Circulating Immune Complexes in Patients with Leprosy

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The occurrence of immune complexes in the serum from rats infected with *M. leprae-murium* and 38 patients with leprosy were studied by the polyethylene glycol precipitation complement consumption (PEG-CC) test and the results were compared in the various forms of the disease. Circulating immune complexes (CIC) were significantly increased in the sera from rats infected with *M. leprae-murium* compared to normal control rats (P<0.005). There were no significant differences between the the level of CIC in the sera from lepromatous leprosy patients and that from tuberculoid leprosy patients, but in the sera from patients with erythema nodosum leprosum (ENL) the level of CIC was significantly increased (P<0.005). And although we couldn’t find a correlation between the level of CIC and bacterial indices in lepromatous leprosy patients, CIC tends to decrease after negative conversion of their bacterial indices.

These findings suggested that the detection of CIC can be of some practical interest in the early diagnosis of ENL and can be a valuable assessment in following the therapy after negative conversion of their bacterial indices.

Key Words: Circulating immune complexes, PEG-CC test, leprosy

Leprosy is a chronic infectious disease caused by *Mycobacterium leprae*. The disease represents a wide, continuous spectrum of clinical manifestations ranging from lepromatous to tuberculoid leprosy (Ridley and Jopling, 1966). Studies of immune mechanisms in leprosy have shown that in lepromatous leprosy patients humoral immunity is increased compared to a normal population or to tuberculoid leprosy patients. (Almeida, 1970), but cellular immunity is decreased (Godal et al, 1971; Hans et al, 1971; Mendes et al, 1974). These findings indicate that the immunologic reaction of the host against *M. leprae* may play a major role in the development of clinical manifestations (Sansonetti and Lagrange, 1981).

The immune complexes composed of soluble mycobacterial antigens and corresponding antibodies are thought to be responsible for tissue lesions in leprosy, particularly for those in erythema nodosum leprosum (ENL). Indeed, there is some evidence of an involvement of immune complexes in the pathogenesis of 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 and 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 circulating immune complexes has also been suspected by the frequent occurrence in leprosy sera of substance which precipitates with C1q in agarose (Moran et al, 1972; Rojas-Espinosa et al, 1972; Gelber et al, 1974). These circulating immune complexes occur frequently, in particular during infectious disease and in association with autoimmunity. Although typical manifestations of immune complex are a much rarer event, they may play an important role in some human diseases (Zubler and Lambert, 1978).

Several assay systems have recently been developed for the detection of soluble immune complexes in circulation. These include C1q-binding test (Nydegger et al, 1974) or C1q-deviation test (Sobel et al, 1975) based on the interaction between immune complex and C1q, PEG-CC test (Harkiss and Brown, 1979) based on the ability of immune complex to fix complement, solid-phase conglutinin binding test (Eisenberg et al, 1977) based on the interaction with conglutinin, and polyclonal rheumatoid factor inhibition assay (Cowdery et al., 1975) or monoclonal rheumatoid factor inhibition assay (Gabriel and Agenlo, 1977) based on the interaction with antiglobulins. Other approaches have been to utilize cellular reactivities with immune complex such as platelet aggregation test (Myllyla et al, 1973) and Raji cell assay (Theofilopoulos et al, 1974). Bjorvatn et al (1976) applied C1q binding test for the detection of CIC in leprosy and reported that the serum Clq binding activity was found to be increased, as compared to the normal value, in most sera from patients with ENL and uncomplicated lepromatous leprosy, and also in the sera from patients with tuberculoid leprosy, but there were no significant differences of C1q binding activities between the various leprosy patient subgroups. But Wager et al (1978) reported that the positivity of the CIC detected by platelet aggregation test was significantly higher in patients with lepromatous leprosy than in tuberculoid leprosy. These findings suggest that there are some correlations between the CIC and clinical manifestations of leprosy. Therefore, the detection and the quantification of CIC in leprosy patients can be used in clinical practice.

In the present study, we have attempted to ascertain whether CIC detected by the PEG-CC test (Harkiss and Brown, 1979) is associated with occurrence of ENL in patients with lepromatous leprosy and to find out the possibility of whether the quantification of CIC can provide useful indices in following therapy.

