SERODIAGNOSTIC TESTS FOR LEPROSY

U. SENGUPTA

Central JALMA Institute for Leprosy, Agra, India

ABSTRACT

All of the specific serodiagnostic tests developed by now only are able to detect advanced stages of the disease. None of these tests can efficiently diagnose leprosy at a very early stage of the disease including TT/BT cases. However, 35 kD based SACT and/or PGL-1 (ND-O/NT-P) based ELISA could be applied for confirmation in doubtful situations wherein cardinal signs of leprosy are not fulfilled. In addition, both of these tests can also be applied for efficiently monitoring patients on chemotherapy.

The reason for any of the specific tests not becoming useful for detection of early cases of leprosy might be due to the presence of insignificant level of antibody even below the background level of the population. It has been noted by us that majority of tuberculoid leprosy cases have a low level of antibody against the specific and whole *M.leprae* antigens (unpublished data). Therefore, attempts have been made to look for the presence of local antibody in the lesions of leprosy patients. It is expected that these studies would lead to the understanding into the mechanism of antibody secretion in early stages of the disease and would help in development of a specific serological test for early leprosy

KEY WORDS : *M.leprae*, leprosy, diagnosis

INTRODUCTION

Leprosy is still one of the major public health problems in India and more than 60% of the world leprosy patients are in India (1). The disease is diagnosed easily by its cardinal signs. However, in early stages of the disease, many a times cardinal signs are not satisfied and so there is a necessity of a laboratory test to confirm the diagnosis of leprosy. An attempt has been made here to give an account for the effort that has been put forth into the development of a serological test for the diagnosis of the disease.

Serological Tests Radioimmunoassay Using *M.leprae* Antigen

Based on the reactivity of a major antigen 7 of *M.leprae* which shows a cross reactivity with BCG 60, the test was developed as a radioimmunoassay by radiolabelling *M.leprae* antigen 7 with I¹²⁵. Using this assay level of *M.leprae* antibodies have been measured. While a high level of antibody was noted in lepromatous (borderline

lepromatous/lepromatous:BL/LL) patients, a low level was noted in tuberculoid (borderline tuberculoid/tuberculoid: BT/TT) patients.

Later Harboe et al (2) further modified the test by preabsorbing the test sera with BCG, *M.avium* and *M.nonchromogenicum*. However, in spite of the above cross-absorption, variation in the quantum of antibody due to the variability in the quantity of antibody present in the population will make the test non-specific.

Fluorescent Leprosy Antibody Absorption (FLA-ABS) Test

Abe et al (3) developed the FLA-ABS test using *M.leprae* as antigen.

The test is performed by preabsorption of serum with cardiolipin, lecithin, BCG and *M.vacciae* to neutralize the cross-reactive antigens before the indirect immunofluorescence assay.

The test is positive in approximately 90% to 100% of BL/LL and 70% to 80% of TT/BT patients. However, a large proportion of normal individuals and contacts also show positivity with

this test. Some studies have shown that FLA-ABS positive and lepromin negative individuals in the endemic region are at higher risk than the FLA-ABS positive and lepromin positive individuals in contracting the disease.

Recently, this test was further developed in an ELISA system in which sonicated *M.leprae* instead of whole *M.leprae* was used as antigen. Here also it was shown that antibody levels gradually increased from TT through LL and 100% of patients were positive to the test except the TT type of leprosy in which the positivity was only upto 66.6%. However, this test is subjected to criticism for not being the same as the FLA-ABS test. Here, *M.leprae* cell walls are disintegrated due to sonication and so the antibodies react with the internal cellular components of *M.leprae*. Whereas, in FLA-ABS test *M.leprae* antibodies react with only cell wall components.

Because of the importance of the assay several research groups in the world carried out a multicentric study using a preabsorbed lepromatous serum pool. This preabsorbed serum was found to cross react with *M.bovis* (BCG) and *M.nonchromogenicum*.

The test would remain still variable because of the use of the same quantity of antigen used for absorption of every serum sample which is well known for its variable content of antibodies. Further, because of this variability of absorption, in some samples cross-reactivity for other mycobacterial species would exist posing problem in the specificity of the test.

