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# Immune complexes and complement hypercatabolism in patients with leprosy*

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SUMMARY

The occurrence of immune complexes in the serum and the level of the C3 breakdown product C3d in the plasma from patients with leprosy were studied by quantitative methods and the results were compared in various forms of the disease. These studies were performed on sixty-two samples from twenty-six patients. The serum $^{125}$I-C1q binding activity was found to be increased by more than 2 s.d., as compared to the normal values, in most of the sera from patients with erythema nodosum leprosum (ENL) (80%) and uncomplicated lepromatous leprosy (82%), but also in the sera from patients with tuberculoid leprosy (58%). *In vitro* studies suggested that immune complexes involving mycobacterial antigens were present in leprosy sera. An increased C3d level ($>$2 s.d.) was also found in most of the plasma from patients with ENL (70%), but rarely in the plasma from patients with uncomplicated lepromatous leprosy (18%) and never in tuberculoid leprosy patients' plasma. The absence of a significant correlation between the $^{125}$I-C1q binding activity and the C3d level in leprosy patients may suggest that extravascular immune complexes are involved in the complement activation occurring in ENL. The quantitation of C3d in plasma may be of some practical interest in the early diagnosis of ENL complications of leprosy.

## INTRODUCTION

The various forms of leprosy represent a wide, continuous spectrum of clinical manifestations (Ridley & Jopling, 1966). Recent studies indicated that cellular and humoral immune reactions against *Mycobacterium leprae* may be involved in the development of some of the pathological manifestations (Godal, 1974). The formation of immune complexes may be expected in lepromatous leprosy from the concomitant occurrence of large amounts of mycobacteria and of corresponding antibodies. Indeed, there is some evidence of an involvement of immune complexes in the pathogenesis of erythema nodosum leprosum (ENL), mainly based on clinical and tissue studies. Certain disease manifestations of ENL, such as albuminuria or skin lesions, are similar to those encountered in serum sickness, or in the experimental Arthus reaction (Waters & Ridley, 1963). Deposits of immunoglobulins and C3, and sometimes mycobacterial antigens have been demonstrated by immunofluorescence in such ENL lesions (Wemambu *et al.*, 1969). The presence of immune complexes in circulating blood has also been suspected and serological studies have revealed the frequent occurrence in leprosy sera of substances which precipitate with C1q in agarose (Moran *et al.*, 1972; Rojas-Espinosa, Mendez-Navarrete & Estrada-Parra, 1972; Gelber *et al.*, 1974). However, the complement level has been consistently found to be normal or elevated (Saitz, Dierks & Shepard, 1968; Wemambu *et al.*, 1969; Malaviya *et al.*, 1972; Petchclai *et al.*, 1973; Gelber *et al.*, 1974).

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The purpose of the present work was to apply to the study of leprosy quantitative methods for the detection of immune complexes in serum and for the demonstration of complement activation products in plasma. The serological results were compared in the various forms of the disease and the correlation with the clinical activity of the disease in ENL was investigated. A radioimmunological method, the [$^{125}$I]C1q binding test, was used for the quantitation of immune complexes (Nydegger *et al.*, 1974; Zubler *et al.*, 1976a). Complement activation was studied by measuring the C3d levels with an immunochemical method (Perrin, Lambert & Miescher, 1975). It is known that activation of C3 results in a fragmentation of the native molecule (mol. wt 200,000) into C3b (mol. wt 190,000) and C3a (mol. wt 10,000). C3b is then cleaved by the C3b inactivator into C3c (mol. wt 150,000) and C3d (mol. wt 35,000). By measuring the C3 level by usual methods, one cannot recognize if an increased synthetic rate is masking an increased catabolism of the molecule. By selective quantitation of the small C3d fragment, a hypercatabolism of C3 may be demonstrated, independent of the C3 synthesis rate.

