50 was found to be reduced in

those patients having lepromatous leprosy who were suffering from ENL and also in the patients having tuberculoid leprosy. Table 2 illustrates the early complement component profile in 15 normal adults, in 25 patients with lepromatous leprosy including 10 cases with ENL, and in 20 cases with the tuberculoid form of the disease. The lepromatous group consisted of 21 polar and 4 borderline lepromatous (BL) forms, while the tuberculoid group consisted of 14 polar and 6 borderline tuberculoid (BT) types. The mean Clq level remained unaltered in the patients with tuberculoid leprosy as compared to normal controls, and the fall of the mean Clq level in the sera of the patients with lepromatous leprosy with or without ENL was not statistically significant. The level of C4 did not show any alteration in either form of illness. However, there was a significant change in the levels of C3 in the sera of the patients with both forms of leprosy. It is emphasized that all of our estimations of serum C3 levels were performed on aged sera. There was no significant alteration in the early complement component levels in the patients with

TABLE 1. *Total hemolytic serum complement levels in normal adults and leprosy patients.*

| Groups | No. of subjects | CH50[a] mean ± S.D. (range) | Statistical evaluation |
|---|---|---|---|
| 1. Normal adults | 20 | 80 ± 26 (44 - 138) | — |
| 2. LL with or without ENL | 10 | 60 ± 29 (28 - 31) | $p > 0.05$ (NS) |
| 3. Tuberculoid leprosy (TT) | 10 | 55 ± 19 (21 - 79) | $p < 0.05$ (S) |

[a] Mean serum CH50 was decreased in both groups of leprosy patients, but the reduction was statistically significant only in the tuberculoid leprosy patients. CH50 level is expressed in units per ml serum.

TABLE 2. *Early and middle complement component profile in the sera of normal adults and leprosy patients.*

| Groups | No. of subjects | Clq | C3 | C4 | C5 |
|---|---|---|---|---|---|
| | | mean ± S.D. (range) | | | |
| 1. Normal adults | 15 | 137 ± 48 (75 - 215) | 211 ± 38 (101 - 300) | 31 ± 10 (20 - 57) | 11.2 ± 5.4 (8 - 25) |
| 2. a) LL without ENL | 15 | 112 ± 45 (65 - 180) | 159 ± 35 (60 - 235) | 29 ± 16 (9 - 56) | 12 ± 3 (6 - 22) |
| | | $p > 0.1$ (NS) | $p < 0.001$ (S) | $p > 0.4$ (NS) | $p > 0.6$ (NS) |
| b) LL with ENL | 10 | 133 ± 38 (102 - 187) | 155 ± 38 (115 - 248) | 33 ± 12 (12 - 51) | 12 ± 1.6 (11 - 15) |
| | | $p > 0.8$ (NS) | $p < 0.001$ (S) | $p > 0.6$ (NS) | $p > 0.5$ (NS) |
| 3. TT | 20 | 145 ± 34 (80 - 192) | 141 ± 24 (80 - 230) | 32 ± 16 (12 - 91) | 14 ± 4 (8 - 24) |
| | | $p > 0.4$ (NS) | $p < 0.001$ (S) | $p > 0.9$ (NS) | $p > 0.2$ (NS) |

Among the early serum complement components, only the mean C3 level was significantly reduced in both types of illness. However, there was no significant change in serum Clq, C4 and C5 levels. ENL had no significant effect on the early complement components. The Clq level is expressed as units percentage in comparison with a WHO reference standard serum 67/97. The C3, C4 and C5 levels are expressed in mg per 100 ml serum.

ENL. Their mean serum levels of Clq, C3, C4, and C5 were 133 units per 100 ml, 155 mg per 100 ml, 33 mg per 100 ml, and 12 mg per 100 ml, respectively. Table 3 depicts the levels of C8 and C9 in the sera of normal controls and leprosy patients. The mean C8 level was reduced in the patients with lepromatous leprosy although this change was not statistically significant; however, a significant decrease was observed in the tuberculoid sera. The mean C9 levels were decreased in both forms of the illness, however, the change was only found to be statistically significant in patients with tuberculoid leprosy. The mean Cl-inactivator levels remained unchanged in the sera obtained from patients with both types of leprosy (Table 4). Figure 1 shows no significant correlation between CH50 and C3 levels in 20 leprosy patients ($r = 0.21$).

