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Skin test activity of an antigen fraction prepared from *Mycobacterium leprae* compared with standard lepromin and tuberculin PPD in leprosy patients

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Summary The reactivity of a cell wall antigen fraction, MLW1, prepared from *Mycobacterium leprae*, which induces strong lymphocyte responses *in vitro*, was compared in the lymphocyte stimulation test (LST) and the 48-hr skin test reaction in leprosy patients. A strong LST response was usually accompanied by a strong skin test response and *vice versa*. As a skin test reagent MLW1 was compared with standard lepromin and tuberculin PPD, and a significant correlation ($r = 0{\cdot}79$, $p < 0{\cdot}001$) was found between MLW1 and standard lepromin. Being a purified and highly-active preparation that can be standardized based on protein concentration, MLW1 should be considered as an alternative to lepromin in the early reaction.

Introduction

The standardization of lepromin is based on the number of acid fast bacilli/ml.[1] In contrast, the standardization of tuberculin PPD is based on functional activity assayed in experimental animals. Another disadvantage of lepromin compared to tuberculin, which has been shown in both experimental animals[2] and man[3,4], is that repeated testing leads to sensitization of the test subject.[5] A skin test reagent which does not induce sensitization and is easy to standardize, like tuberculin, would be a great improvement in leprosy.

A cell wall antigen preparation from *Mycobacterium leprae* called MLW1,

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producing only one line corresponding to *M. leprae* antigen 7 in crossed immunoelectrophoresis, induced strong *in vitro* lymphocyte responses in patients with tuberculoid leprosy and in healthy contacts of leprosy patients, while lymphocytes from lepromatous leprosy patients and non-exposed controls did not respond.[6] Like PPD, MLW1 also consists of solubilized antigens in which the amount of protein can be determined. Thus, it can be standardized on the basis of biological activity per μg protein as PPD for use as a skin test reagent. However, since MLW1 is known to contain crossreacting antigenic determinants, a marked contribution of such determinants to the skin test activity of the preparation would reduce its usefulness as a skin test reagent.

In the present study the ability of MLW1 to stimulate lymphocytes *in vitro* was compared with its skin test activity in leprosy patients. Its potency and specificity as a skin test reagent were compared with standard lepromin and tuberculin PPD.

Materials and methods

PATIENTS

All the patients in this study attended the All Africa Leprosy and Rehabilitation Training Centre (ALERT) in Addis Ababa, Ethiopia. Thirty-four patients, 16 females and 18 males, with a median age of 24 years (range 13 to 39), were included. They were all classified clinically according to the Ridley-Jopling scale,[7] and, in addition, 13 patients were also classified histologically. There were two patients with tuberculoid/borderline tuberculoid leprosy (TT/BT), 21 with BT, 6 with borderline lepromatous (BL) and 5 with lepromatous leprosy (LL). Ten of the patients had been treated with dapsone (DDS, 100 mg/day) for a period varying from 1 month to 5 years; the others were untreated.

SKIN TESTING

The following three antigens were injected intradermally on the same forearm with the injection sites at least 4 cm apart in a volume of 0·1 ml: 1 The lepromin (obtained from Dr W F Kirchheimer, Carville, Louisiana) was armadillo derived and contained $1{\cdot}6 \times 10^7$ bacilli. 2 A fractionated preparation of *M. leprae* of armadillo origin, called MLW1[6], 0·2 or 2 μg protein. 3 Tuberculin purified protein derivative (PPD) from Statens Seruminstitut, Copenhagen, Denmark, Batch RT23, 0·04 μg protein (2 TU). Reactions were read after 48 hr; the induration was measured with a ruler and the mean of the longitudinal and transverse diameter recorded. An induration of 5 mm or more was considered a positive reaction.