## MATERIALS AND METHODS

*Patients studied.* Thirty-nine serum samples from thirteen patients with ENL (two serum samples or more per patient, obtained at intervals of 1 week or more); eleven sera from seven patients with uncomplicated lepromatous leprosy and twelve sera from six patients with tuberculoid leprosy were studied. The control groups included ten patients with active pulmonary tuberculosis and five clinically healthy individuals from Ethiopia. In addition, samples from thirty blood donors from Geneva were studied. Leprosy and tuberculosis patients were diagnosed and treated at the Addis Ababa Leprosy Hospital and Tuberculosis Center respectively. The age distribution of the different groups was similar, with means ranging from 23–27 years. All leprosy patients were under treatment with DDS or clofazamine. For the treatment of ENL, most patients received in addition a combination of two or more of the following drugs: stibophen, chloroquin, acetylsalicylic acid, chlorpromazine and prednisolone.

*Collection and storage of serum and plasma samples.* For collection of serum, blood was allowed to clot at ambient temperature for 1–2 hr, was then centrifuged at 1500 *g* for 15 min and the serum was stored at −70°C. To obtain plasma, blood was collected in plastic tubes containing EDTA (20 mM final concentration), centrifuged at 1500 *g* for 15 min and the supernatant was immediately stored at −70°C. Serum and plasma samples were shipped to Geneva on dry ice and were kept at −70°C for up to 3 months.

*[$^{125}$I]C1$^{3}$q binding test.* The modified [$^{125}$I]C1q binding test for the detection of soluble immune complexes was used (Zubler *et al.*, 1976a): briefly, [$^{125}$I]C1q is mixed with native test serum and then free C1q is separated from C1q bound to complexes by a precipitation with polyethylene glycol (PEG). Results are expressed as percent [$^{125}$I]C1q precipitated as compared to the radioactivity precipitated with trichloroacetic acid (TCA) in control tubes. The mean of duplicated tests is calculated and represents the C1q binding activity (C1q-BA) of the sample. Sucrose gradient fractions have also been tested, and the specific C1q-BA in such gradient fractions was obtained after a correction for the non-specific C1q-BA observed in gradient fractions obtained with NHS (Zubler *et al.*, 1976b). In some experiments, the test was performed on sera or sucrose gradient fractions which had been incubated in presence of mycobacterial antigens. As controls, samples were tested after incubation in presence of 0·9% NaCl.

*Complement studies.* These were carried out on EDTA-plasma. Haemolytic activity (CH50) was quantitated in a continuous flow system (Nydegger *et al.*, 1972). C1q, C4, C3 and properdin factor B were quantitated by single radial immunodiffusion (Perrin, Lambert & Miescher, 1974). The C3 breakdown product, C3d, was quantitated in a two-step procedure (Perrin, Lambert & Miescher, 1975): in the first step, C3 and the high molecular weight fragment, C3c, were precipitated with PEG. In the second step, the C3d was measured in the PEG supernatant by single radial immunodiffusion using anti-C3d antigen antiserum. The values for CH 50, C1q, C4 and factor B were expressed in per cent of a normal plasma pool. The values for C3 and C3d were expressed in mg%.

*Density gradient studies.* Ultracentrifugations were performed with a SW65-Ti rotor in a Spinco L2-65B preparative ultracentrifuge. Samples of serum or PEG fraction obtained from serum (0·8 ml) were layered on 4·2-ml sucrose gradients, 10–40% (w/v) in 0·1 M borate, 0·1 M EDTA, pH 8·3, and centrifuged at 34,000 rev/min for 17 hr at 7°C. In some experiments, serum (0·4 ml) was first incubated in presence of mycobacterial antigens (0·8 ml); thereafter 1·2-ml portions were layered on 3·8-ml gradients and centrifuged at 43,000 rev/min for 13 hr at 7°C. The techniques used for the preparation of gradients, the collection of gradient fractions and for obtaining OD patterns from gradients with a u.v. flow cell, have been described (Zubler *et al.*, 1976b). IgG and IgM were used as 7s and 19s sedimentation markers respectively.

In some experiments, a PEG precipitation was done in order to concentrate the C1q binding material before ultracentrifugations (Zubler *et al.*, 1976b). For the present study, 1 vol. of serum was mixed with 4 vol. of a 4% (w/v) PEG (MW 6000) solution in borate-EDTA buffer, pH 8·3. The mixture was kept at 4°C for 1 hr, centrifuged at 2000 *g* for 30 min, the precipitate was homogenized and resuspended with borate-EDTA buffer in 1/3 or 1/6 of the original volume of serum.