It is interesting to compare the complementograms of the patients with leprosy with those of the patients with nephritis (Tables 1, 2, 5). The reduction in CH50 was more spectacular in the latter groups of patients, especially in patients with AGN and LN. Moreover, the levels of all the early complement components including Clq, C3 and C4 were much more reduced in the patients with AGN and LN than in the patients with both

TABLE 3. *Distal complement component profile in the sera of normal adults and leprosy patients.*

| Groups | No. of subjects | C8 mean ± S.D. (range) | C9 mean ± S.D. (range) |
|---|---|---|---|
| 1. Normal adults | 6 | 142 ± 28 (112 - 182) | 163 ± 54 (95 - 217) |
| 2. Lepromatous leprosy (LL) | 10 | 106 ± 41 (35 - 238) | 113 ± 57 (38 - 250) |
| Statistical evaluation *vs* Group 1 | | $p > 0.05$ (NS) | $p > 0.05$ (NS) |
| 3. Tuberculoid leprosy (TT) | 6 | 89 ± 57 (0 - 180) | 83 ± 67 (25 - 197) |
| Statistical evaluation *vs* Group 1 | | $p < 0.05$ (S) | $p < 0.05$ (S) |

TABLE 4. *Cl-inactivator levels in the sera of normal adults and leprosy patients.*

| Groups | No. of subjects | Cl-inactivator[a] mean ± S.D. (range) | Statistical evaluation |
|---|---|---|---|
| 1. Normal | 12 | 114 ± 19 (85 - 135) | |
| 2. Lepromatous leprosy (LL) | 14 | 119 ± 25 (79 - 165) | $p > 0.05$ (NS) |
| 3. Lepromatous leprosy (LL) with ENL | 7 | 125 ± 30 (68 - 150) | $p > 0.05$ (NS) |
| 4. Tuberculoid leprosy (TT) | 12 | 108 ± 12 (52 - 135) | $p > 0.05$ (NS) |

[a] No change in the level of serum Cl-inactivator was observed in either group of leprosy patients. The Cl-inactivator level is expressed as units percentage in comparison to a WHO reference standard serum 67/97.

forms of leprosy. On the contrary, only C3 and CH50 were reduced in the patients with MPGN. Figure 2 depicts the distribution of C4 and Clq in the patients with various diseases who had reduced C3 levels. When C3 levels of the patients were below the lower limit of the control group they were considered to be reduced.

**Composition of cryoglobulin.** Table 6 depicts the composition of cryoglobulins present in the sera of 16 leprosy patients. IgG was the most frequently detected (10 of 16 samples) immunoglobulin in these cryoprecipitates. Two samples from lepromatous cases with ENL showed mixed types of cryoglobulin, one from a similar patient showed IgG as well as C3, while the composition of three samples remained undetermined. It is interesting to point out that the cryoprecipitates from three serum samples contained fibrinogen.

**Study of alternate pathway of complement activation.** Table 8 shows that the levels of the serum factor B in the lepromatous cases have not decreased significantly. However, the levels of its breakdown products (Ba) have increased remarkably in them, specifically in the ENL patients, which indicates activation of C3-PA-convertase or GBase (factor D).

