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# The Epidemiological Evaluation, in Burma, of the Skin Test Reagent LRA6; a Cell-free Extract from Armadillo-derived *Mycobacterium leprae*. Part 1: Leprosy Patients[1]

Michael J. Shield, John L. Stanford, Gallego Garbajosa, Philip Draper, and Richard J. W. Rees[2]

Old tuberculin and purified protein derivative (PPD) preparations from mycobacteria have been used as skin test reagents for many years. In particular, preparations from *Mycobacterium tuberculosis* have been used to measure the amount of natural tuberculosis infection in a community and the extent to which a cell-mediated response has been induced by BCG vaccination.

More recently, ultrasonicated extracts of live mycobacteria from a variety of species have been used in a number of skin test studies. These preparations, referred to as "new tuberculins," have greatly increased antigenic specificity compared with old tuberculins and PPDs and have been used successfully in several countries ([7, 13, 15, 17, 18]) to investigate the degree to which populations have been sensitized to environmental mycobacteria.

Until recently the inability to culture *M. leprae* has not allowed a comparable *M. leprae*-derived antigen to be prepared for use in epidemiological studies. Two factors, the discovery that *M. leprae* can be grown abundantly in the armadillo ([4]) and the development of suitable techniques for the separation and purification of the bacilli from the host tissues, have changed this situation. Batches of infected tissue from this source have been used, not only for making lepromin ([6]), but also for providing purified *M. leprae* for use in the production of ultrasonicated extracts. A skin test reagent so prepared from one of the first batches (LRA4) was used in a pilot study to assess the optimum dosage to be administered. A similar preparation from a subsequent batch (LRA6) was used in the epidemiological study described below, which was carried out on the population of a high leprosy endemicity area around Mandalay in Burma. Being a cell-free extract, it is comparable to the "new tuberculins" described above. It differs markedly from other and older skin test reagents, i.e., lepromins and leprolins, and was called LRA6 to denote that it was batch # 6 of Rees' leprosy reagent from armadillo origin. Its use as a skin test reagent in leprosy patients is presented in the first part of this paper. The work described was carried out as part of a more extensive study which included the use of "new tuberculins" of a number of other mycobacterial species.

## MATERIALS AND METHODS

### Reagents used and their administration

The *M. leprae* reagent (LRA6) was prepared from the livers and spleens of experimentally infected armadillos. The tissues had been stored at −20°C and were sterilized by gamma radiation (2.5 Mrad from $^{60}Co$) before processing. Bacteria were purified by the method of Draper ([3]) which involves homogenization, differential centrifugation, treatment with Triton ×100 and pronase (and with collagenase in the case of the spleen) and banding on sucrose den-

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[2] M. J. Shield, M.R.C.Path., Senior Lecturer in Bacteriology, Wright-Fleming Institute of Bacteriology, St. Mary's Hospital Medical School, Praed Street, London W2, England. J. L. Stanford, M.D., Reader in Bacteriology, Middlesex Hospital Medical School, London W1, England. G. Garbojsa, Team Leader, WHO Leprosy/BCG Trial, Burma. P. Draper, D.Phil., Scientific Staff; R. J. W. Rees, F. R. C. Path., Head, Laboratory for Leprosy and Mycobacterial Research, The National Institute for Medical Research, Mill Hill, London, England.

sity gradients. They were suspended in 0.05% (w/v) Tween 80 and broken ultrasonically at 20 kHz for 20 min at 4°C with an MSE 150 watt ultrasonic disintegrator using a 20 mm diameter probe and a tip displacement of 4 $\mu$m. Particles were removed by centrifuging at 35,000 $\times$ $g$ for 30 min. The supernatant was filtered with 0.45 $\mu$m and (twice) with 0.22 $\mu$m Millipore filters and standardized to a final protein concentration of 2 $\mu$g/ml determined spectrophotometrically according to the method of Warburg and Christian ([23]). This dose was chosen as being the one, as assessed by the pilot study, that produced the optimal size of response. In subsequent studies ([19]) where cell-free extracts of *M. leprae*-A have been used, a high concentration (10 $\mu$g/ml) has been employed. This brings the response sizes into a comparable range with those achieved with other "new tuberculins."