LYMPHOCYTE STIMULATION TEST

Blood was drawn for the LST on the same day as skin testing was performed, but prior to the injection of the antigen. Mononuclear cells were isolated and cultured as previously described.[6] Briefly, 10^5 cells/well were stimulated with 1 MLW1, the same preparation as used in skin testing, and 2 tuberculin PPD, Batch RT33, Statens Seruminstitut, and then cultured in triplicates for 6 days. Proliferation was measured as ^{3}H-thymidine incorporation. The median counts per minute (cpm) for each triplicate was used and the degree of stimulation expressed as Δcpm = cpm of stimulated culture − cpm of unstimulated control. According to our previous study[8] an individual with an LST response of Δcpm $\geqq$ 5000 was defined as a responder.

Results

The antigen preparation MLW1 was tested as a skin test reagent in patients with various clinical forms of leprosy at two doses, either 0·2 or 2 μg per skin test site. Figure 1 compares in 23 patients the capacity of MLW1 to induce a 48-hr skin reaction with its capacity to stimulate lymphocytes *in vitro* ($r = 0{\cdot}66$, $p < 0{\cdot}001$) at a concentration of 0·1 μg/ml which was shown before to give the highest median response in the BT group.[6] In 11 of the patients a positive LST response was accompanied by a positive skin test response and *vice versa*. Five patients (1

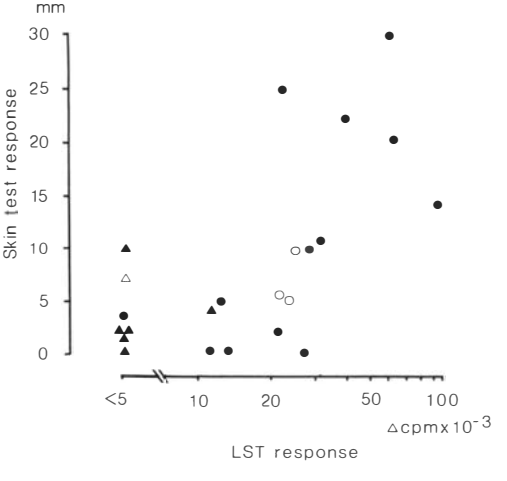

Figure 1. *In vitro* lymphocyte responses to 0·1 μg/ml and skin test responses to 0·2 μg (○, △) and 2 μg (●, ▲) of MLW1 in patients with various clinical forms of leprosy (TT/BT, n = 2; BT n = 14; ●, ○), (BL, n = 4; LL, n = 3; ▲, △). Lymphocyte stimulation is shown as net counts per minute (Δcpm = cpm of stimulated culture − cpm of unstimulated control) after incorporation of ^{3}H-thymidine, and skin test activity as diameter of induration in mm read at 48 hr.

TT/BT, 3 BT; 1 BL) with moderately strong LST responses (11,000 Δcpm to 27,000 Δcpm) showed a negative skin test response, while two patients (2 BL) with moderate skin test response (7 and 10 mm) showed very weak LST responses, 2300 Δcpm and 1500 Δcpm, respectively. In the 16 patients (2TT/BT, 13 BT, 1 BL) who were responders in the LST (Δcpm $\geqq$ 5000) the strength of the responses correlated better ($r = 0{\cdot}70$, $p < 0{\cdot}01$) than in the 13 patients (2 TT/BT, 9 BT, 1 BL, 1 LL) who showed a positive skin test response ($\geqq$ 5 mm) ($r = 0{\cdot}50$, $p < 0{\cdot}10$).

In 14 of the patients with TT/BT and BT leprosy, the skin test and LST were performed with both MLW1 and PPD, and in Figure 2(A) and (B), the potencies of these two reagents are compared in both tests. Based on the protein concentrations which were used, the ratio between the doses of MLW1 and PPD was 1:10 in the LST and 50:1 in the skin test. There were three patients who showed stronger responses to MLW1 than to PPD in the LST (Figure 2(A)), and two of these were among the seven patients (with a positive skin test response to MLW1) who showed stronger skin test responses to MLW1 than to PPD (Figure 2(B)), showing that antigenic specificity was expressed in both tests.

