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# Solubilization of Preformed Immune Complexes in Sera of Patients with Type 1 and Type 2 Lepra Reactions[1]

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A significant perturbation in the serum complement system during an erythema nodosum leprosum (ENL) episode has been reported earlier ([14, 16]). At the onset of reactions in multibacillary leprosy, massive amounts of circulatory mycobacterial breakdown products are released which contribute to the formation of serum immune complexes ([20]) in the vascular and extravascular compartments ([7, 12]). They are solubilized in the presence of complement, and these in turn are eliminated by the phagocytic system. Furthermore, solubilization is significantly influenced by C3-convertases, $\overline{C4b, 2a}$ and $\overline{C3b, Bb}$, P. Immune precipitates undergoing solubilization in serum have been observed to contain a large amount of C3b and C4 peptides ([9]). A deficiency in any of these important complement components may prevent dissolution of immune aggregates in plasma, and thus further precipitate them in various tissues during the inflammatory processes. We have endeavored to evaluate the serum complement activity of leprosy patients with or without ENL by measuring their serum solubilization capacity on preformed immune precipitates, and we have also looked for a basis for serum complement dysfunction in the disease. Patients suffering from type 1 reactions have also been included in the study.

## MATERIALS AND METHODS

Twenty-seven leprosy patients (21 males and six females) were studied. Their ages ranged from 18 to 74 years with a mean of 37 years. Their durations of illness varied from 3 to 40 years. The primary diagnosis for each one of them was made through clinical, bacteriological, histopathological and immunological features ([17]). There were 23 lepromatous (LL), 2 borderline borderline (BB), and 2 borderline lepromatous (BL) patients. Four patients received dapsone and clofazimine; 5 patients received rifampin, clofazimine, and dapsone; 1 patient received clofazimine, prothionamide, and dapsone; and 14 received only dapsone. The treatment for the remaining three patients was not known to us. Of these 27 patients, 7 patients developed type 2 reaction, and 2 BB patients and 1 BL patient had downgrading reactions; 1 BL-LL patient had an upgrading reaction. These 11 patients with reactions were admitted to the Dermatology Department of the Lok Nayak Jai Prakash Narayan Hospital in New Delhi, India, for follow-up study. The remaining 16 cases were living in the Leprosy Home, Shahdara, Delhi, and they did not develop any reactions. To control the reactions of these 11 patients in addition to the multidrug therapy, 5 patients were given 40 mg of prednisolone; 2 were given prednisolone and clofazimine; 1 was given prednisolone, clofazimine, and chloroquine; 2 were prescribed an augmented dose of clofazimine (300 mg); the remaining 1 was given clofazimine and aspirin. Paired samples were taken from these 11 reactional patients. Initial blood samples (10 ml) were collected at the onset of the reaction; subsequent samples were taken after remission of ENL after 4 weeks. All serum samples were stored at

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−70°C. In addition, 21 serum samples were collected from normal individuals as controls.

Anti-bovine serum albumin antiserum (anti-BSA) was raised in healthy rabbits by injecting BSA (Sigma Chemical Co., St. Louis, Missouri, U.S.A.). BSA was labeled with $^{125}I$ by the chloramine T method ([8]). A quantitative precipitin curve was constructed to determine the equivalence zone between the antibody preparation and the BSA. In order to obtain the experimental immune precipitates, rabbit anti-BSA antibody and $^{125}I$-labeled BSA at four times antigen excess were incubated for 1 hr at 37°C and subsequently kept overnight at 4°C ([5]). The resulting precipitates were washed three times with cold phosphate buffered saline (PBS) and then suspended in PBS.

Solubilization was carried out by incubating a mixture of 0.1 ml of test esra, 0.1 ml of cold normal saline, and 20 μl of $^{125}I$-BSA-anti-BSA immune complexes containing about 2 μg of protein as per the technique of Takahashi, *et al.* ([18]). The mixtures were then incubated for 2 hr at 37°C. The reaction mixtures were frozen, mixed with 10 mM EDTA-saline, and thereafter were centrifuged at 900 × *g* for 30 min. The precipitates so obtained were measured for their radioactivity in a gamma counter. The procedure was also repeated for the controls. The solubilization capacity of a serum sample was calculated on the basis of the amount of radioactivity found in the supernatant of the reaction mixture expressed as a percentage of the total radioactivity of the preformed immune complex added.

