INTERNATIONAL JOURNAL OF LEPROSY

Volume 67, Number 3
Printed in the U.S.A.
(ISSN 0148-916X)

CORRESPONDENCE

This department is for the publication of informal communications that are of interest because they are informative and stimulating, and for the discussion of controversial matters. The mandate of this JOURNAL is to disseminate information relating to leprosy in particular and also other mycobacterial diseases. Dissident comment or interpretation on published research is of course valid, but personality attacks on individuals would seem unnecessary. Political comments, valid or not, also are unwelcome. They might result in interference with the distribution of the JOURNAL and thus interfere with its prime purpose.

A Study on the Methods for Early Serological Diagnosis of Leprosy and Their Potential Use

TO THE EDITOR:

As is well known, leprosy is a chronic infectious disease caused by *Mycobacterium leprae.* It principally affects the peripheral nerves and the skin. In persons suffering from lepromatous leprosy, leprosy is a generalized disease affecting several systems and many organs. Recent studies have indicated that, although multidrug therapy (MDT) has been implemented, the leprosy detection rates still have not declined and in some areas of the world have even increased, as has been the case with leprosy relapse due to drug resistance. Hence, leprosy is still a disease which will continue into the next century.

In 1997, the World Health Organization (WHO) Expert Committee on Leprosy pointed out that continued leprosy research was needed (WHO working paper; Seventh Meeting of the WHO Expert Committee on Leprosy, 26 May–3 June 1997, Geneva). According to the WHO Expert Committee on Leprosy's research agenda, the tests to measure *M. leprae* infection is still an important project. The tests for the serological diagnosis of leprosy (TSDL), especially ELISA based on phenolic glycolipid-I (PGL-I) and their artificial products of epitopes as antigens, have been standardized. However, their practical evaluations still have not been determined. We have systematically evaluated these TSDL, and report the results in this communication.

MATERIALS AND METHODS

Sera for detection. Sera were collected from 644 cases with various types of leprosy (155 LL, 168 BL, 95 BB, 114 BT, 112 TT) classified according to Ridley-Jopling criteria; 160 patients with tuberculosis; 570 normal controls (NC) in a nonendemic area of leprosy; 884 household contacts (HC), 3603 random population (RP), 369 leprosy patients clinically cured with dapsone (LPD), and 8 multibacillary patients to be treated with MDT (MPM).

Antigens. Materials used included: Whole *M. smegmatis* (Ms), prepared in our laboratory; whole *M. leprae* (WML), provided by Dr. Douglas; PGL-I, ND-O-BSA and ND-P-BSA, provided by Dr. Brennan; NT-O-BSA and NT-P-BSA, provided by Dr. Buchanan. HRP-IgA, -IgG, -IgM and -IgAGM were provided by Dr. Douglas. FITC-IgG was provided by Dr. Abe.

The TSDL evaluated, a total of 12 technologies, were as follows:

1. Fluorescent leprosy antibody absorption test (FLA-ABS), according to Abe's technology ([3]).

2. Indirect enzyme-linked immunosorbent assay (ELISA) based on PGL-I as an antigen (PGL-I-ELISA), according to Brennan's technology ([6, 7]).

3. ELISA based on *M. smegmatis* as an antigen (Ms-ELISA), according to Douglas' technology ([4]).

4. ELISA based on WML as an antigen (ML-ELISA), according to Brett's technology ([9]).

5. ELISA based on ND-O-BSA, NT-O-BSA, ND-P-BSA and NT-P-BSA as antigens separately (ND-O-BSA-ELISA, NT-O-BSA-ELISA, ND-P-BSA-ELISA and NT-P-BSA-ELISA), basically according to Brennan's procedures; some parameters have been optimized by us ([2, 6]).

6. Serum antibody competition test (SACT), basically according to Klatser's and Shinha's procedures; some parameters have been optimized by us ([2]).

7. Monoclonal antibody specific binding assay (McAb/SBA), established by Wu, Q., *et al.* ([5]).

8. Latex agglutination test (LAT), established by Wu, Q., *et al.* ([1]).

9. Gelatin particle agglutination test (GPAT) (Fujerebio, Inc., Tokyo, Japan), provided by WHO.

Statistical analysis. The cut-off value, i.e., normal value, was determined by the percentile method; evaluations for various methods were conducted with the /N ratio, Student's *t* test, correlation analysis, individual agreement, sensitivity, specificity, Youden's Index, likelihood ratio (LR), etc.

RESULTS

Results of 12 TSDL evaluations. A total of 12 methods were established according to the original author's procedures and/or our procedures (names and procedures in Materials and Methods). Results for the evaluations were as follows:

From the data obtained based on parameters of sensitivity, specificity, practicability and correlation among them on a large scale, the results indicated that: a) For detecting the antibody level ND-O-BSA-ELISA is the best, PGL-I-ELISA is second. Ms-ELISA may be used as a primary tool for screening infection with *M. leprae* in order to save specific artificial products as antigen. b) LAT, GPAT, and ND-O-BSA ELISA were comparable in detecting results; LAT and GPAT are simple, more rapid and more suitable for use in the field. c) artificial products were better than natural antigens, especially easier to coat and to standardize. d) McAb-SBA were more suitable for detecting paucibacillary (PB) cases because a higher detection rate was obtained in PB patients.