In Figure 3 skin test activity of standard lepromin and MLW1 is compared in patients throughout the leprosy spectrum. Fourteen of the patients were tested with a dose of 0·2 μg MLW1 per skin test site, and three of these were negative to MLW1 and positive to lepromin. The remaining 20 patients were tested with a dose of 2·0 μg MLW1, and three of these were positive to MLW1 and negative to lepromin. A dose of MLW1 between 0·2 and 2·0 μg appeared to correspond in

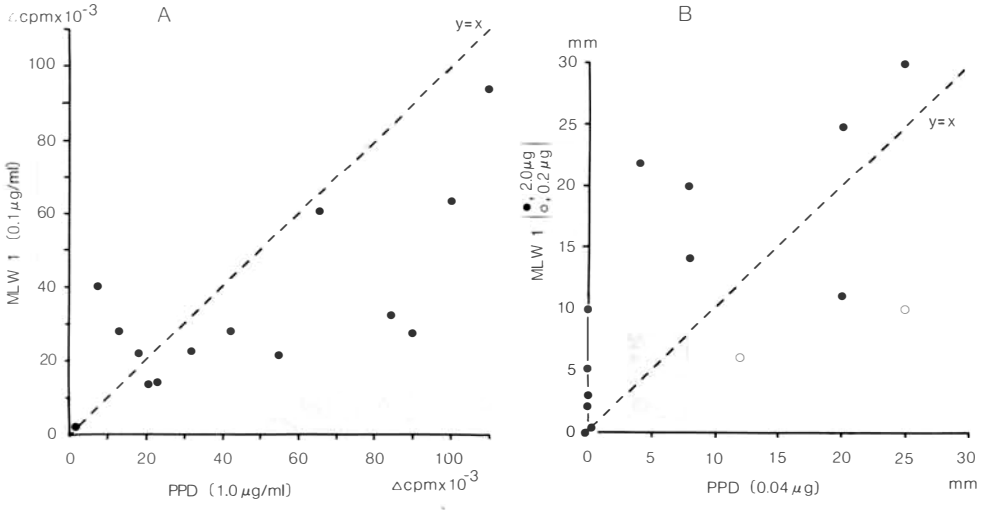

Figure 2. *In vitro* lymphocyte stimulation (A) and skin test activity (B) in 14 patients with TT/BT and BT leprosy. They were skin tested with a dose of 2 μg of MLW1 (●) except for two patients who were tested with 0·2 μg (○), while the strength of PPD was 2 TU (0·04 μg). The lines of identity, y = x, are stippled. For further explanation see legend to Figure 1.

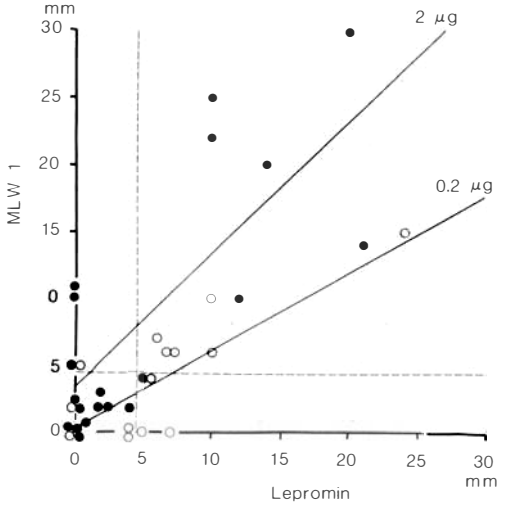

Figure 3. Correlation plot of skin test activities with MLW1 (2 μg, ●; 0·2 μg, ○) and lepromin in 34 patients with various clinical forms of leprosy (TT/BT, n = 2; BT, n = 21; BL, n = 6; LL, n = 5). The lines of regression at both doses are solid and the dividing lines between positive and negative responses are stippled. For further explanation see legend to Figure 1.