To investigate the role of some important complement components on the solubilizing capacity of fresh normal human sera, the above test was repeated by adding graded quantities of monospecific anti-human C3, C4 (Meloy Laboratories, Inc., Springfield, Virginia, U.S.A.) and factor B antisera (Kent Laboratories, Redmond, Washington, U.S.A.) in the reaction mixtures. To study the solubilization capacity of sera after inactivation of complement, the serum samples were heated at 56°C for 30 min. These samples were obtained from normal, LL, and ENL patients. An attempt was also made to perform a reconstitution experiment with lepromatous sera by adding various amounts of fresh normal sera. Finally, we carried out studies to reveal the role of mycobacterial breakdown products on the process of solubilization by adding various amounts of lepromin (World Health Organization, Geneva, Switzerland) to the reaction mixture.

## RESULTS

The capacity of normal sera collected from 21 human volunteers to solubilize the immune precipitates consisting of $^{125}I$-BSA-anti-BSA complex varied from 60% to 92% with a mean ± S.D. of 81.3 ± 9.1%. It ranged from 30% to 53.5% (mean 41.05 ± 6.1%) in ten lepromatous sera—a statistically highly significant difference ($p < 0.001$) (Fig. 1). Thus, complement-mediated immune-complex (IC) solubilization in LL patients was markedly reduced by about 50.6% when compared to normal subjects.

The sera of 11 patients suffering from type 1 reactions (4 patients) and from type 2 reactions (7 patients) had an average solubilization capacity of 41.9 ± 12.1% and a range of 14.6% to 57.5%. However, in one of them (ENL) its value was as low as 14.6% (Fig. 2). The mean solubilizing capacity of the patients' sera during the reaction was considerably lower than that of the controls, but it was not significantly different from that of LL patients without ENL (mean 41.05 ± 6.1%). After clinical remission of the reaction, the patients mostly showed no appreciable increase in the IC solubilization, having a mean of 45.6 ± 8.6% (Fig. 2).

Of the 11 patients, 7 showed an increase and the remaining 4 showed a decrease. Out of the 7 samples showing an increase in the percentage of solubility of IC following remission of reaction, 4 samples were collected from ENL patients and the remaining 3 were obtained from one BL patient undergoing downgrading reaction, one BB patient having downgrading reaction, and one LL patient undergoing upgrading reaction (LL → BT). Of the 4 samples showing a decrease, 3 were taken from ENL patients and 1 was obtained from a BB patient undergoing downgrading reaction. Interestingly, one patient showed a sharp rise in IC solubilization capacity from 14.6% to 51.4%. He was 29 years old and had been suffering

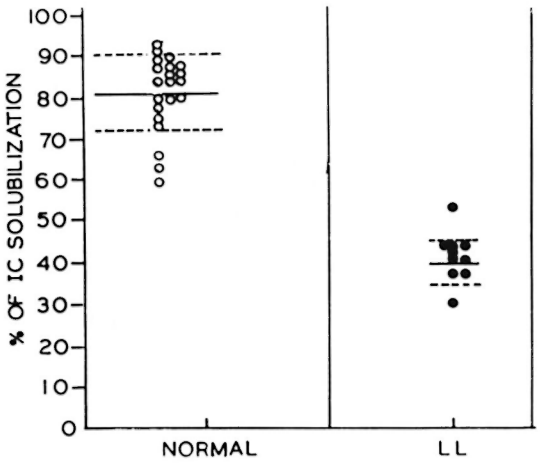


FIG. 1. Profile of IC solubilization capacity (ICSC) of sera from normals and lepromatous patients; mean value of the latter was found to be significantly reduced when compared to controls. (——) = mean; (– – –) = standard deviation.