*Experiments with mycobacterial antigens.* For such experiments, an antigen preparation of *M. leprae* from armadillo tissue, homogenized by sonication, was used at a protein concentration of 3 mg/ml (Kronvall *et al.*, 1976).

*Statistical evaluation.* Statistical evaluation was carried out according to Stud ent's *t*-test and by linear regress ion analysis by the method of the least squares.

## RESULTS

### [125I]C1q binding activity in serum samples

The [125I]Clq binding activity (Clq-BA) was measured on thirty-nine serum samples from thirteen patients with ENL, eleven sera from seven patients with uncomplicated lepromatous leprosy (L. leprosy)

TABLE 1. [125I]Clq binding activity and complement levels in patients with leprosy and in controls

| | Sera tested (no) | [125I]-Clq binding activity (%)* | Complement levels | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Clq (%)† | C4 (%)† | Factor B (%)† | C3 (mg%) | C3d (mg%) | CH50 (%)† |
| Normal values (Blood donors, Geneva) | 30 | 7·0±2·5‡ | 100±18 | 100±21 | 100±14 | 135±21 | 0·8±0·5 | 100±15 |
| Ethiopian controls | 5 | 9·2±4·1 | 107±28 | 119±18 | 96±22 | 130±36 | 0·6±0·5 | 101±23 |
| Leprosy: ENL (total) | 39 | 18·7±8·7 | 127±29 | 112±36 | 128±39 | 160±48 | 2·3±0·8 | 111±27 |
| ENL acute | 12 | 20·6±6·3 | 112±29 | 115±42 | 163±44 | 188±58 | 2·4±0·4 | 115±39 |
| ENL convalescent | 12 | 16·5±6·4 | 126±30 | 106±33 | 107±29 | 146±41 | 2·0±0·9 | 104±31 |
| Lepromatous (without ENL) | 11 | 27·5±17·7 | 139±28 | 102±42 | 127±30 | 140±48 | 1·3±0·7 | 104±30 |
| Tuberculoid | 12 | 14·5±6·1 | 123±22 | 106±41 | 111±28 | 127±23 | 0·8±0·4 | 97±29 |
| Tuberculosis | 10 | 12·8±7·0 | 152±21 | 117±23 | 104±17 | 157±31 | 0·9±0·5 | 122±24 |

* Per cent radioactivity precipitated.
† Per cent of a normal plasma pool.
‡ These are mean values± 1 s.d.

and twelve sera from six patients with tuberculoid leprosy (T. leprosy). For controls, thirty serum samples from blood donors from Geneva, five sera from healthy Ethiopians and ten sera from ten patients with active pulmonary tuberculosis (Tbc) who were also Ethiopian, were tested. The mean values for Clq-BA which have been found in these patient groups are shown on Table 1. As compared to the Clq-BA in the normal controls (blood donors), the mean Clq-BA was significantly increased in the sera

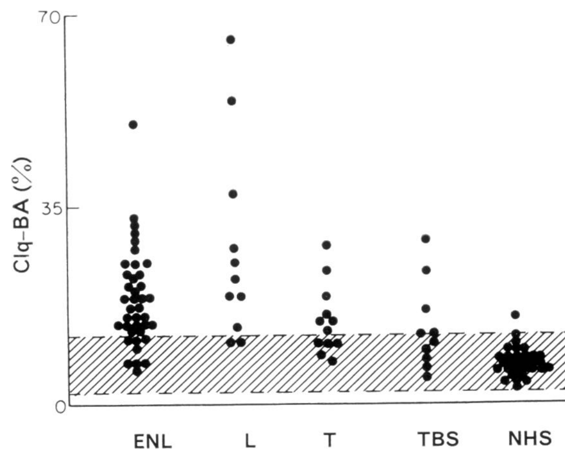


FIG. 1. [125I]Clq binding activity (Clq-BA) in the serum from patients with erythema nodosum leprosum (ENL), uncomplicated lepromatous leprosy (L), tuberculoid leprosy (T), pulmonary tuberculosis (TBC) and from healthy blood donors (NHS). The normal range (±2 s.d.) for the Clq-BA is indicated.