MATERIALS AND METHODS

Experimental animals: A total of 27 male rats, weighing about 85 grams, were divided into two groups, an infected group and a control group. $10^8$ to $10^9$ Mycobacterium leprae were inoculated into the testicles of the group to be infected. After inoculation, every two months some rats from both the infected and control groups were sacrificed and the sera were collected and stored at $-20^\circ$C.

Patients: Serum samples from 32 patients with lepromatous leprosy and 6 patients with tuberculoid leprosy were collected. All patients were diagnosed and treated at the World Vision Special Skin Clinics.

Complement: A pool of fresh guinea pig sera was used as a complement source. It was
divided into 0.3ml aliquots and kept at -20°C.

**Sensitized sheep erythrocytes (EA):** The sheep was bled under sterile conditions into a sterile tube containing heparin. The blood was mixed with the same volume of Alsever's solution and stored at 4°C before use (Alsever and Ainslie, 1941). The blood was washed with the diluent, modified barbital buffer (MBB) pH 7.3 (Hirsch et al., 1980), three times and then made up to a 10 percent erythrocyte suspension. It was adjusted to 0.5 percent suspension and mixed with the same volume of hemolysin (Difco, 1:1000) and kept at room temperature for 30 minutes. After that, it was washed twice with MBB and finally made up into a 0.25% EA suspension (Weir, 1978).

**Polyethylene glycol (PEG):** PEG 6000 (Iwai Kagaku Co. Ltd) was dissolved in borate buffer, pH 8.4 to give a stock solution of 12.5%, and a wash solution of 2.5% and stored at 4°C.

**Polyethylene glycol precipitation-complement consumption (PEG-CC) test:** To detect circulating immune complex, the PEG-CC test (Harkis and Brown, 1979) was performed (Fig. 1). Fifty microliters of borate buffer and 50 μl of 0.2M ethylenediamine tetra acetic acid (EDTA) were added to 0.3ml of test or control serum, and the solutions briefly mixed. 0.1ml of 12.5% PEG was added and the tubes were briefly vortexed, and then left 4°C for 90 min. After spinning at 1700g for 15 min at 4°C, the supernatants were discarded and the pellets washed by adding 1.0 ml of 2.5% PEG. The tubes were vortexed to resuspend the pellets, then spun again at 1700g for 15 min at 4°C. After aspirating the supernatant, the pellets were resuspended in 50 ul of warm MBB by vortexing. They were then assayed functionally by measuring their ability to consume complement. Fifty-microliters of pooled guinea pig serum were added as a source of complement and incubated at 37°C water bath for 30 min. The titer of 0.3 ml of control or test serum
50 μl of borate buffer
50 μl of 0.2 M EDTA

--- Add 0.1ml of 12.5% PEG

Left at 4°C for 90 mins.

Centrifugation at 1,700 g for 15 mins. at 4°C

Sediment Supernatant
| Discard |

--- Add 1ml of 2.5% PEG

Resuspend & centrifugation at 1,700 g for 15 mins at 4°C

Sediment Supernatant
| Discard |

Resuspend with 50 μl of 37°C MBB

Add 50 μl of complement and left at 37°C water bath for 30 mins.

Add 0.5ml of 0.25% EA and left at 37°C water bath for 30 mins.

Centrifugation at 700 g for 10 mins.

Check the hemolysis percentage with spectrophotometer at 530 nm.

Fig. 1. Method for polyethylene glycol precipitation-complement consumption test.
Samples giving values of 25 or more %CC were considered as positive in PEG-CC test.

RESULTS

1. CIC in rats infected with *M. lepraemurium*

The results of assaying sera from infected and control groups for CIC are shown in Fig. 2 and Table 1. After induction of infection by inoculation into the rat testicles with *M. lepraemurium*, the serum CIC was assayed every two months. Normal control sera gave a value of 11.1±12.3%CC (mean±s.d.). The upper limit of normality was taken as 2 s.d. above the mean, i.e. 36%CC. In the infected group, eleven out of fifteen (73%) samples were positive, with a mean value of 61.3±27.8%CC (mean±s.d.). The positivity of CIC was significantly higher in the infected group (p<0.005), but no significant differences in the level of CIC were observed between groups having a different duration of infection.