## DISCUSSION

Since our leprosy patients belonged to the lower socio-economic strata, we selected for our control group age and sex-matched normal adults from a similar class of society. The study of serum complement in both sexes in the normal group showed no significant difference ($p > 0.05$). The mean CH50 of ten normal males was 89 units per ml, with a range from 66 to 120 units per ml; the mean value for ten normal females was 70 units per ml with a range from 44 to 138 units per ml. Further, a separate study (unpublished) of the complement profile in the same number of males and females from a high socio-economic group (medical students, doctors and their wives) showed that differences between the early complement components (e.g., Clq, C3, C4, C5) in the two groups were also insignificant. Thus, the mean serum Clq, C3 and C4 levels in ten males were 130 units per 100 ml, 102 mg per 100 ml, and 25.8 mg per 100 ml, respectively; and in ten females were 144 units per 100 ml, 114 mg per 100 ml, and 23 mg per 100 ml, respectively. A similar study with the terminal complement components, e.g., C8 and C9, in both sexes was not possible due to insufficient supply of these monospecific antisera. The *in vivo* fixation of complement is most commonly heralded by a depression of the total hemolytic complement activity of the serum. Our present study of serum complement profiles in leprosy patients revealed that the mean CH50 was reduced in patients having lepromatous leprosy with ENL. A significant decrease was also observed in the patients with tuberculoid leprosy. On the other hand, no such reduction of CH50 was noticed in the lepromatous cases without ENL (Table 1). Depression of CH50 in leprosy patients has also been recorded by other authors ([20, 23]). Among the early complement components, the C3 level was significantly reduced in both lepromatous and tuberculoid forms of leprosy. Similar depres-

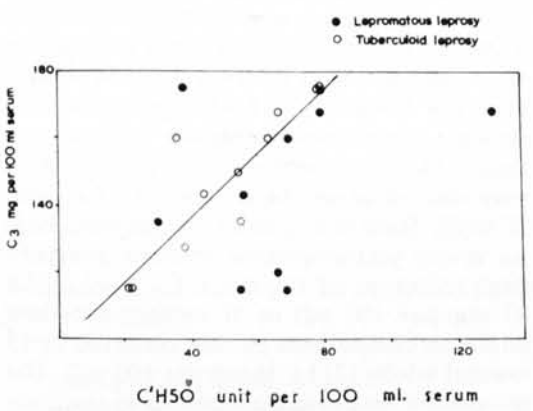


FIG. 1. Correlation of CH50 and C3 in leprosy.

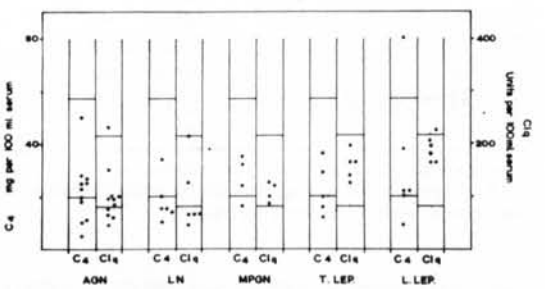


FIG. 2. C4 and Clq levels in patients with various diseases who had low C3 levels: AGN = acute glomerulonephritis; LN = lupus nephritis; MPGN = membrano-proliferative glomerulonephritis; L. Lep. = lepromatous leprosy; T. Lep. = tuberculoid leprosy. The horizontal lines denote the upper and lower limits of complement components in normal subjects.

TABLE 5. *Early complement component profile in the sera of patients with nephritis.*

| Groups | No. of subjects | CH50 Units per ml serum mean (range) | Clq Units per 100 ml serum mean (range) | C3 Mg per 100 ml serum mean (range) | C4 Mg per 100 ml serum mean (range) |
|---|---|---|---|---|---|
| 1. Normal | 15 | 80 (44 - 138) | 137 (75 - 215) | 211 (101 - 300) | 31 (20 - 57) |
| 2. Acute glomerulonephritis (AGN) | 12 | 24 (10 - 58) | 109 (44 - 230) | 28 (5 - 72) | 22 (5 - 50) |
| 3. Lupus nephritis (LN) | 7 | 29 (0 - 76) | 103 (44 - 212) | 41 (0 - 102) | 18 (10 - 34) |
| 4. Membrano-proliferative glomerulonephritis (MPGN) | 6 | 44 (0 - 100) | 137 (88 - 200) | 72 (0 - 185) | 35 (18 - 56) |

Total hemolytic complement decreased in the patients belonging to all three types of nephritis. Among the early complement components, the mean C3 level was also decreased in all three forms of patients, but the mean Clq and C4 levels were reduced only in acute glomerulonephritis and lupus nephritis and not in membrano-proliferative gomerulonephritis.