Enzyme Linked Immunosorbent Assay (ELISA) Using Phenolic Glycolipid-1 (PGL-1) or its Terminal Sugars.

PGL-1 was found to be a specific lipid antigen of *M.leprae*. Further, it was noted that the specificity lied on the terminal sugars of the lipid. Therefore, these sugars, natural disaccharides (ND) and natural trisaccharides (NT) of PGL-1 were synthesised. Both ND and NT were further conjugated with BSA (ND-O-BSA or NT-P-BSA) for use in the ELISA.

In BL/LL patients, presence of a high level of anti PGL-1 IgM antibody was demonstrated. About 90% to 95% of BL/LL patients and 25% to 60% of TT/BT patients were found to be positive for PGL-1 antibodies. Healthy controls from U.K., U.S.A., India and several other countries were

negative for the antibodies. However, cross-reactivity with normal control sera from a few countries have been reported.

Nevertheless, the sera from tuberculosis patients and other mycobacterial diseases did not show any reactivity. When ND-O-BSA/NT-P-BSA were used instead of PGL-1 as antigen the test results were almost similar as noted in the PGL-1 ELISA. It was further noted that there was a steady increase in both IgM and IgG antibodies as the disease progressed from TT through LL indicating a direct relationship between antibody levels and the bacterial load, i.e., bacterial index (BI). Although, such a correlation was not always evident, most of the above studies have confirmed that for any specific value of BI the antibody levels have tremendous variations showing a wide scatter which have been discussed adequately (4).

Although, a recent field study (5) could not establish the relationship between the prevalence rates of leprosy and PGL-1 antibody but most of the case controlled studies in BL/LL patients indicated that there is a good correlation between the decline of BI, clinical score and the antibody levels during the course of chemotherapy.

A study in a contact population in which positivity was found to be 11.2% compared to that of 1.7% in the control group. Further, in a 2 year follow up study in contacts, they noted an attack rate of 8.3% in the seropositives compared to that of 0.4% in the seronegatives. A recent study indicated that although there was elevated levels of PGL-1 antibody in hyperendemic population, it did not predict either a future clinical disease or individuals at high risk in developing the disease. Further, when the assay was applied in a large population in a hyperendemic area, seropositivity rates in contacts and noncontacts were almost similar indicating that PGL-1 antibody level would not be a useful indicator in detecting population at risk.

The above studies clearly indicated that PGL-1 (ND-O/NT-P) serology could only be useful for screening patients and for the follow-up of patients on chemotherapy. Hence, attempts were made for developing a simpler test in a passive haemagglutination test, *M.leprae* gelatin particle agglutination test (MLPT) and latex agglutination test. Recently, the gelatin based agglutination test was marketed in the form of a kit (SERODIA-leprae, Fujirebio Inc., Japan) and it was mentioned that the test can reliably detect multibacillary pa-

tients. However, several authors (3,4) pointed out its limitations in diagnosing leprosy in hyperendemic areas.

Other Lipids and Carbohydrates

Other lipids like dimannosylated phosphatidylinositol and sulfolipid-IV and a carbohydrate, lipoarabinomannan have been used. However, due to their cross-reactivity with other mycobacterial species, their use as specific diagnostic tests have remained limited.

Serum Antibody Competition Test (SACT) or Inhibition Assay (IA) using Monoclonal Antibody (MAb) Against Specific Epitopes of *M. leprae*.

Sinha et al (6) using MAb (ML04) (identifies a specific epitope on 35 kDa antigen of *M. leprae*) established a SACT assay. The SACT assay which was established using I125 ML04 has been developed in an ELISA (7). This test has been adopted in various laboratories in the world. It detects almost 100% of active BL/LL patients, mostly with high inhibition dilution 50 titre. More than 40% of TT/BT patients and about 10% of healthy contacts have been found to be positive for the assay. The importance of MLO4 based SACT along with other serological assays for evaluation of clinical activity of neuritic leprosy has been established. While correlating with number of patches or number of nerve involvement in paucibacillary leprosy, the antibody levels showed a direct correlation with the extent of lesion. Further, in a long term follow-up study of BL/LL patients under chemotherapy 35 kDa based SACT correlated best with the reduction of BI and the clinical score.