Blood from the earlobes may be substituted for venous blood, and the blood may be absorbed onto a filter paper strip and dried at room temperature (filter paper blotting dried blood spot); the dried spot can be reconstituted by adding a suitable amount of water for the detection tests. Its advantages are: the equipment for collecting blood is simpler, the blood is easier to collect, transport, store and keep the antibody reactivity; this method is cheaper, making a large-scale study possible.

After systematically evaluating the two kinds of inexpensive blocking agents (skim milk and egg albumin), we discovered their optimizing conditions for TSDL. They not only increased the specificity and sensitivity of the TSDL but they also made them more simple and more economical.

Studies on practicability. Our studies on practicability included: 1) Sero-immunological epidemiology. In ND-O-BSA-ELISA, the increase or decrease of the optical density (OD) value has a positive correlation with the bacterial index (BI) and the order of positive rates was: a) in various types of leprosy: LL>BL>BB>BT>TT; b) in household contacts (HC), random population (RP), normal controls of endemic areas (ENC), and normal controls of nonendemic areas (NNC): HC>RP>ENC>NNC. 2) In populations with subclinical *M. leprae* infection: the highest risk group was between the ages of 15 and 25 with an increase or a persistence of high OD values prior to the onset of disease. 3) OD values gradually decreased over time following treatment and these declines paralleled declines in the BI. 4) In clinically cured leprosy cases after dapsone therapy, an increase or a persistence of high OD values in the ND-O-BSA-ELISA preceded the onset of leprosy relapse.

DISCUSSION

Basic evaluation for TSDL. For sensitivity, specificity, speed, simplicity and economy the ND-O-BSA-ELISA and the GPAT are recommended. The former was

more sensitive than the latter. The latter is more simple and more rapid than the former, thus more suitable for use in the field. The other assays can be used under certain conditions. Although none of the TDSL could be used independently to confirm diagnosis or as typing tools for leprosy, each of them except the Ms-ELISA can provide support for the diagnosis of leprosy, especially in its early stages and for excluding suspected cases, and for infection with other mycobacteria, as well as for follow up after completion of treatment, early detection of relapse and even for distinguishing relapse from reversal reaction (WHO working paper; Seventh Meeting of the WHO Expert Committee on Leprosy, 26 May–3 June 1997, Geneva). Additionally, TSDL can be used for sero-epidemiological studies to detect subclinical infection with *M. leprae* and for populations at high risk, for monitoring changes in the intensity of *M. leprae* exposure, and to determine epidemiological trends, extent, and transmission of infection with *M. leprae.* They will increase our knowledge of the epidemiology of *M. leprae* infection. TSDL do not detect all clinically apparent infections because the majority of paucibacillary patients do not demonstrate humoral response. This is an obstacle of TSDL. In order to solve this problem, the development of a new, more sensitive and more specific assay is very necessary.

Limitation of TSDL. TSDL of leprosy are clearly useful, although in a limited way. The reasons that the results differ from study to study may be that: a) all antigens' sensitivity and specificity are still not satisfactory; b) TSDL still need to be optimized and standardized. At present, different laboratories in the world conduct TSDL with different procedures and agents, especially quality control of sera collected from leprosy patients needs to be identified. As is well known, the effectiveness of blocking agents, their optimum concentrations and the sort of microplate influences on cut-off values often increase and make the OD values vary quite a bit among samples tested. c) it is difficult to collect the ideal sera for testing, e.g., samples of sera from various types of untreated leprosy patients and normal controls from a nonendemic area of leprosy cannot be obtained to fulfill the quality requirements of TSDL.

Strategies for studying methods for detection of *M. leprae* infection. Strategies for studying these methodologies include: a) before creating a satisfactory method, one ought to study the methods presently available for optimization and standardization and apply them to as many available ranges as possible; b) one should study the potential use of combining TSDL with the polymerase chain reaction; and c) one should create a new, more sensitive, more specific method based on serological and molecular biology principles; an ideal confirmation test especially needs to be developed.