Skin tests were performed by the intradermal injection of 0.1 ml of the reagent into the volar aspect of the forearm using 1 ml disposable tuberculin syringes (Gillette scimitar) and 25 gauge needles.

Twenty-one other "new tuberculins" (from 19 mycobacterial species) were also employed in the survey. All were prepared from viable organisms grown on Sauton's medium by the method of Paul, *et al.* ([8]). Persons aged 11 years and older received LRA6 and three other "new tuberculins." Only one other reagent was given at the same time to those of 10 years or younger.

The transverse and longitudinal diameters of induration of the skin test responses were read at 72 hr by one reader (M.J.S.) and the mean diameter in millimeters calculated. Responses of 5 mm or more were regarded as positive. Chi-square was calculated and probability values for significant differences between groups were derived from tables.

**Areas of study**

The majority of persons studied were indigenous to the Irrawaddy Valley region north of Mandalay, where the Burmese Government and the World Health Organization (WHO) are conducting a joint BCG trial against leprosy ([2]). This is a chiefly rural area with some 100,000 inhabitants. Leprosy prevalence is high although the incidence and prevalence of the disease vary considerably from village to village. Marked differences were noted in the mode of life, social behavior and type of work undertaken by men and women. Women spent more time at home engaged in cottage-style industries while men, from around 14 years of age upwards, spent more time away from the village in pursuit of their agricultural work. The movement of males between villages was an everyday occurrence, while movement of females occurred more as a group event for such activities as attendance at marriage or religious ceremonies. Such basic differences between the sexes with respect to their social behavior may well account for some of the observed differences with regard to skin test responses and leprosy prevalence.

The WHO trial area is physically divided into east and west bank communities by the natural barrier provided by the Irrawaddy River. This is especially marked during the monsoon months and it was in this monsoon and pre-monsoon period that our study was carried out.

**Patients tested**

All the leprosy patients tested came from the area described, with the exception of 13 tested at Rangoon's leprosarium and 30 female tuberculosis patients from the Aung Sang Tuberculosis Hospital in Rangoon. The following clinical types of leprosy were recognized: tuberculoid (TT), borderline tuberculoid (BT), borderline (BB), borderline lepromatous (BL), lepromatous (LL and LI), and indeterminate (I). These groups to which the patients were assigned are defined by the Ridley-Jopling classification criteria ([9]). However, it must be pointed out that while the Ridley-Jopling classification is based on both clinical and histological criteria, only a few of the patients in this trial had been biopsied. The histological data were thus incomplete and groupings therefore had to be made on clinical features alone.

Data concerning the leprosy incidence and prevalence for each of the disease types in each of the villages of the WHO trial area were available from the WHO records. Each of the persons tested had name, age, sex and BCG vaccination status recorded. Patients, in addition to being classified as described above, had the duration of their dis-

TABLE 1. *Patient groups studied and the number and percentage of positive reactors (≥5 mm induration) to LRA6.*