from patients with ENL ($P<0{\cdot}001$), L. leprosy ($P<0{\cdot}005$) and T. leprosy ($P<0{\cdot}005$). The highest mean value was found in L. leprosy. No significant difference in Clq-BA was observed between the various leprosy patient groups. The mean Clq-BA was also increased in the Tbc patients ($P<0{\cdot}005$). Only in L. leprosy was the mean Clq-BA significantly higher when leprosy was compared to Tbc ($P<0{\cdot}05$). There was no significant difference between the mean Clq-BA in Ethiopian controls and that found in blood donors from Geneva. The values for Clq-BA in individual patient's sera are shown on Fig. 1. As compared to the normal controls, the Clq-BA was increased by more than 2 s.d. in 80% of ENL sera, 82% of L. leprosy sera, 58% of T. leprosy sera and 30% of Tbc sera.

*Complement levels in plasma samples*

Various complement parameters were measured in plasma samples obtained from patients who had sera tested. First, the total haemolytic complement activity (CH50) and the levels of Clq, C4, C3 and properdin factor B were quantitated. The mean values which were observed in the various patient groups

FIG. 2. C3d levels in the plasma from patients with erythema nodosum leprosum (ENL), uncomplicated lepromatous leprosy (L), tuberculoid leprosy (T), pulmonary tuberculosis (TBC) and from healthy blood donors (NHS). The normal range ($\pm 2$ s.d.) for the C3d level is indicated.

are shown on Table 1. As compared to the normal controls, all complement levels measured were increased to various extents in the leprosy and the Tbc patients. One should mention that the mean Clq levels were significantly increased in all these patient groups ($P<0{\cdot}01$). In individual patients, none of the complement values significantly correlated with the Clq-BA. Secondly, the C3d fragment of C3 was quantitated in the plasma samples. The mean C3d levels which were observed in the various patient groups are also shown on Table 1. The mean C3d level was significantly increased in ENL ($P<0{\cdot}001$), it was elevated at the limit of significance in L. leprosy ($P = 0{\cdot}05$) and was not significantly different from the normal value in T. leprosy and Tbc. The mean C3d level was also significantly elevated in ENL when compared to L. and T. leprosy ($P<0{\cdot}001$). The C3d levels in individual patient's plasma are shown on Fig. 2. As compared to the normal control, the C3d levels were increased by more than 2 s.d. in 70% of ENL plasma, 18% of L. leprosy plasma and in none of the T. leprosy or Tbc plasma.

*Correlation between the Clq-BA and the C3d level*

By linear regression analysis, a significant correlation was found between the Clq-BA and the C3d level in patients with ENL, but the correlation coefficient was not very high ($r = 0{\cdot}359$, $P<0{\cdot}05$). When studying all the patients with leprosy, the correlation between the Clq-BA and the C3d level was not significant ($r = 0{\cdot}243$, $P>0{\cdot}05$ (Fig. 3).

*Clq-BA, C3d level and clinical activity in ENL*

From the patients with ENL, two to seven serum samples have been obtained at intervals of 1 week or

more for prospective follow up study. The Clq-BA and the C3d level have been compared to the clinical activity of the disease. From twelve ENL patients, serum samples have been obtained during both an acute bout of ENL and a period of convalescence. The Clq-BA and/or C3d levels were found to be lower during the convalescence in eight patients. However, the mean Clq-BA and C3d level were only insignificantly lower during the convalescence in comparison with the acute phase of ENL (Table 1). It should be noted that, in fact, the Clq-BA as well as the C3d level, remained rather constant within individual patients during the follow-up study. However, in a prolonged follow-up of one patient, the Clq-BA and the C3d level were regularly found to be decreased during periods of convalescence, as observed following three different bouts of ENL. All the other complement levels studied were lower during the convalescence. No conclusions could be drawn from this study with respect to the effects of various drug regimes.

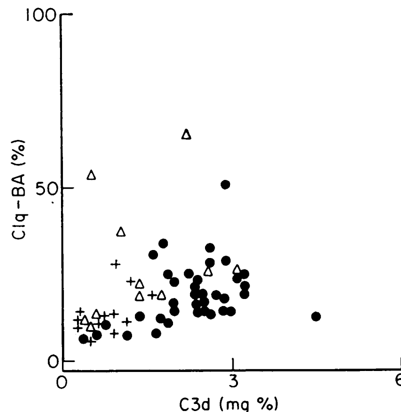


FIG. 3. Correlation between the [$^{125}$I]Clq binding activity (Clq-BA) and the C3d level in patients with leprosy: (●) erythema nodosum leprosum; (△) uncomplicated lepromatous leprosy; (+) tuberculoid leprosy.