While screening healthy contacts, 10% positivity was noted in this population. In a two year follow-up study of a contact population Sinha et al (8) noted that a larger proportion of contacts developed clinical disease from the antibody positive group than from the antibody negative group. However, a large field study conducted in a hyperendemic area already indicated that the

SACT would not be useful as a diagnostic test for detection of future cases because of its low level of positivity in healthy contacts (Gupte et al, manuscript under preparation). In a recent comparative study between 35 kDa based SACT and PGL-1 based MLPT it was proved that SACT is more specific than MLPT.

Other Proteins Suggested for Serodiagnostic Tests

Although, several recombinant proteins have been used in the serology of leprosy but none of these was sensitive enough to identify significant numbers of advanced cases of leprosy. However, recently 135 kD fusion protein (referred to as LSR antigen/protein) has been extensively used to study the seroreactivity of this antigen and it was noted that while only 50-60% of stable LL patients' sera reacted with LSR lysate, 98% sera from type 2 reactional patients reacted with this protein.

Recently, 29/33 kDa doublet delipidified cell wall proteins have also been used for diagnosis. However, their use in a large number of serum samples are needed to form a definite opinion for their ultimate use in the diagnosis of leprosy.

Nerve Antigen for Serodiagnosis

Nerve involvement leading to its damage is a primary and early feature of leprosy. Due to the sequestration of nerve antigens at a very early stage, it is expected that these antigens would lead to production of antibodies in the host immune system. Based on this understanding many workers attempted to measure the antibody level in patients using either whole nerve extract or any one of the major components of nerve like basic myelin protein, sphingolipids, cerebrocides as antigens in an indirect ELISA system. However, majority of these studies failed to demonstrate the presence of antineural antibodies in a significant number of leprosy patients and so it was not possible to develop any meaningful serological test which could help in the diagnosis of leprosy.

REFERENCES

1. Noordeen, S. K. (1995) Elimination of leprosy as a public health problem, progress and prospects. Bull WHO 73, 1-6.
2. Harboe, M., Closs, O., Rees, R.J.W. and Walsh, P. (1978) Formation of antibodies against

- Mycobacterium leprae* antigen 7 in armadillo. J. Med. Microbiol, 11, 525-35.
3. Abe, M., Minagawa, F., Yashino, Y., Ozawa, T., Sakawa, K. and Saito, T. Fluorescent leprosy antibody absorption (FLA-ABS) test for detecting sub-clinical infection with *Mycobacterium leprae*. Int. J. Lepr. 48, 109-19.
 4. Sinha, S., Mc Entegart, A., Girdhar, B.K., Bhatia, A.S. and Sengupta, U. (1989) Appraisal of two *Mycobacterium leprae* specific serological assays for monitoring chemotherapy in lepromatous (BL/LL) leprosy patients. Int. J. Lepr. 57, 24-32.
 5. Fine, P.E.M., Ponnighaus, J.M., Burgess, P., Clarkson, J.A. and Draper, C.C. (1988) Seroepidemiological studies of leprosy in Northern Malawi based on an enzyme linked immunosorbent assay using synthetic glycoconjugate antigen. Int. J. Lepr. 56, 243-254.
 6. Sinha, S., Sengupta, U., Ramu, G. and Ivanyi, J. A. (1983) serological test for leprosy based on competitive inhibition of monoclonal antibody binding to the My2a determinant of *Myco-bacterium leprae* Trans. Roy. Soc. Trop. Med. Hyg. 77, 869-871.
 7. Mawatha, J., Moreno, C., Sengupta, U., Sinha, S. and Ivanyi, J. (1988) A comparative evaluation of serological assays for lepromatous leprosy. Lepr. Rev. 59, 195-199.
 8. Sinha, S., Ramu G., Girdhar, B.K. and Sengupta, U. (1985) A monoclonal antibody based competition radioimmunoassay for monitoring anti-*M.leprae* antibodies in leprosy patients and contacts. Ann.Nat.Acad.Med.Sci.(India), 21, 1.
 9. Prakash, O., Beuria, M.K., Girdhar, B.K., Katoch, K. and Sengupta, U. (1997) Efforts in diagnosing leprosy using serological techniques. J Biosci.22,111-6.