| Population group | Male | | Female | | Total | | Mean age in years | | |
|---|---|---|---|---|---|---|---|---|---|
| | No.+/ total | (%+) | No.+/ total | (%+) | No.+/ total | (%+) | Male | Female | Total |
| Leprosy patients | | | | | | | | | |
| Tuberculoid (TT) | 36/77 | (47%) | 30/85 | (35%) | 66/162 | (41%) | 22.0 | 23.9 | 23.0 |
| Borderline tuberculoid (BT) | 0/6 | (0%) | —[a] | | 0/6 | (0%) | 21.0 | —[a] | 21.0 |
| Borderline (BB) | 0/4 | (0%) | 0/5 | (0%) | 0/9 | (0%) | 43.5 | 40.6 | 41.9 |
| Borderline lepromatous (BL) | 0/10 | (0%) | 1/6 | (17%) | 1/16 | (6%) | 40.3 | 33.8 | 37.9 |
| Lepromatous (LL & LI) | 2/72 | (3%) | 1/26 | (4%) | 3/98 | (3%) | 44.1 | 46.4 | 44.7 |
| Lepromatous with tuberculosis | 0/10 | (0%) | 1/3 | —[b] | 1/13 | (8%) | 46.4 | 65.3 | 50.8 |
| Indeterminate | 3/15 | (20%) | 3/15 | (20%) | 6/30 | (20%) | 16.8 | 18.8 | 17.8 |
| Tuberculosis patients | —[a] | | 3/30 | (10%) | 3/30 | (10%) | —[a] | 35.3 | 35.3 |

[a] None tested.
[b] Too few results to give meaningful percentage.

ease and the extent and site of their lesions noted.

Enough ancillary data concerning 143 of the 162 TT patients tested with LRA6 were available for further analyses to be performed, since all but one of the 162 had developed their disease since the commencement of the WHO/Burmese trial. These 143 patients were grouped according to their predominant type of lesion and a correlation was made with the type of leprosy index case contacted prior to the patient developing his/her disease. Three predominant types of lesion were recognized clinically, these being:

a) "Neural" leprosy—involvement of a single nerve, e.g., the ulnar or the common peroneal, without clinical skin involvement.
b) Single "skin" lesions—without clinical evidence of nerve involvement.
c) "Multiple" lesions—nerve and skin affected with two or more dermatomes involved.

This latter "multiple" lesion group had been classified as TT in the trial area and might have been considered as BT by others. In the results presented below this group has been included in two separate analyses; first with the rest of the TT patients and second with the BT group.

The 143 TT patients (which include the "multiple" lesion group) were also assessed on the basis of the type of index case they had contacted prior to developing their disease. Three forms of contact were recognized: with either bacilliferous (LL and BL) patients, non-bacilliferous patients (TT, BT or I), or no known contact (i.e., no close association with leprosy patients other than by living in an endemic area).

## RESULTS

The skin test results shown are for LRA6 alone. The total numbers of persons tested in each of the groups in the study together with the mean ages of the persons within the groups and the number and percentage of positive reactors to LRA6 are shown in Table 1.

A histogram (Fig. 1) shows the results, in millimeters, obtained with LRA6 when tested in TT patients; results for the two sexes are shown separately.

The distribution of the 143 TT patients according to the two groups of criteria, type of lesion and nature of contact, is shown in Table 2. In Table 3, using the same framework, the percentage of positive responders to LRA6 among these 143 patients is shown.