*Characterization of the Clq binding material in leprosy*

In order to investigate the size of the Clq binding material, the sera from two patients with ENL and one patient with L. leprosy exhibiting an increased Clq-BA, were subjected to ultracentrifugation analysis, using sucrose density gradients (10–40% w/v). After ultracentrifugation, the Clq-BA was measured in each of seven serial gradient fractions. The Clq-BA was always encountered in gradient fractions 3 and 4 from the bottom of the tubes containing about 10–26s material (Fig. 4).

Some experiments were performed in order to investigate the nature of the Clq binding material in leprosy serum. The assumption was made that the increased Clq-BA in such serum may be related to the presence of immune complexes involving antigens from *M. leprae*. It is known that immune complexes in great antigen excess do not bind Clq efficiently. The Clq-BA was therefore studied to see whether it decreased after an incubation of leprosy sera in presence of an *M. leprae* antigen preparation (Ml-ag). Sera were incubated overnight at 37°C in presence of either Ml-ag or 0·9% NaCl for controls. The Ml-ag protein concentration was 12 mg/ml serum. In experiments with three ENL and three L. leprosy sera, the Clq-BA was found to be increased after an incubation with Ml-ag (26±19%, mean Clq-BA±1 s.d.) as compared to controls with NaCl (17±18%). When T. leprosy sera, Tbc sera, normal human sera (NHS) or NHS containing various amounts of heat-aggregated human immunoglobulins (AHI) were used in these experiments, the Clq-BA was found to be the same (maximal difference of 2%) in samples with Ml-ag as in controls with NaCl. The serum of one patient with ENL was subjected to ultracentrifugation analysis after an incubation with either Ml-ag or NaCl. The results are shown on Fig. 5. When the serum was incubated with NaCl, the maximal Clq-BA was found in gradient fractions containing about 12–20s material; but when the serum was incubated with Ml-ag, the maximal Clq-BA was found in gradient fractions containing smaller, 8–16s material. The total Clq-BA in all the fractions of the respective gradients was about the same after the incubation with either Ml-ag or NaCl.

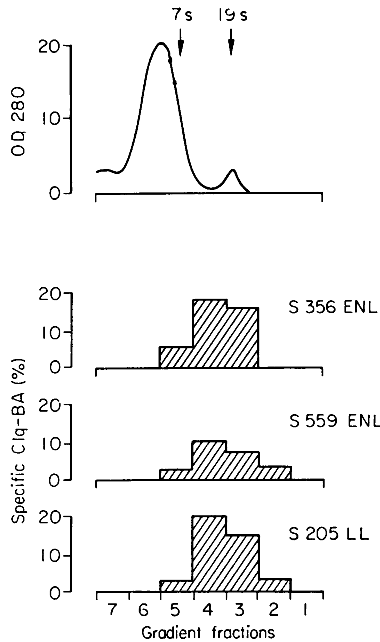


FIG. 4. Specific [$^{125}$I]C1q binding activity (C1q-BA) measured in individual sucrose gradient fractions obtained by separation of sera from patients with erythema nodosum leprosum (ENL) and uncomplicated lepromatous leprosy (LL): at the top of the figure the optical density (OD) pattern obtained with a serum and the positions of the 7s and 19s markers are indicated. C1q-BA is represented by the columns on the lower part of the figure.

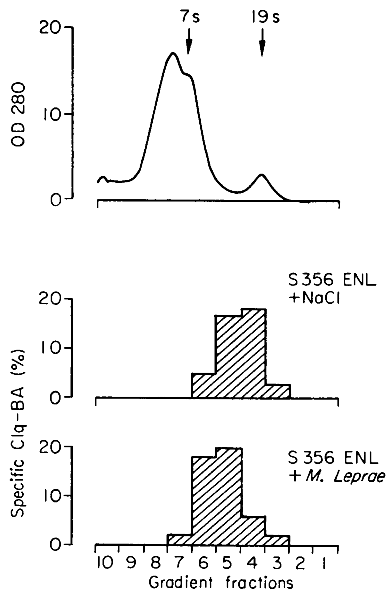


FIG. 5. Specific [$^{125}$I]C1q binding activity (C1q-BA) measured in individual sucrose gradient fractions obtained by separation of the serum from a patient with erythema nodosum leprosum (ENL) after an incubation of the serum with either an *M. leprae* antigen preparation or 0·9% NaCl for the control: at the top of the figure the optical density (OD) pattern obtained with the serum and the positions of the 7s and the 19s markers are indicated, C1q-BA is represented by the columns on the lower part of the figure.