SUMMARY

This is a serial study. In this series we have established 12 methods for the early serological diagnosis of leprosy, including the FLA-ABS test, ELISAs with artificial products (ND-O-, ND-P-, NT-O-, NT-P-BSA; PGL-I, whole *M. leprae* and *M. smegmatis*), monoclonal antibody specific binding assay (McAb/SBA), latex agglutination test (LAT), and MLPA. These methods were compared with each other on a large scale in leprsoy patients and in the field. The results indicate that 1) Excellent results were obtained when ELISAs were conducted with skim milk or egg albumin as the blocking agent and by using blood from earlobes instead of from venipuncture. 2) Accordng to the four "S" standard (sensitivity, specificity, simplicity and speed), among the 12 methods the ND-O-BSA-ELISA (ND-ELISA) is the best and the MLPA is more suitable for use in the field because it is simple and rapid. 3) In the ND-ELISA, the increase or decrease of the OD value has a positive correlation with the BI, and the order of positive rates was a) in various types of leprosy: LL>BL>BB>BT>TT; b) in household contacts (HC), random population (RP), normal controls in endemic areas (ENC) and normal controls in nonendemic areas (NNC): HC>RP>ENC>NNC. 4) In a population with subclinical *M. leprae* infection, the highest risk group was between the ages of 15 and 25 and had an increase or a persistence of high OD values prior to onset of disease. 5) OD values gradually decreased over time fol-

lowing treatment and these declines paralleled declines in the BI. 6) In cases cured with dapsone therapy, there was an increase or a persistence of high OD values in ND-ELISA prior to the onset of a leprosy relapse.

In conclusion, we have compared and evaluated 12 immuno-assays and have shown that the ND-ELISA is the most practical one for use in investigating sero-immunological epidemiology, subclinical infection with *M. leprae,* early detection of disease, monitoring of antimicrobial therapy, and even for the prediction of leprosy relapse.

—Qinxue Wu
Xinyu Li
Yueping Yin
Huiwen Shu
Wanhui Wei
Qi Liu
Ganyun Ye

Institute of Dermatology
CAMS and PUMC
Nanjing 210042
Jiangsu Province
China

REFERENCES

1. Wu., Q.X., Li, X. Y., Wei, W. H., Shen, J. P., Sheng, D. M. and Ye, G. Y. [Potential of gelatin agglutination test in the study of leprosy.] China Lepr. J. **9** (1993) 138–141.
2. Wu, Q. X., Li, X. Y., Yin, Y. P. and Hou, W. [A new SACT method and its comparison with PGL-I-ELISA.] China Lepr. J. **12** (1996) 108–112.
3. Wu, Q. X., Ma, Z. X., Shu, H. W., Zhou, L. L., Liu, Q., Ye, G. Y., Wang, T. J. and Ma, B. K. [Comparison of FLA-ABS test employing sera from venous blood and blood from earlobes of 79 cases of leprosy.] Acta Acad. Med. Sin. **7** (1985) 69–71.
4. Wu, Q. X., Wei, W. H., Liu, Q., Li, X. Y., Xue, Z. Y. and Ye, G. Y. Ms-ELISA for detection of serum antibody level in patients—establishment of Ms-ELISA.] Acta Acad. Med. Sin. **10** (1988) 451–455.
5. Wu, Q. X., Xue, Z. Y., Li, X. Y., Wei, W. H. and Ye, G. Y. [Detection of specific circulating immune complexes in leprosy patients by mouse monoclonal antibody against phenolic glycolipid-I.] Acta Acad. Med. Sin. **11** (1989) 293–297.
6. Wu, Q. X., Ye, G. Y. and Li, X. Y. Serological activity of natural disaccharide octyl bovine serum albumin (ND-O-BSA) in sera from patients with leprosy, tuberculosis and normal controls. Int. J. Lepr. **56** (1988) 50–55.
7. Wu, Q. X., Ye, G. Y., Li, X. Y., Liu, Q. and Zhou, L. L. A preliminary study on serological activity of a phenolic glycolipid from *M. leprae* in sera from patients with leprosy, tuberculosis and normal controls. Lepr. Rev. **57** (1986) 129–136.
8. Wu, Q. X., Ye, G. Y., Yin, Y. P., Li, X. Y., Liu, Q. and Wei, W. H. Rapid serodiagnosis for leprosy—a preliminary study on the latex agglutination test. Int. J. Lepr. **58** (1990) 328–333.
9. Wu, Q. X., Ye, G. Y., Zhou, L. L., Shu, H. W., Liu, X. Y., Ma, Z. X. and Li, Z. W. Determination of antibodies in dried blood from earlobes of leprosy patients by enzyme-linked immunosorbent assays—a preliminary report. Int. J. Lepr. **53** (1985) 565–570.

Vaccination of Mice Against the Leprosy Bacillus with Skin-Test Antigens

To the Editor:

The specter of approximately 700,000 new leprosy cases a year, despite a prevalence of only about 805,000 ([11, 12]), haunts us. Many, perhaps the majority, of these cases are detected in the course of intensive case finding ([11]), and are single-lesion cases which are difficult to diagnose definitively. Measurement of the true incidence of leprosy requires new diagnostic tools, more sensitive than physical examination supported at times by slit-skin smear ([9]). Neither serology, even the modern, user-friendly, sensitive phenolic glycolipid-I (PGL-I)-based kits ([5]), nor gene-amplification based on a variety of primers provides the necessary sensitivity ([10]). A promising approach to the diagnosis of asymptomatic leprosy is provided by the availability of two new skin-test antigens—MLSA-LAM (soluble antigens of *Mycobacterium leprae*