In Table 2 there is an association be-

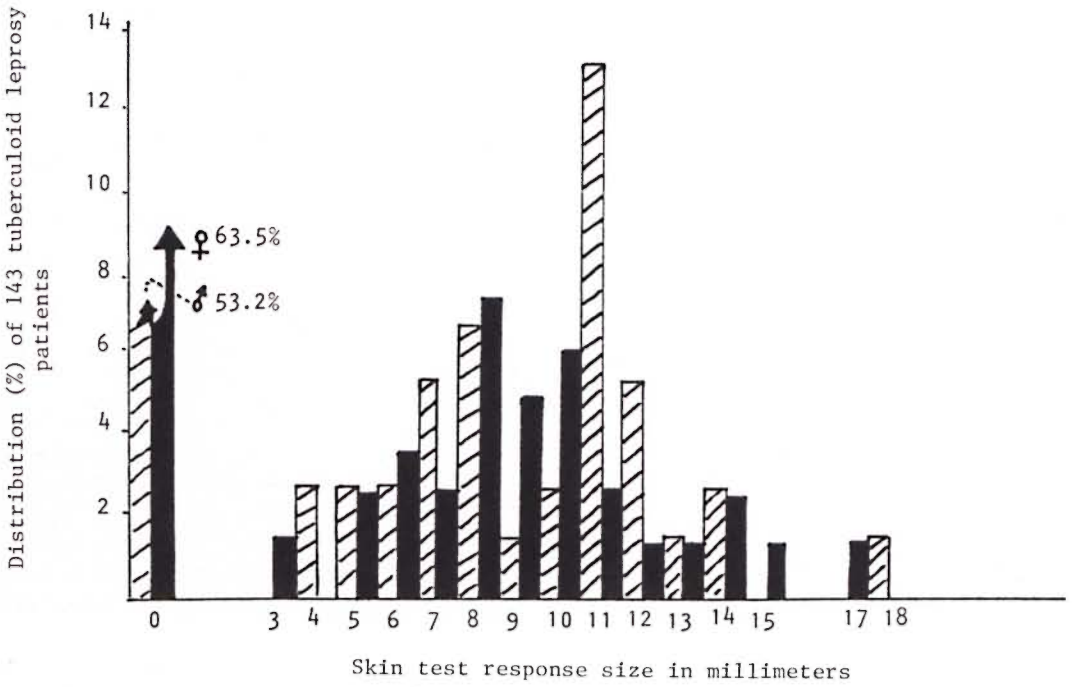


FIG. 1. Skin test responses to LRA6 (2 μg/ml) using 77 male (▨) and 85 female (■) tuberculoid leprosy patients. The two percentage figures beside the vertical arrows in the 0 mm column indicate the percentages of male (♂) and female (♀) non-responders.

tween the type of index case contacted prior to the development of disease and the type of tuberculoid lesion that develops. This is demonstrated by the statistically significant difference between the 52% of the "multiple" lesion group that had a bacilliferous contact as compared with the 13% and 16%, respectively, that had such prior contact in the single "skin" lesion and "neural" leprosy groups ($p < 0.01$ in both cases). In Table 3 the responses to LRA6 vary not with the type of index case contacted but with the type of lesion exhibited by the patient. Patients with "neural" leprosy gave a significantly greater percentage of positive reactors (18/32 or 56%) than the single "skin" lesion group (33/94 or 35%) ($p < 0.05$). The numbers tested in the "multiple" lesion group (6/17 or 35% positive) were not large enough for the observed difference in percentage positivity between "neural" and "multiple" lesion groups to attain statistical significance. When the "neural" leprosy group was analyzed further, a correlation between the duration of the lesion and the LRA6 positivity was found. This is shown in Figure 2. No such correlation was found in the other two groups.

TABLE 2. *Distribution of 143 tuberculoid (TT) leprosy patients according to type of lesion and type of index case contacted prior to developing disease.*

| Predominant site of patient's lesion | Nature of index case contacted | | | Total |
|---|---|---|---|---|
| | Bacilliferous (LL & BL) | Non-bacilliferous | No known contact | |
| Skin (single) | 12 (13%) | 22 (23%) | 60 (64%) | 94 (100%) |
| Neural | 5 (16%) | 9 (28%) | 8 (56%) | 32 (100%) |
| Multiple | 9 (52%)[a,b] | 4 (24%) | 4 (24%) | 17 (100%) |

[a] Significantly higher than the "skin" group in contact with bacilliferous cases, $p < 0.01$, chi-square test.
[b] Significantly higher than the "neural" group in contact with bacilliferous cases, $p < 0.01$, chi-square test.

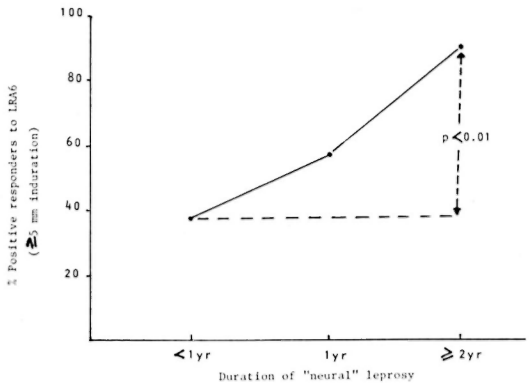


FIG. 2. The percentage positive skin test reactions among tuberculoid leprosy patients with involvement of a single nerve and no clinical skin involvement ("neural" cases) in relation to the duration of the neural lesion.