Since one possibility was that the increase in Clq-BA in ENL and L. leprosy sera observed in presence of Ml-ag was the result of a reaction of Ml-ag with free anti-mycobacterial antibodies, the Clq binding material was largely separated from free antibodies before investigating the effect of an incubation with Ml-ag on the Clq binding activity. The Clq binding material from 2 ENL and one L. leprosy sera was partially purified and concentrated using PEG, and then fractionated on sucrose density gradients. This procedure led to a two to four times increased Clq-BA in 10–26s gradient fractions when compared to the Clq-BA in respective gradient fractions obtained with the original untreated sera. When such gradient fractions were incubated in presence of Ml-ag, the Clq-BA was found to be strongly reduced as compared to the Clq-BA after incubation with NaCl. (Table 2).

TABLE 2. Effect of an incubation with *M. leprae* antigens on the [$^{125}$I]Clq binding activity of partially purified Clq binding material from leprosy sera

| Sera used for purification of Clq binding material | Specific [$^{125}$I]Clq binding activity (%) | |
|---|---|---|
| | When incubated with NaCl | When incubated with Ml-ag* |
| S 356 ENL | 41† | 21 |
| S 559 ENL | 46 | 17 |
| S 205 L. leprosy | 49 | 26 |
| NHS | 0 (9·4‡) | 0 (11·2‡) |
| NHS+AHI (0·2 mg/ml)¶ | 29 | 30 |

* Partially purified Clq binding material was incubated with *M. leprae* antigens (Ml-ag) for 3 hr at 37°C; the Ml-ag protein concentration was 4 mg/ml sucrose gradient fraction material.

† Mean values of duplicate experiments.

‡ Values for 'non-specific' [$^{125}$I]Clq precipitation in sucrose gradient fraction obtained with NHS, after an incubation with NaCl or Ml-ag respectively.

¶ AHI = Aggregated human immunoglobulins.

## DISCUSSION

There is now good evidence that the immune reactions against *M. leprae* may play an important role in the pathogenesis of leprosy, while *M. leprae* itself exhibits little *in vivo* toxicity (Godal, 1974). Lepromatous leprosy is characterized by the concomitant occurrence of large amounts of *M. leprae* and of corresponding anti-mycobacterial antibodies (Kronvall *et al.*, 1976). Therefore, one might expect the formation of immune complexes as well as an activation of the complement system triggered by such immune complexes. Deposits of immunoglobulins and C3 have been detected by immunofluorescence in skin lesions (Wemambu *et al.*, 1969) and renal glomeruli (Shwe, 1972) in patients with L. leprosy or ENL. Furthermore, the existence of circulating immune complexes has been suggested by the finding of Clq precipitins in agarose (Agnello, Winchester & Kunkel, 1970) in the sera from patients with L. leprosy (Moran *et al.*, 1972; Rojas-Espinosa *et al.*, 1972; Gelber *et al.*, 1974). Some authors found a higher incidence of such Clq precipitins in agarose in ENL than in uncomplicated L. leprosy (Moran *et al.*, 1972; Gelber *et al.*, 1974). This observation was not confirmed using a quantitative estimation of immune complexes with the Clq binding radioassay. Indeed, in the present investigations, an increased [$^{125}$I]Clq binding activity (Clq-BA) was demonstrated frequently in the sera from patients with ENL and with uncomplicated L. leprosy, but was also found in sera from patients with T. leprosy.