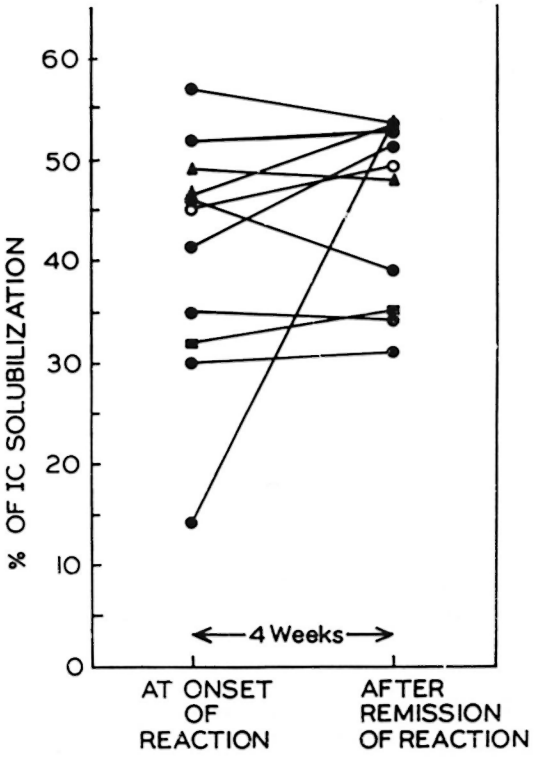


FIG. 2. A follow-up study of ICSC of the sera from leprosy patients at the onset of type 1 and type 2 reactions and after their clinical remission. ●——● = LL with ENL (7 patients); ▲——▲ = BB ⌊ (2 patients); ■——■ = BL ⌊ (1 patient); ○——○ = LL ⌈ (1 patient).

from lepromatous leprosy. He had developed nodular ENL lesions, his fingers degenerated, had joint pains, and had low-grade fever. He was given 60 mg of prednisolone every alternate day to control his ENL reaction.

Heat inactivation of the test sera caused a decrease in the solubilizing capacity in normals as well as in LL and ENL patients (Fig. 3). It varied from 30% to 40%. Dayer, *et al.* [3] have reported about 11% solubilization in heated human sera. Our results record a higher value; possibly crossreactivity between human serum albumin (HSA) and BSA may result in antigen excess leading to solubilization of the IC. It appears that heat inactivation could still save some solubilizing capacity of the sera. Antisera against human C3, C4, and factor B had a significant suppressive action on solubilization (The Table), suggesting that optimum levels of C3, C4, and factor B are essential for IC solubilization.

The addition of normal serum factors to the lepromatous sera marginally enhanced the extent of solubilization from 53% to 62% (Fig. 4). Even after adding an equal volume of fresh serum to the lepromatous serum, no significant increase of the solubilization capacity of the deficient sera was seen. Figure 5 shows that the presence of mycobacterial cleavage products in serum significantly reduces the serum's IC solubilization capacity. It was observed experimentally that by incorporating a very high quantity of lepromin breakdown fragments in the serum, a reduction in solubilization as high as 50% to 70% could be achieved.

## DISCUSSION

The present study describes IC solubilization levels in sera from leprosy patients with type 1 and type 2 reactions. The mean solubilization capacity of sera from LL patients with or without reaction was significantly reduced as compared to controls (Fig. 1). In addition, BB and BL patients with downgrading reactions and BL-LL patients with upgrading reactions also showed reduced solubilization levels. Further, the ENL and type 1 reactional patients were followed up and neither of these groups showed any significant improvement in the mean solu-

