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DIFFICULTIES IN THE EARLY SERODIAGNOSIS OF LEPROSY

Leprosy is diagnosed clinically by the presence of any two of the following signs, *ie.*, anaesthetic skin lesions, thickened nerve and presence of acid fast bacilli in the smears of skin lesions[1]. Clinicohistologically leprosy has been classified into two distinct polar forms, *viz.* tuberculoid and lepromatous. In the tuberculoid pole the macrophages are able to kill *Mycobacterium leprae* due to heightened cell mediated immunity (CMI) induced by T cells (Th1 type of immunity) while in the lepromatous pole due to specific suppression of CMI *M.leprae* grow freely in the macrophages along with a heightened antibody response induced by B cells (Th2 type of immunity). Between these two polar forms the disease manifests in different forms depending on the immunological status of the host. Therefore, the disease has been finally classified on the basis of histological, microbiological and immunological parameters on a scale described by Ridley and Jopling[2] as mentioned below: tuberculoid (TT), borderline tuberculoid (BT), borderline (BB), borderline lepromatous (BL) and lepromatous (LL). Although in the recent past several serological tests have been established to diagnose the above clinical conditions, the development and use of the specific serological assays which are of significant relevance are described below.

Specific Serological Assays

Attempts have been made by several workers[3-8] to establish specific serological tests for the diagnosis of leprosy. These are serodiagnostic assays such as Antigen based radioimmunoassay (RIA)[3], Fluorescent antibody absorption (FLA-ABS) test[4], Phenolic glycolipid-1 (PGL-1) based enzyme linked immunosorbent assay (ELISA)[5-7] and, 35 kDa based RIA/ELISA[8].

Antigen 7 based RIA

Two dimensional gel immunoelectrophoresis of *M.leprae* antigens revealed that antigen 7 is one of the major antigenic components of *M.leprae*[9]. This antigen cross reacted strongly with antigen BCG 60 and could be labelled adequately with ^{125}I. Harboe *et al*[10] carried out extensive studies with this antigen and noted that when rabbits[11] and armadillos[12] are injected with whole *M.leprae* antigens, the antibodies are produced mainly against antigen 7. Further, it was noted that in armadillos the antigen 7 antibody levels correlated with the clinical signs of leprosy[12].

While determining antibody levels in leprosy patients, the highest levels were observed in lepromatous patients, lower in tuberculoid patients and lowest in contacts of leprosy patients[3]. However, wide variations in the levels were observed in the same types of leprosy patients[13,14]. The antibody activity was noted in most of the classes of immunoglobulins (IgG, IgA, IgM)[13,15,16]. On chemotherapy the lowering of antibody levels were noted in lepromatous patients. Although the assay provided a great deal of

information, the specificity of the assay was in doubt due to its cross reactivity with BCG 60. To make the assay more specific the investigators modified the assay by preabsorbing the test sera with BCG, *M.avium* and *M.nonchromogenicum*[12]. As the specificity of the assay was doubtful, further modification of the assay were not done. Moreover due to application of the test to a limited number of people no conclusion could be drawn about its usefulness.

FLA-ABS test

Abe *et al*[17,18] standardised an immunofluorescent assay by using preabsorbed test sera with mycobacteria (BCG, *M.vacae*), cardiolipin and lecithin. Using this test more than 90% of LL and 80% of TT/BT patients were shown positivity[17,19]. The test was subjected to criticism when it was noted that even after preabsorption the test sera cross-reacted with *M.lepraemurium*, *M.bovis* (BCG) and *M.nonchromogenicun*[20]. Any serological test which would require preabsorption of antibody would remain variable because of the presence of an unknown quantity of antibody in the sera. Therefore, this test was also not found useful for the diagnosis of leprosy.

PGL-1 based ELISA

After the demonstration of the specificity of PGL-1[21] and its terminal sugars[22], PGL-1 or BSA-conjugated terminal sugars (natural disaccharide/natural trisaccharide) were used as antigens in the ELISA[5-7,23-27]. Using these assays approximately 90 to 95% of BL/LL and 50 to 60% of TT/BT patients were found to be positive. Further, it was noted that in individual groups there were wide variations in the levels of antibodies as determined by the variations in optical densities.

35 kDa based RIA/ELISA

Sinha *et al*[8] using monoclonal antibody (Mab) MLO4 (identifies a specific epitope on 35 kDa antigen of *M.leprae*) established a serum antibody competitioin test (SACT) or inhibition assay. The SACT which was established using ^{125}I MLO4 was developed later in an ELISA[28,29]. This test was adopted in various laboratories in the world. It detects nearly 100% of active BL/LL patients. However, more than 50% of TT/BT patients were found to be negative for the test[8,30-34].

Local Immunity in Leprosy Skin Lesions

From available informations it is evident that though the specific serological assays were able to diagnose the advanced stages of the disease (BL/LL), these assays failed to identify the disease at the early stages (TT/BT) of the spectrum. To understand the reasons for seronegativity in early stages of the disease serum samples were further obtained from BT patients for assessment of antibody levels against 35 kDa, PGL-1 and as well as whole *M.leprae* antigens. It was noted that 30 to 40% of BT patients were not positive for antibodies to specific antigens and also for antibodies against whole *M.leprae* antigens[35]. These findings clearly indicated that in the early stages of the disease (TT/BT) B-cell stimulation is not at a level which could ultimately result in a significant antibody response against the background level of antibody already present in the endemic population. Therefore, a study was undertaken to find out the local immune response in the lesions. Initially an organtypic culture of skin lesion was standardised[35]. Elution from these cultures established that 96% of the skin lesions of leprosy patients of all types secreted *M.leprae* specific antibody against 35 kDa antigen of *M.leprae*[35]. Further, skin cultures were carried out with TT/BT lesions and it was observed that these lesions produced antibodies which peaked at 24 hours and started declining after 48 hours[36]. These findings confirmed that in a large proportion of TT/BT patients there was no systemic *M.leprae* antibody response but there was a local antibody response in the skin lesion indicating an induction of Th2 type of immune response locally in the granuloma where Th1 type of immune response induced CMI is a predominant feature. To establish the coexistence of Th1 and Th2 types of immune response in the same granuloma kinetic studies were undertaken for measuring the of responses of Th2 inducing interleukins (IL-4,IL-6) and Th1 inducing cytokines (IFNg,TNFa) from the BT skin lesions. It was noted that while BL/LL patients liberated high levels of IL-4 and low levels of IFNg and TNFa, the BT/TT skin lesions secreted high levels of IFNg as well as high levels of IL-4 and IL-6. Recently, using immunophenotypic markers TT/BT lesions have been found to contain not only large numbers of T cells and macrophages but also B cells in the granulomas (CJIL unpublished observations).

Conclusions

It is evident from the above that specific serodiagnostic assays are not enough to establish diagnosis of all forms of leprosy. These tests failed to identify 30 to 40% of TT/BT leprosy patients. Studies on local immunity in the skin lesions indicated that a large proportion of TT/BT lesions produce antibodies locally without inducing significant proliferation of B cells which is a prerequisite for a systemic antibody response. Till now, the search

for specific antigens of *M.leprae* has been made by using sera of leprosy patients. Recently several new mycobacterial antigens have been identified using proteomic research. It is hoped that such research would lead to identification of many more *M.leprae* specific antigens which might be useful for the establishment of an early serodiagnostic test for leprosy.

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