TABLE 6. *Composition of cryoglobulins in the sera of leprosy patients.*

| Serum no./ leprosy type | | ENL | Immunoglobulins and other plasma proteins[a] |
|---|---|---|---|
| 1 | Lepromatous | + | IgG, C3 |
| 2 | " | + | Unclassified |
| 3 | " | + | IgA |
| 4 | " | + | IgG |
| 5 | " | + | IgG, IgM |
| 6 | " | + | IgG, IgA, IgM |
| 7 | " | + | IgG |
| 8 | " | + | IgG |
| 9 | " | — | Unclassified |
| 10 | " | — | Fibrinogen |
| 11 | " | — | Unclassified |
| 12 | " | — | IgG, fibrinogen |
| 13 | " | — | IgG, fibrinogen |
| 14 | " | — | IgG |
| 15 | " | — | Fibrinogen, ceruloplasmin |
| 16 | Tuberculoid | — | Unclassified |
| 17 | " | — | IgG |

[a] Detected in the cryoglobulins by immunodiffusion and immunoelectrophoretic technics.

sion of serum C3 levels in leprosy patients has been reported earlier from this laboratory ([25]). There was no significant difference between the C3 levels in patients with or without ENL. The nutritional status of an individual may affect the total hemolytic complement level ([8]); however, the patients in the present series do not show any evidence of such nutritional deficiency because the serum albumin levels of the patients have been found to be within normal limits ([7]).

The normal variation of C3 levels is multifactorial. In a separate study from this laboratory (unpublished), we found that the mean C3 level in 14 normal adults from the lower socio-economic strata was 188 ± 26 mg per 100 ml, and in 20 normal adults belonging to the higher socio-economic class (medical students, doctors and their wives) it was only 108 ± 25 mg per 100 ml ([25]). This difference is significant and might be due to associated parasitic and helminthic diseases as well as chronic inflammatory processes ([27]), which are common among the subjects belonging to the lower socio-economic group. Both leprosy patients and the normal controls belong to the low socio-economic strata. Thus, the high C3 levels in our control group supports the above concept and the significant low C3 levels in both types of leprosy indicate hypocomplementemia in these patients. The nutritional status of an individual may also influence the C3 level ([8]). An earlier study from this laboratory (unpublished) on severe undernutrition revealed a significant reduction of the mean C3 level (153 ± 43 mg per 100 ml) in 31 undernourished adults in comparison to that observed in 15 normal adults (211 ± 38 mg per 100 ml). The geographic location may also have some influence on the serum C3 level as observed by

Dutta *et al* ([10]). They recorded significantly high C3 levels in the normal subjects of Ladakh, situated at an altitude of 1,200 feet in the Himalayan region. However, a study of complement profile in the normal population of the Indian subcontinent is still lacking in the literature. This is a serious lacuna for the study of complement in various diseases.

The presence of immune complexes in leprosy patients has been shown by some authors. Cryoglobulins present in the sera of these patients have been suggested to be immune complexes ([4, 24]). However, our data (Table 6) has shown the presence of C3 along

TABLE 7. *Serum levels of factor B (GBG) and Ba, its breakdown product (GAG) in normal subjects and lepromatous patients.*

| Groups | No. of subjects | Factor B[a] mean ± S.D. (range) | Ba[b] mean ± S.D. (range) |
|---|---|---|---|
| 1. Normal | 10 | 136 ± 37 (68 - 175) | 73 ± 48 (25 - 160) |
| 2. Lepromatous leprosy (LL) | 15 | 126 ± 25 (72 - 166) | 128 ± 33 (85 - 162) |
| 3. Lepromatous leprosy with ENL | 3 | 120 (102 - 135) | 220 (162 - 280) |
| Statistical evaluation | 2 vs 1 | $p > 0.25$ (NS) | $p < 0.05$ (S) |

[a, b] Serum factor B and Ba have been expressed as percentage of a WHO reference serum 67/97, which has been taken as 100 units per 100 ml.