<table>
<thead>
<tr>
<th>Months after inoculation</th>
<th>Control group</th>
<th>Test group</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>%CC**</td>
<td>No.</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>5.5±5.5</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>36.2</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>7.4±7.4</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>0.0±0</td>
</tr>
<tr>
<td>9</td>
<td>2</td>
<td>22.0±2.4</td>
</tr>
<tr>
<td>11</td>
<td>2</td>
<td>0.5±0.5</td>
</tr>
<tr>
<td>13</td>
<td>1</td>
<td>25.8±</td>
</tr>
</tbody>
</table>

Total 12 11.1±12.3 15 61.3±27.8

* number of samples.
** mean ± S.D.
Results are expressed as % complement consumption (% CC).

2. CIC in patients with leprosy

The CIC was assayed on thirty-two serum samples from lepromatous leprosy patients and six serum samples from tuberculoid leprosy

![Fig. 2. Detection of immune complexes in murine leprosy. Sera from rats which had been inoculated with *M. lepraemurium* were collected every two months after inoculation and were assayed for percentages of complement consumption by PEG-CC test. Control groups were indicated as open circles (○) and test groups as closed circles (●). The upper limit of normal rats is indicated (----).](image)
Fig. 3. Detection of immune complexes in 38 patients with leprosy. Patients were divided into two groups: lepromatous type (L) and tuberculoid type (T). The upper limit of healthy persons is indicated (---);
Mean %CC±SD: 42.0±36.3 in total leprosy patients.
43.6±37.9 in L group
33.2±24.4 in T group

Fig. 4. Detection of immune complexes in lepromatous leprosy patients which divided into four groups according to their complications.
Group I: Sixteen patients without ENL within 6 months
Group II: Eleven patients with ENL at that time of sampling or within 6 months
Group III: Four patients with neuralgia only
Group IV: One patient with chronic ulcer on foot for 20 years

3. Correlation between CIC and ENL in lepromatous leprosy patients

Sixteen patients who have not experienced ENL during the past 6 months before serum sampling, eleven patients who have experienced ENL within 6 months before serum sampling, four patients with neuralgia only, and one patient with chronic foot ulcer for 20 years were assayed for CIC (Fig. 4). Four out of sixteen (25%) serum samples from patients without ENL were positive, and ten out of eleven (90%) serum samples from patients with ENL were positive. So the serum samples from patients with ENL showed significantly higher positivity (p<0.005) than those from patients without ENL.

4. Correlation between CIC and bacterial index in lepromatous leprosy patients

There are no correlations between CIC and bacterial indices.

Fig. 5. Detection of immune complexes in lepromatous leprosy which divided into four groups according to their bacterial indices.
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bacterial indices in lepromatous leprosy patients (Fig. 5). In the patients whose bacterial indices have been negative for a year or less, three out of five (60%) were positive, and in the patients whose bacterial indices have been negative for more than two years, two out of nine (22%) were positive. The positivity of CIC after negative conversion showed a tendency to decrease but not significantly (p>0.1) (Fig. 6).

DISCUSSION

Polyethylene glycol (PEG) is a synthetic polymer and can be used to fractionate plasma proteins according to molecular size (Polson et al, 1964). Creighton et al (1973) reported that immune complexes in plasma can be selectively precipitated by using PEG. Harkiss and Brown (1979) reported that immune complexes are precipitated by a low concentration of PEG (2.5%), while only very small amounts of monomer immunoglobulin come down in the pellet, and the precipitated immune complexes can be quantitated by measuring the anti-complementary activity of immune complexes (PEG-CC test).