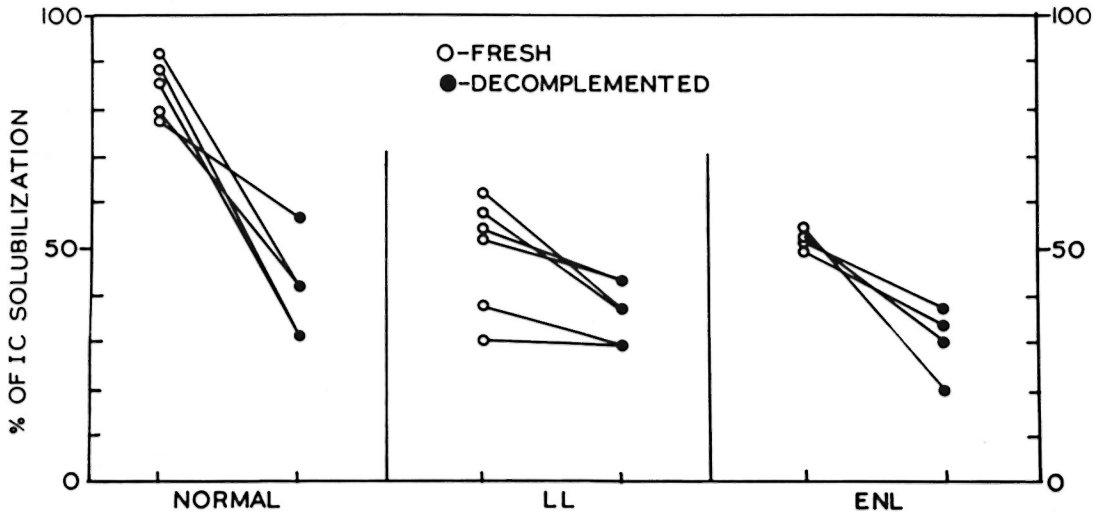


FIG. 3. Reduction in ICSC of sera from normal subjects and from patients with or without ENL following heating at 56°C for 30 min. Decrease of ICSC was much more in normal sera (84.1% to 40.1%) as compared to that in lepromatous sera (48.3% to 37.7%) as well as in ENL sera (52.2% to 28.8%).

bilization capacity of their sera after subsidence of the reaction (Fig. 2). However, one patient, having a very low solubilizing capacity of 14.6% during the onset of ENL, had significant restoration of solubilization (to 51.4%) but did not reach a normal value. In a recent study by Ramanathan, *et al.* ([11]) it was reported that, even after remission of reaction, ENL patients maintained a significant lowering in their IC solubilization level and that BT patients with type 1 reactions were also shown to have decreased solubilizing capacity. These findings support our present results. However, unlike our observations, the mean solubilization levels of LL patients were reported to be unaltered in their study.

The solubilization of IC is a complement-dependent phenomenon ([18]). Increased complement utilization appears to be an important event in LL patients with or without ENL since complement activation is facilitated by high levels of serum complexes persisting in the sera of these patients ([4, 7, 15]). It seems likely that inadequate replenishment of complement components may be the basis for the low solubilization of IC in the sera obtained from patients suffering from lepromatous leprosy. This is borne out by the fact that the level of circulating immune complexes (CIC) in ENL patients following remission is not significantly restored ([15]).

The basis for decreased solubilization in the BB and BT patients in reaction is not clear, since the bacterial load in these patients is relatively small. It has been shown recently by Furukawa, *et al.* ([4]) that positivity and mean values of CIC in borderline and tuberculoid leprosy patients are as high as those in the lepromatous group. It is plausible that reduced solubilization in BB and BL patients in reaction may have some association with their serum complexes. Polyclonal B-cell activation is a salient feature of leprosy wherein idiotype-antiidiotype antibody aggregates form a major fraction of total circulating immune complexes ([13]).

To identify the involvement of complement components in IC solubilization, the latter was studied in the presence of anti-C3, anti-C4, and anti-factor B antibodies (The Table). It appears that some levels of C3, C4, and factor B are likely required for optimum solubilization. A better method would have been to add purified complement components (C3, C4, and factor B) to the patients' sera and study its solubilization level.