With regard to the "multiple" lesion group (Tables 2 and 3), an alternative method of analysis may also be necessary if these patients were in fact BT rather than TT as has been suggested earlier. When the LRA6 results from these 17 "multiple" lesion patients are added to those of the 6 BTs shown in Table 1, this enlarged BT category gives 6 positives (26%) out of the 23 tested with LRA6. This leaves 51 positive reactors (40%) to LRA6 in the remaining 126 TT patients shown in Table 3, the "multiple" lesion group having been removed. The difference between the 26% positivity in this enlarged BT category and the 40% in the remaining TT patients is not, however, statistically significant.

BCG vaccinated and unvaccinated TT patients showed the same degree of sensitization to LRA6 (data not shown). Despite this however, the distribution of tuberculoid lesions, according to whether or not the patients had received BCG vaccination, did vary. This is shown in Table 4. The 259 TT patients included in this table are all those skin tested for whom the BCG vaccination status was known, whether or not LRA6 was one of the skin tests used. Only 6% of the "multiple" lesion group had been BCG vaccinated prior to the development of leprosy compared with 25% of both of the other groups. This difference between the "multiple" lesion category and the other two groups is statistically significant, being $p < 0.01$ for "multiple"/single "skin" lesion, and $p < 0.05$ for "multiple"/"neural" leprosy, respectively.

None of the LL patients had received BCG but this may purely have been due to the higher average age of this group (Table 1) and reflects the fact that BCG vaccination was not widely administered in Burma before the 1950s.

TABLE 4. *Distribution of lesions in 259 tuberculoid leprosy patients according to BCG vaccination status.*

| Predominant site of patient's lesion | No. (%) BCG vaccinated | No. (%) unvaccinated | Total (100%) |
|---|---|---|---|
| Skin (single) | 40 (25%) | 121 (75%) | 161 |
| Neural | 16 (25%) | 48 (75%) | 64 |
| Multiple | 2 ( 6%)[a] | 32 (94%) | 34 |

[a] Significantly lower than the "skin" group, $p < 0.01$, chi-square test. Significantly lower than the "neural group, $p < 0.05$, chi-square test.

## DISCUSSION

The results obtained with LRA6 are close to what one might expect of an *M. leprae*-

TABLE 3. *Percentage of positive responders (≥5 mm induration) to LRA6 among 143 tuberculoid (TT) leprosy patients according to type of lesion and type of index case contacted prior to developing disease.*[a]

| Predominant site of patient's lesion | Nature of index case contacted | | | Total |
|---|---|---|---|---|
| | Bacilliferous (LL & BL) | Non-bacilliferous | No known contact | |
| Skin (single) | 3/12 (25%) | 6/22 (27%) | 24/60 (40%) | 33/94 (35%) |
| Neural | 3/5 (60%) | 4/9 (44%) | 11/18 (61%) | 18/32 (56%)[b] |
| Multiple | 3/9 (33%) | 1/4 (25%) | 2/4 (50%) | 6/17 (35%) |

[a] Data presented as a number of positive responders to LRA6/total number in group (% positive responders).
[b] Significantly higher than the "skin" group, $p < 0.05$, chi-square test.

derived skin test preparation, the highest positivity being in TT patients and the lowest in LL ones. Even though no direct comparison is being made between LRA6 and the skin test results obtained with "new tuberculins," it is worth noting that only LRA6 gave such a marked degree of difference between the two ends of the leprosy spectrum. The added influence of tuberculosis did not alter the lack of response to the leprosy reagent among the LL patients.