In the present study, twenty-one out of thirty-eight (55%) patients with leprosy were positive. This positivity ratio is somewhat lower in comparison with 76% in Clq binding test (Bjorvatn et al, 1976) and 67% in Clq deviation test (Tung et al, 1977), but higher compared with the 41% in the platelet aggregation test (Wager et al., 1978) and 7% in Raji cell assay (Tung et al, 1977). These discrepancies appear to be due to a difference in the mechanisms of the assays applied for the detection of CIC; Raji cell assay is based on the cellular reactivity of Raji cell to complement component and specifically detects immune complexes which bind activated complement components, and platelet aggregation test is based on the interaction between CIC and Fc receptor of platelets, but Clq binding test, Clq deviation test and PEG-CC test are based on the interaction of the CIC and complement (Zubler and Lambert, 1978). And the second reason for the lower positivity in our study might be that most of the patients in this experiment, were bacteriologically negative patients, but in other experiments performed by Clq binding test or Clq deviation test they included only those patients with active leprosy. The third reason might be the difference in defining the positivity.

As described previously in materials and methods we took the point of anti-complementary activity of pooled control sera as O%CC, the sera from leprosy patients showing the anti-complementary activity above O%CC in this PEG-CC test might have higher anti-complementary activity than in normal controls, but in this experiment the upper limit of normality was taken as 2 s.d. above the mean, i.e. 25%CC (Harkiss and Brown, 1979).

There are no significant differences in CIC between the types of leprosy, lepromatous
and tuberculoid, and this observation is compatible with the results from Clq binding test (Bjorvatn et al., 1976) and the Clq deviation test (Tung et al., 1977). But this analysis with small numbers of patients with tuberculoid leprosy should be interpreted with caution. But Wager et al. (1978) reported that the CIC in lepromatous leprosy patients were higher than in tuberculoid leprosy patients in the platelet aggregation test, but the clinicopathological significance of the higher level of CIC in lepromatous leprosy patients is not yet fully understood. From the data presented in this report the CIC positivity in the patients with ENL are significantly higher than without ENL (Fig. 4). These results suggest that CIC from PEG-CC testing may help to predict the appearance of ENL. In addition to it, in systemic lupus erythematosus (SLE) one of the immune complex mediated diseases (Koffler et al., 1971), the group containing sera from patients with active SLE, the CIC positivity and mean value were higher than in the remission group (Harkiss and Brown, 1979)

A good correlation was found between CIC levels and disease activity of SLE. There is no correlation between CIC and bacterial indices in lepromatous leprosy patients in this study (Fig. 5), and this finding can be observed in rats infected with M. lepraemurium. After infection with M. lepraemurium, the CIC shows a tendency to increase but considering the data of normal control rats, there are no changes in CIC according to the duration of infection (Table 1). In considering the observation that the number of bacilli inoculated into the testicle apparently doubled each 12.2 days during the period through 14 weeks, and thereafter the rate of multiplication declined (Hanks and Backerman, 1950), it is concluded that there are no correlations between CIC and the increment of the number of bacilli in rat testicles.

And in the sera from lepromatous leprosy patients, whose bacterial indices became negative, CIC are still high and become decreased at least 2 years later after negative conversion indicates that M. leprae which persisted in various organs can induce the formation of CIC although bacterial indices become negative, and/or patients with lepromatous leprosy have a defect in eliminating CIC from the circulation due to the decreased cell-mediated immunity. This finding is compatible with the report that the transient appearance of detectable CIC occurs in measles virus or rubella virus infection, but in the patients having prolonged CIC formation, immune complex-associated virus-specific antigen are detected (Ziola et al., 1983).

From the data presented in this study, one can predict that M. leprae or its products can exist after the bacterial index becomes negative in lepromatous leprosy patients and the detection of CIC by PEG-CC test can be used for following the therapy after negative conversion of their bacterial indices. Further investigations on the immunogenetical role of HLA-DR antigens or associated gene(s) for eliminating the CIC from the circulation are under study.

REFERENCES


Creighton WD, Lambert PH, Miescher PA: Detection of antibodies and soluble antigen-antibody com-
Hanks JH, Backerman T: The tissue sites most favorable for the development of murine leprosy in rats and mice. Int J Lepr 18:185, 1950
Ridley DS, Jopling WH: Classification of leprosy according to immunity, a five group system. Int J Lepr 34:253, 1966.

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