Several data support the hypothesis that the detected [$^{125}$I]Clq binding material, which sedimented in a 10–25s range, consists of immune complexes involving *M. leprae* antigens. The decrease of the Clq-

BA of partially purified C1q binding material to about half of the control value after an incubation with *M. leprae* antigens (Ml-ag) suggests that immune complexes involving such antigens were present in the sera. The Ml-ag did not non-specifically interfere with the Clq binding test and the addition of Ml-ag may lead to the formation of complexes with a higher antigen-antibody ratio, and with a lower efficiency to bind C1q (Nydegger *et al.*, 1974). Such experiments can only be carried out using partially purified C1q binding fractions, since it requires a preliminary separation of free antibodies which could form new complexes upon addition of MI-ag. Indeed, an increased C1q-BA results from the incubation of whole sera with Ml-ag, probably indicating its reaction with an excess of anti-mycobacterial antibodies. The finding of a decreased sedimentation velocity of the C1q-BA after the incubation of a serum with Ml-ag probably represents a decrease in the size of previously existing complexes upon addition of more antigen, as well as the formation of new, small complexes. Antigen-antibody systems other than those involving mycobacterial antigens may also be present in the leprosy sera. Bonomo & Dammacco (1970) found, in leprosy sera, mixed cryoglobulins with IgM exhibiting anti-IgG activity. In addition, although the interference of DNA and bacterial lipopolysaccharides is very limited in the [$^{125}$I]C1q binding test (Zubler *et al.*, 1976a), one cannot rule out that other biological substances which bind C1q may influence the present investigation. Moran *et al.* (1972) reported the possibility of a direct reaction of certain mycobacterial antigens with C1q in agarose.

In previous studies, normal or increased levels of all of the complement components studied have generally been observed (Saitz *et al.*, 1968; Wemambu *et al.*, 1969; Malaviya *et al.*, 1972; Petchclai *et al.*, 1973; Gelber *et al.*, 1974). In the present study, these findings have been generally confirmed. The increased levels of C1q, C3 and properdin factor B during ENL probably reflect an increased synthesis of these components in association with the inflammatory syndrome. Such modifications have been frequently observed in infectious diseases (Schur & Austen, 1968). However, the quantitation of breakdown products of complement components such as the C3d catabolic fragment of C3 has been shown to provide a possibility for the evaluation of complement activation independently of the synthetic rate of these components in human disease, such as systemic lupus erythematosus (SLE) and glomerulonephritis, as well as in *in vitro* complement activation studies (Perrin *et al.*, 1975). The usual haemolytic or immunochemical methods for measuring the plasma level of complement components provide only a static profile of the complement system and an increased synthetic rate can mask an increased catabolism. The finding of increased C3d levels in 70% of ENL plasma probably indicates an *in vivo* activation of C3. One should note that C3d levels were within the normal range in the control group of patients with tuberculosis although C3 plasma levels were as elevated as in ENL.

The generation of C3d in plasma may result from an activation of C3, a central component of the complement system, by the classical as well as by the alternative pathway, and either pathway may be activated by immunological or non-immunological mechanisms (Müller-Eberhard, 1974). In certain clinical conditions, such as SLE or rheumatoid arthritis, increased C1q-BA values in serum were frequently observed (Nydegger *et al.*, 1974; Zubler *et al.*, 1976a, b) and there is a very significant correlation between the C1q-BA and the C3d level (Lambert & Zubler 1976; Nydegger *et al.* 1976). This suggests that C1q binding complexes in the serum are involved in the complement activation. In the present study, only a poor correlation between the C1q-BA and the C3d level was found in ENL. In addition, while C3d levels are similar to those observed in SLE by Perrin *et al.* (1975), C1q-BA values are lower in ENL than in SLE. It is therefore not likely that the increased C3d levels in ENL were only due to complement activation by immune complexes present in the circulation. One should consider the possibility that extra-vascular immune complexes are involved in the complement activation occurring during ENL and that the C3d catabolic fragments detected in the plasma from patients with ENL would be diffusing from extra-vascular spaces. The results from tissue immunofluorescence studies by Wemambu *et al.* (1969) support such a hypothesis. In the light of the known biological effects of complement breakdown products (Cochrane & Koffler, 1973), the demonstration of a hypercatabolism of C3 in ENL suggests that an activation of the complement system may be involved in the pathogenesis of this clinical condition. Furthermore, the quantitation of C3d in plasma may be of some practical interest in the early diagnosis of ENL complications of leprosy.

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