In a recent study, a marked decrease of C3b receptors on the erythrocytes of LL patients has been reported ([19]). It appears that

THE TABLE. *Decrease of IC solubilizing capacity of normal fresh human sera by addition of antibodies against several complement components.*

| Fresh normal human sera added (μl) | Anti-complement component anti-sera added (μl) | $^{125}$I-BSA-anti-BSA preformed immune complex (μl) | % of IC solubilized in presence of 1:10 diluted anti-complement component anti-sera | | |
|---|---|---|---|---|---|
| | | | Anti-human C3 | Anti-human C4 | Anti-human factor B |
| 100 | 0 | 20[a] | 82.4 | 83.5 | 88 |
| 100 | 10 | 20 | 63.36 | 82.5 | 78 |
| 100 | 25 | 20 | N.D.[b] | 72.5 | 74 |
| 100 | 50 | 20 | 54.5 | 65.2 | 68.2 |
| 100 | 100 | 20 | 47.8 | 63.5 | 43.87 |

[a] Total volume was made up to 220 μl in all tubes by addition of normal saline. Results clearly show that C3, C4, and factor B help in solubilization of IC. The observed decrease of solubilization of sera might be due to reduced concentrations of C3, C4, and factor B.

[b] N.D. = not done.

these cells are unable to participate in the clearance of CIC from blood. This may further account for the reduced IC solubilization in LL patients with or without ENL. The composition of CIC in leprosy patients is well known ([2, 10, 15]). Of those components, enhanced levels of C-reactive protein (CRP) and rheumatoid factor (RF) may interfere with IC solubilization, CRP by inducing the consumption of complement and RF affecting complement fixation by having a molecular interaction with C3 ([1, 3, 6]). Solubilization may, therefore, be considerably impeded during ENL. An attempt was made to study the reconstitution of deficient solubilizing capacity of lepromatous sera by normal sera. The addition of normal serum factors to sera obtained from LL patients resulted in a marginally enhanced solubilization of only 53% to 62% (Fig. 4). Since reduced solubilization in the deficient patients' sera could not be restored by the ad-

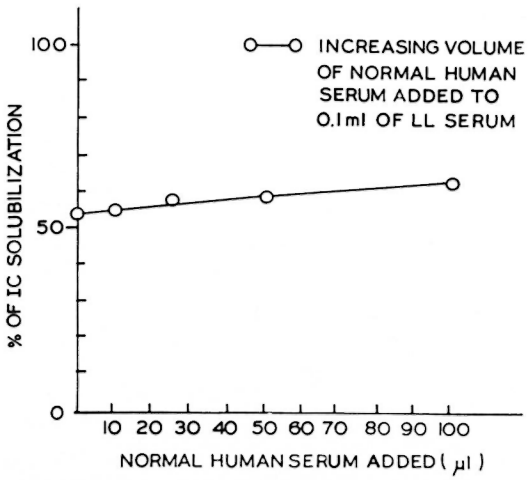


FIG. 4. An attempt to reconstitute reduced ICSC of LL with ENL sera with the addition of a fresh normal human serum sample. To 0.1 ml of the test serum having ICSC as 53% was added different amounts of a fresh normal human serum and the SC of the mixture was determined. No significant restoration of SC of the test sample could be observed; only a marginal increase of ICSC from 53% to 62.2% was registered when the test serum and normal serum were in equal volumes.

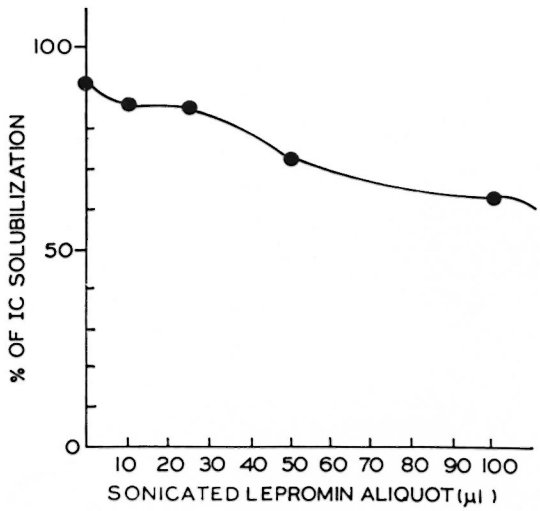


FIG. 5. Influence of sonicated lepromin A ($4 \times 10^7$ *M. leprae*/ml) on IC solubilization. Lepromin A was sonicated and the sonicate was diluted with saline so as to obtain 17.1 mg protein/ml. Different amounts of lepromin sonicate were incubated with 0.1 ml of fresh normal human serum at 37°C for 1 hr. The incubation mixture was immediately assayed for IC solubilization.