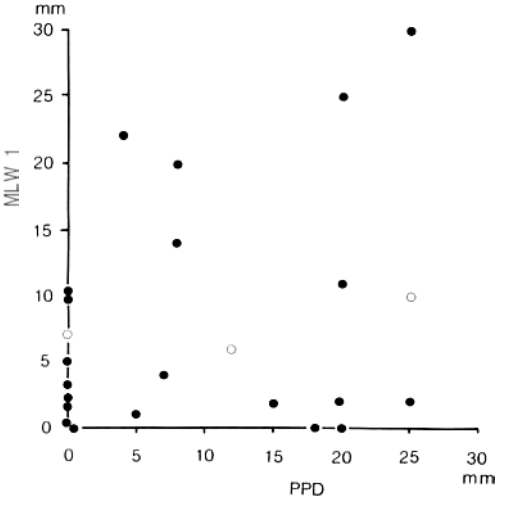

Figure 4. Correlation plot of skin test activities with MLW1 (2 μg, ●; 0·2 μg, ○) and tuberculin PPD, 2 TU, in 24 leprosy patients (TT/BT, n = 2; BT, n = 14; BL; n = 5; LL, n = 3). For further explanation see legend to Figure 1.

strength to standard lepromin. A correlation was found in skin test activity between lepromin and MLW1 ($r = 0{\cdot}79$, $p < 0{\cdot}001$).

Previously, we demonstrated[6] that tuberculin PPD and MLW1 show different antigenic specificities in the LST, and in the present study no correlation ($r = 0{\cdot}26$) was found between MLW1 and tuberculin PPD in the 24 patients tested with both antigens (Figure 4). Apparently, antigenic determinants which may be common to MLW1 and PPD did not greatly influence the skin test activity of MLW1.

Discussion

Using various antigens, skin test and LST responses have been reported by many workers to correlate well,[9–14] and soluble antigens have given better correlations than particulate antigens.[15] In leprosy, a crude antigen consisting of *M. leprae* bacilli of either human or armadillo origin has been most commonly used both in the skin test and the LST assays. Leprosin A, a skin test reagent made by Stanford *et al.*,[16] is a total sonicate of the bacilli, and since it consists of solubilized antigens, its protein content can be determined. But, in contrast to the MLW1 preparation which contains mainly one antigenic component, Leprosin A consists of various antigenic components. The MLW1 preparation has previously been shown to be a particularly potent stimulator in the LST.[6] Looking at individual responses both in leprosy patients and healthy contacts of leprosy patients, they were higher for MLW1 than for a preparation of whole *M. leprae* bacilli of human origin.[8] In the present study we have compared the potency of MLW1 to stimulate lymphocytes *in vitro* with its potency to induce an early skin reaction in patients with leprosy. It seems that the MLW1 preparation can also induce a fairly strong reaction in patients with a positive LST response. The finding of a negative skin test response in some of the patients with a moderate to strong LST response is in agreement with others who reported the LST to be more sensitive than the skin test in tuberculosis.[17–19]

Antibodies to the ML7 antigen have been shown to cross-react extensively with the BCG60 antigen,[20] and these antigens appear to be the major constituents of the MLW1 preparation and tuberculin PPD, respectively. When the LST responses to MLW1 of healthy contacts of leprosy patients and of non-exposed controls were compared, they were completely separated,[6] and all the individuals in these two groups showed higher responses to PPD than to MLW1, in contrast to the group of patients with TT and BT leprosy where one third showed higher responses to MLW1 than to PPD.[21] In spite of the extensive cross-reaction which may be expressed to a varying extent in the individual responses, these results show that MLW1 and PPD are recognized as different antigens. With regard to the comparisons of the skin test and LST responses in the present study, it seems that the different antigenic specificity of MLW1 and PPD can be expressed in the skin test results, even though the relative doses of the two antigens used in these two tests are not directly comparable.

Because of the known cross-reactivity with other mycobacterial antigens, the MLW1 preparation should not be regarded as a specific reagent in tests for DTH to *M. leprae* until it has been further tested. However, one may compensate for a certain lack of specificity by using MLW1 in combination with other antigens like tuberculin PPD and looking at the relative responses in the skin test and LST.[6] Further elucidation of its specificity is needed to evaluate its role as a prospective skin test reagent in leprosy.

Acknowledgments

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