TABLE 8. *Mechanism in serum of complement activation in glomerulonephritis and leprosy.*

| Groups | Total no. patients | No. showing reduced CH50[a] | No. showing reduced C3[a] | No. showing reduced Clq[b] | No. showing reduced C4[b] | No. showing both reduced Clq and C4[b] | Mechanism of complement activation |
|---|---|---|---|---|---|---|---|
| 1. Post streptococcal acute glomerulonephritis (AGN) | 12 | 7 (58%) | 11 (92%) | 4 (36%) | 4 (36%) | 4 (36%) | Classical as well as alternate pathway |
| 2. Lupus nephritis (LN) | 7 | 4 (67%) | 6 (86%) | 4 (67%) | 4 (67%) | 4 (67%) | Classical pathway |
| 3. Membranoproliferative nephritis (MPGN) | 6 | 3 (50%) | 4 (67%) | 0 | 1 (16%) | 0 | Only alternate pathway |
| 4. Tuberculoid leprosy | 35 | 4 (40%)[c] | 17 (48%) | 1 (6%) | 8 (48%) | 1 (6%) | Mainly alternate pathway |
| 5. Lepromatous leprosy | 39 | 4 (40%)[c] | 12 (30%) | 1 (2.5%) | 1 (2.5%) | 1 (2.5%) | Mainly alternate pathway |

[a] Percentage of total number of patients.
[b] Percentage of number of patients with reduced serum C3 levels.
[c] Serum CH50 was estimated in only ten patients.

with other immunoglobulins in only 1 of 17 samples, while IgG was present in 10 samples. Cryofibrinogen was also detected in three patients. Thus, it appears that the cryoprecipitates present in lepromatous sera probably represent another type of immunoglobulin aggregate.

Since the complement activation following the classical pathway is associated with the formation of immune complexes, Clq and C4 would have been consumed in it. It is probable that C3 proactivator (C3-PA) activated by the aggregates of immunoglobulins ([29]) present in the sera of leprosy patients (Table 6) lead to the inflammatory reaction at the C3 level, thus providing an alternate pathway for the reactivity of the complement system in leprosy. Our studies on C3PA during activation of the bypass (C3-PA-convertase or GBGase) have confirmed the functioning of the alternate pathway (Table 7), because although the levels of serum factor B have not decreased significantly in the lepromatous cases, the levels of its breakdown products (Ba) have increased remarkably in them, specially in ENL cases. Parallel findings have recently been reported by Bjorvatn *et al* ([3]), who recorded no decrease of serum factor B level in lepromatous patients and had emphasized the importance of the study of the levels of breakdown products of the proteins of the complement system ([3]). Also no depression of Clq and C4 levels in the sera of the leprosy patients (Table 2) and definite lowering of these two components in AGN and LN (Table 5) lend additional support to this assumption.

Recently Laham and his co-workers ([16]) demonstrated that stimulated lymphocytes produced an activating factor which activates Cl and subsequently together with its natural substrates, C4 and C2, modulated lymphocyte response. It is possible that this feedback control loop between cellular immunity and complement system might be lacking in leprosy due to the deficiency of lymphocytic functions ([11]).

The physiologic role of complement components from C5 to C9 remains unanswered, although clearly there must be a role to account for the evolutionary persistence of these complex factors ([31]). Thus, the unchanged serum C5 levels in the leprosy groups (Table 2) are not in accordance with the complement consumption either by direct or by alternate pathways. However, the unexpected high serum C5 levels in leprosy patients, who showed significantly low serum C3 levels, might be due to the "acute phase" of inflammatory processes in the lepromatous process ([15]).

There was a significant reduction of C8 and C9 levels only in patients with tuberculoid leprosy (Table 3); however, these levels remained unchanged in the lepromatous patients (Table 3). Petchclai *et al* ([18]) recorded the levels of C8 and C9 in both groups of leprosy patients and observed no distinct change in comparison with the control group. The significantly low level of serum C8 levels in only patients with tuberculoid leprosy (Table 3) may be due to the cytolysis of macrophages containing *M. leprae* by lymphocytes, which involves direct activation of C8 by the lymphocytes on the surface of the target cells ([31]). Evidently, such processes might not have taken place in the lepromatous patients due to the nonfunctional lymphocytes. The levels of Cl-inactivation at the Cl level in leprosy patients appears to be normal ([12]). Recently, Bach-Mortensen *et al* ([2]) reported a rise of the Cl-inactivator level in carcinoma, lymphomas, leukemia and certain viral diseases which reflected activation of the disease. Thus, our data showed that there was no rise of Cl-inactivator level due to the active lepromatous process (Table 4).