The overall positive responses to LRA6 among the TT patients (Fig. 1) show a well defined unimodal distribution but with a trend for males to give slightly larger reactions than females and a slightly greater percentage of positive reactors. There is little difference between the histograms for TT patients and the normal population ([14]) other than in amplitude.

The unexpected finding of highest LRA6 positivity in the "neural" leprosy type of tuberculoid patients is difficult to interpret, but it is interesting that Barnetson, *et al.* ([1]) in a study of 24 "borderline" leprosy patients in reversal reaction showed that lymphocyte transformation test (LTT) responses to two different types of *M. leprae* preparation varied according to the site of the reversal lesion encountered. Raised LTT responses to the cytoplasmic antigens correlated with nerve lesions; whereas raised LTT responses to whole washed bacilli, and much smaller responses to cytoplasmic antigens, correlated with skin lesions. LRA6, being an ultrasonicate preparation, contains much cytoplasmic antigen and may therefore have detected neural lesions more readily. The additional finding that the higher the level of sensitization to LRA6 the greater the duration of "neural" leprosy (Fig. 2) might be the result of recurrent release of, and sensitization to, cytoplasmic antigen during the repeated attacks of immunologically mediated nerve damage. The clinical significance of these results could be that the immune mechanism correlating with the positive LRA6 response is protective against the development of skin lesions in many cases but is, perhaps, the mechanism of nerve damage. In a future study it might well be worth paying particular attention to the time course of LRA6 responsiveness to see whether the proportions of immediate, Arthus, Jones-Mote, 48 hour and 72 hour responsiveness vary in the different forms of tuberculoid disease.

The results concerning the "multiple" lesion group of tuberculoid patients (Tables 2 and 3) are of particular interest. First, from an epidemiological viewpoint, the association between the development of multiple tuberculoid lesions and prior contact with bacilliferous patients suggests that excessive contact with *M. leprae* leads to the development of the more severe form of disease. Secondly, the statistically significant difference between the "multiple" lesion group and the two TT categories (single "skin" lesion and "neural") with respect to BCG vaccination rates suggests that, in the absence of BCG vaccination, patients are more likely to progress towards the lepromatous end of the spectrum. If one postulates that it is the development of anergy to the Group i (common) mycobacterial antigens ([22]) that leads to this progression or downgrading, then it would seem possible that immunization with BCG blocks the emergence of this suppressor mechanism.

Testing with LRA6 alone only appears to detect this subtle progression towards anergy in a crude fashion. Percentage positivity to LRA6 in the "multiple" lesion group was no different from that in the single "skin" lesion TT patients (Table 3). Hence, the LRA6 responses do not reflect what the epidemiological information given above indicates; namely, the association that exists between excessive exposure to *M. leprae* in the form of contact with bacilliferous index cases and the development of more disseminated forms of tuberculoid disease. However, if one accepts that the "multiple" lesion group is in fact closer to the BT patients, then the 14% less positivity found in the enlarged BT category (6/23 or 26.7%) (see Results section) compared with the true TT patients (51/126 or 40%) does show a trend towards anergy. The numbers were too small to attain statistical significance on this particular point, however.

Once the BB, BL and LL stages of disease are reached, LRA6 responses are conspicuously absent (Table 1). The negative reactions found among the LL patients are readily explicable in terms of the current immunological concepts of the disease. They fit in well with the accepted negativity obtained, for example, with standard Mit-

suda lepromin in lepromatous leprosy. In the borderline phases of the disease there is evidence to suggest that the anergy differs from that found at the LL end of the spectrum. Previous studies have shown that among borderline groups, skin test unresponsiveness is of a non-specific type; whereas the state of anergy in lepromatous leprosy is limited to *M. leprae* and a few similar organisms ([8, 13]). The mechanisms of these two types of unresponsiveness may be different; the specific type probably being due to suppression triggered by Group iv (species specific) antigens of particular species, whereas the non-specific type is probably due to compartmentalization of the relevant cells outside the circulation ([10]), or a suppressor mechanism triggered by Group i (common) mycobacterial antigen ([21]). As pointed out in these previous reports, the detection of these two different types of anergy by means of skin testing techniques can only be achieved when multiple skin tests are employed utilizing reagents prepared from a variety of different mycobacterial species. Non-specific anergy to a range of mycobacteria can then be detected. Testing with LRA6 on its own, although it detects anergy in both borderline and LL patients, cannot distinguish between the non-specific anergy of the former and the specific anergy of the latter.