dition of normal serum, it is possible that some anticomplementary materials were present in the deficient patients' sera. Therefore, we studied the effect of *Mycobacterium leprae* breakdown products on the solubilization capacity of normal sera. The presence of mycobacterial cleavage products in serum caused a significant reduction in IC solubilization capacity (Fig. 5). It is interesting to note that when a very high quantity of lepromin breakdown fragments were added to the serum, a substantial reduction (50% to 70%) in solubilization occurred. Earlier Saha, *et al.* ([14]) had observed that even after the control of ENL by therapy, the serum C3d level did not decline, suggesting continued complement consumption. The additional leprosy bacilli into normal human serum could bring about a significant complement consumption through the alternative pathway ([16]). Therefore, it seems likely that the presence of a large quantity of circulating bacterial products in the sera of ENL patients forestalls complement-mediated solubilization of serum immune complexes.

In conclusion, it is suggested that reduced solublization capacity may be due to enhanced levels of circulatory serum complexes, or cleavage products of *M. leprae,* or both, in sera of different types of leprosy patients. A reduced serum complement level may be the required condition for the reactional state of the leprosy patient, but the initiation of the reaction may not depend upon a quantum change in IC metabolism. ENL, or type 1 reaction, may be prompted by a change in cellular reactivity which is determined by immunomodulatory complement-activation products and the basal immune status of the patient.

## SUMMARY

Serum complement activity in leprosy patients has been studied using solubilization of preformed immune complexes as an index. The solubilization capacity of sera from lepromatous patients with or without erythema nodosum leprosum (ENL) as well as from type 1 reactional patients was found markedly reduced as compared to controls. Solubilization did not improve at all in the ENL patients after remission of the reaction phase. The addition of fresh normal sera failed to bring about any significant restoration of solubilizing capacity of the deficient sera. *Mycobacterium leprae* sonicate significantly reduced the solubilization capacity. Our results suggest that circulating mycobacterial breakdown products possibly interfered with the capacity of the ENL patients' sera to solubilize immune complexes.

## RESUMEN

Se estudió la actividad del complemento sérico en pacientes con lepra usando el ensayo de la solubilización de complejos inmunes preformados. En comparación con los controles, se encontró que la capacidad de solubilización de los sueros de pacientes lepromatosos con o sin eritema nodoso leproso (ENL), o con reacciones del tipo 1, estuvo marcadamente reducida. La solubilización no mejoró en los pacientes con ENL después de la remisión de la fase reaccional. La adición de suero fresco normal no restauró la capacidad solubilizante de los sueros deficientes. El sonicado de *Mycobacterium leprae* redujo significativamente la capacidad de solubilización. Los resultados sugieren que los productos de rompimiento micobacteriano circulantes podrían interferir con la capacidad de los sueros de los pacientes con ENL para solubilizar los complejos inmunes.

## RÉSUMÉ

On a étudié chez des malades de la lèpre l'activité en complément du sérum, en utilisant une méthode de solubilisation de complexes immuns préformés comme indicateur. La capacité de solubilisation des échantillons de sérum prélevés chez des malades lépromateux, atteints ou non d'érythème noueux lépreux (ENL) de même que de malades atteints d'épisodes réactionnels de type 1, s'est révélée profondément réduite, quand on la comparait à la capacité de solubilisation observée chez les témoins. La solubilisation ne s'améliorait aucunement chez les malades atteints d'ENL, après la disparition de la phase réactionnelle. L'addition de sérum normal frais n'a pas permis de rétablir de manière significative la capacité de solubilisation dans les échantillons de sérum qui étaient déficients. Des sonicats de *Mycobacterium leprae* ont réduit significativement la capacité de solubilisation. Nos résultats suggèrent que les produits en circulation provenant de la dégradation des mycobactéries pourraient interférer avec la capacité du sérum des malades atteints d'ENL de solubiliser les complexes immuns.

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