Among 25 patients with lepromatous leprosy, 10 individuals who had been suffering from moderate to severe forms of ENL were selected for the study of early complement components. No significant difference in the levels of Clq, C3 and C4 was observed between the patients with or without ENL, albeit the mean CH50 (52 units per ml) was lower in the former. However, the reduction in CH50 did not appear to be statistically significant in the ENL cases. Other reports in the literature also stress that the distribution of serum complement levels among ENL patients is the same as in those without reactions ([20, 26]).

Thus, complement activation was not a significant feature in our reactional cases of lepromatous leprosy. This might be due to the subacute or chronic phase of ENL in our patients at the time of collection of serum samples. It is possible that an acute attack of ENL might lower the complement profile only transiently. Saitz *et al* ([26]) found no distinct depression of CH50 and C2 levels in the sera of their leprosy patients with ENL in

comparison to those without reaction.

Our study of early complement component profiles in the patients with glomerulonephritis and its comparison with that in patients having leprosy is not only interesting but also throws light on the mechanism of complement activation in leprosy as well as in glomerulonephritis. Table 8 shows that of 12 patients with AGN, low C3 levels were observed in 11 cases (92%). Furthermore, of these 11 cases with a low C3 level, Clq and C4 levels were reduced in only four patients (36%). Similarly, of seven patients with LN, six cases (86%) had reduced C3 levels. Of these six patients with depressed C3 levels, four cases (67%) showed both low Clq and C4 levels (Fig. 2), which suggests that these four patients had been suffering from active disease during the time of sample collection ([28]). In contrast, of six patients with MPGN, four (67%) had low C3 levels of which only one had a low C4 level; none showed any change in the Clq level (Fig. 2). Thus, these findings definitely show that hypocomplementemia in AGN and LN was accompanied by a fall in the early reacting components; while in MPGN the early complement components were normal, meaning thereby a predominant involvement of the classical pathway in the former and the alternate pathway in the latter ([21, 34]). However, in three cases of AGN, hypocomplementemia was not associated with low Clq and C4 levels (Table 8), which again indicates that the alternate pathway might play the role in complement activation in some patients with AGN ([21]). Further analysis of the data shows that the reduction of serum C3 was found in only 17 cases (48%) of 35 patients with tuberculoid leprosy; of these 17 patients with low C3 activity, the C4 level was reduced in 8, and the Clq level was found to be depressed in one (Table 8). Thus the classical pathway of complement reactivity might have occurred in only one case. Similarly, of 39 patients with lepromatous leprosy, a low C3 level was observed in only 12 cases (30%); of these, only one case with low C3 levels showed both low Clq and C4 levels. Hence the classical pathway of complement activation did not occur in most of them and perhaps it is not the usual mechanism for complement consumption in leprosy as it is in lupus nephritis. A recent parallel study in leprosy patients from Ethiopia also showed a significant reduction of the C3 component with a normal C4 level, which pointed towards an involvement of the "alternate pathway" ([30]).

A clear understanding of complement activation in leprosy is yet to be achieved although there are several reports describing the complement component levels in these patients ([20, 26]). C3 is an acute phase reactant which is consumed in the process of activation. Thus, the observed C3 level in the leprosy patients appeared to be the result of these parameters, leading to changes in other complement component levels. Reduced synthesis of C3 due to hepatic lesions associated with leprosy may be another possibility ([1]). But, it can be ruled out here since, in a separate study on liver function tests in 33 leprosy patients, the present authors found that the mean prothrombin time (13.5 seconds), the mean serum cholesterol level (144 mg per 100 ml), SGOT (19 IU per liter), and SGPT (8 IU per liter) were all within the normal limits (unpublished). In a recent report highlighting the characteristics of the complement system in the presence of immune complexes in sarcoidosis, the authors have pointed out widespread disturbances in the system although the process of complement activation was not found to follow any definite pathway, classical or alternate ([13]).