There are too few patients with indeterminate leprosy to make significant comment, but in their response to LRA6 alone they appear indistinguishable from healthy persons.

The 10% positivity to LRA6 in the 30 tuberculosis patients tested does not indicate any significant degree of cross-reactivity with *M. tuberculosis* at the strength at which the leprosy reagent was used, and indeed fits with the observed depression of responsiveness to other "new tuberculins" ([13]).

At this point it would seem worthwhile putting the LRA6 responses in perspective in relation to those achieved with tuberculin in tuberculosis and with respect to how they may fit in with mycobacterial immunology. There is obviously a clear difference between the comparatively low percentage of positivity in TT patients and the analogous situation in tuberculosis patients in whom skin test positivity to tuberculin approaches 100%. If, though, the different forms of tuberculoid disease ("skin," "neural," "multiple" lesion) are the result of different immunological effector mechanisms, as seems likely and is suggested by the evidence given above and elsewhere ([1]), and if LRA6 positivity equates with a particular form of response to *M. leprae* (Table 3), then one could hardly expect to find 100% positivity among TT patients.

The mechanisms that govern observed immunological effects in the mycobacterioses and which range from protective immunity on the one hand through to hypersensitivity and finally anergy on the other are just beginning to be unravelled ([11, 12]). As part of this unravelling process, we would like to suggest that in leprosy the amount of contact with the organism rather than its invasiveness is of paramount importance in determining the type of lesion that develops. This is reflected in the epidemiological evidence presented and to some extent by LRA6, positive responses to which are maximal in neural hypersensitivity reactions but become virtually absent at the lepromatous end of the spectrum. By contrast, it is well recognized that in tuberculosis the response to the tuberculin skin test is almost always positive and that among normal populations tuberculin positivity is a useful marker for the detection of subclinical infection. Even so there is evidence ([5, 13, 20]) that a spectrum of disease does exist in tuberculosis so that some grossly infected tuberculosis patients exhibit tuberculin negativity prior to severe debilitation and subsequent death. Owing, though, to the nature of *M. tuberculosis*, the time course and spectrum of tuberculosis is extremely limited compared with that of leprosy. Conversely, although *M. leprae* is perfectly capable of inducing a cell-mediated immunological response, as detected by the positive reactions to LRA6, contact with excessive amounts of antigen results in the development of anergy ([14]). This is consequent upon the particular patterns of antigens presented ([16]) and the low toxicity of *M. leprae* which allows excessive multiplication of the organism in human tissues without death ensuing.

## SUMMARY

A cell-free extract derived from *M. leprae* isolated from armadillos (LRA6) was

used in Burma as a skin test reagent in 334 leprosy and 30 tuberculosis patients.

Positivity to LRA6 was highest in tuberculoid (TT) patients (41%) and lowest in lepromatous (LL) ones (3%). A subgroup of 32 TT patients with predominantly "neural" lesions was found in whom there was 56% positivity to LRA6. In this group there was a statistically significant association between the duration of clinical neural involvement and LRA6 positivity, 88% of patients who had had lesions for two or more years giving positive responses. BCG vaccination was not found to influence patients' LRA6 responses although more disseminated disease of the tuberculoid type was found in unvaccinated persons. There was also a statistically significant association between the development of "multiple" lesion tuberculoid type disease and prior contact with bacilliferous index cases, but no detectable difference in the LRA6 responses between these and TT patients with single "skin" or "neural" lesions.