## SUMMARY

In the present study we have estimated the serum levels of early, middle, and distal complement components, e.g., Clq, C3, C4, C5, C8, and C9 along with Cl-inactivator and CH50 in patients with tuberculoid and lepromatous leprosy and have compared these results with the levels in healthy subjects as well as with levels in patients with other immune complex diseases. We have also analyzed the cryoglobulins present in the sera of these patients; they consisted of either a single or mixed IgG, IgA, IgM or fibrinogen in most instances. The component C3 was found in only one sample. It appears that unlike lupus nephritis, in which complement is activated by direct path in about 30% to 50% of leprosy patients, significant C3 complement consumption takes place primarily via the alternate pathway and is probably initiated by the aggregated immunoglobulins represented in cryoprecipitates. This is further supported by the study of serum factor B and its breakdown product (Ba) in these patients. The question of the role of the mid-

dle and distal complement components, such as C5, C8 and C9, during total hemolytic complement and C3 consumption in leprosy remains unanswered.


## RESUMEN

Se determinaron los niveles séricos de Clq, C3, C4, C5, C8, C9, inactivador de Cl, y del DH50, en pacientes con lepra lepromatosa y tuberculoide. Los valores obtenidos se compararon con los obtenidos en individuos sanos y en pacientes con otras enfermedades por complejos inmunes. También se determinó la presencia de crioglobulinas en los sueros de los pacientes. Estas crioglobulinas estuvieron constituídas por las inmunoglobulinas IgG, IgA e IgM, solas o mezcladas, o por fibrinógeno en la mayoría de los casos. Sólo en una muestra se encontró el componente C3 como constituyente del crioprecipitado. A diferencia de lo que sucede en la nefritis lúpica, donde el complemento se activa por la via clásica, parece ser que en el 30 ó 50% de los pacientes con lepra, donde se observa un consumo importante de C3, el complemento se activa principalmente por la via alterna, probablemente por las inmunoglobulinas agregadas presentes en los crioprecipitados. El estudio del factor B y de su producto de rompimiento (Ba) en el suero de estos pacientes, apoya la activación del complemento por la via alterna. Queda por establecerse el papel que juegan los componentes intermedios y finales del complemento (e.g. C5, C8 y C9) durante el consumo de C3 y del complemento hemolítico total.

## RÉSUMÉ

Dans cette étude, on a procédé à une estimation des taux sériques des constituants précoce, moyens, et lointains du complément, c'est à dire Clq, C3, C4, C5, C8, et C9, ainsi que du CH50 et de l'inactivateur du C1 chez les malades atteints de lèpre tuberculoïde ou lépromateuse. Ces résultats ont été comparés avec ceux obtenus chez des sujets sains, de même qu'avec les taux observés chez les malades souffrant d'autres affections à complexes immuns. On a également analysé les cryoglobulines présentes dans le sérum de ces malades. Ces globulines consistaient, soit à l'état simple, soit mélangé, d'IgG, d'Iga, IgM ou de fibrinogènes, dans la plupart des cas. Le constituant de ces trois n'a été trouvé que dans un seul échantillon. Chez environ 30 à 50% des malades de la lèpre, une utilisation significative du complément C3 prend place par le truchement de la voie de substitution. Ceci est différent de ce que l'on observe dans la néphrite associée au lupus, au cours de laquelle le complément est activé par voie directe. Chez les malades de la lèpre, cette utilisation est probablement mise en route par l'accumulation des immunoglobulines qui sont présentes dans les cryoprecipitats. Cette hypothèse est d'ailleurs renforcée par les résultats obtenus lors de l'étude du facteur sérique B et de ses produits de dégradation (Ba) chez ces malades. La question du rôle des constituants moyens et lointains du complément, tels que C5, C8, et C9, autour de l'utilisation du complément hémolytique complet et du C3, chez les malades de la lèpre, reste cependant sans réponse.


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