The true classification of the "multiple" lesion tuberculoid-type patients (i.e., whether severe TT or BT) was in some doubt and is discussed in the text. However, the overall percentage positivity to LRA6 among unequivocal BT patients and other borderline groups (BB and BL) was 3% and among tuberculosis patients, 10%.

In this study borderline patients were not distinguishable from the LL patients on their LRA6 response alone but evidence from other studies suggests that this relative anergy in both borderline leprosy patients and tuberculosis patients is attributable to a generalized suppression of response to all mycobacteria; whereas anergy in lepromatous disease is specific to *M. leprae*.

Twenty percent of patients with indeterminate leprosy gave positive LRA6 responses and were indistinguishable on this criterion alone from the normal population.

It appears that LRA6, which contains a large proportion of cytoplasmic antigen, may detect a particular form of response to *M. leprae* which accounts for positivity only in a selective group of TT patients. It may thus be a useful tool, either when used alone or in combination with other "new tuberculin" skin test reagents prepared from other mycobacteria, to investigate immunological mechanisms in leprosy and possibly even other mycobacterioses.


## RESUMEN

Se utilizó un extracto libre de células de *M. leprae* aislado de armadillos (LRA6) para determinar la reactividad en piel de 334 pacientes con lepra y de 30 pacientes con tuberculosis de Birmania.

La positividad al LRA6 fue máxima en los pacientes tuberculoides (TT, 41%) y mínima en los lepromatosos (LL, 3%). Se identificó un subgrupo de 32 pacientes TT con lesiones predominantemente neurales en los que hubo un 56% de positividad al LRA6. En este grupo hubo una asociación estadísticamente significativa entre la duración clínica de la afección neural y la positividad al LRA6; 88% de los pacientes con lesiones de 2 ó más años dieron una respuesta positiva. La vacunación con BCG no influyó en la reactividad de los pacientes al LRA6 aún cuando se encontró una enfermedad más diseminada del tipo tuberculoide en las personas no vacunadas. También hubo una asociación estadísticamente significativa entre el desarrollo de la enfermedad tipo tuberculoide, con lesiones múltiples, y el contacto previo con casos bacilíferos pero no hubieron diferencias detectables en las respuestas al LRA6 entre éstos y los pacientes TT con lesiones únicas en piel o nervios.

La clasificación de los pacientes como tuberculoides con lesiones múltiples, ya fueran TT o BT, fue algo dudosa y ésto se discute en el texto. Sin embargo, el porcentaje global de positividad al LRA6 entre los pacientes claramente BT y otros grupos intermedios (BB y BL) fue del 3%, y entre los pacientes con tuberculosis fue del 10%.

En este estudio, los pacientes intermedios ("borderline") no fueron distintos de los pacientes LL en cuanto a su respuesta al LRA6 pero otros estudios sugieren que esta anérgia relativa tanto en los pacientes con lepra intermedia como en los pacientes con tuberculosis, es atribuíble a una depresión generalizada de la respuesta a todas las micobacterias, mientras que la anérgia en la enfermedad lepromatosa es específica para el *M. leprae*.

Veinte porciento de los pacientes con lepra indeterminada dieron respuestas positivas al LRA6 pero fueron indistinguibles, por este único criterio, de la población normal.

Parece que el LRA6, el cual contiene una gran proporción de antígenos citoplásmicos, puede usarse para identificar a una forma particular de respuesta al *M. leprae*, que explica la positividad sólo en un grupo selecto de pacientes TT. El LRA6 puede ser una herramienta útil, usado sólo o en combinación con otros antígenos, para investigar los mecanismos inmunológicos en la lepra y, posiblemente, en otras micobacteriosis.

## RÉSUMÉ

Un extrait libre de cellules, dérivé de *M. leprae* isolés à partir d'armadillos (LRA6), a été utilisé en Birmanie

pour procéder à des épreuves cutanées chez 334 malades atteints de lèpre, et 30 patients tuberculeux.

Les taux de réaction positive au LRA6 était les plus élevés chez les malades tuberculoïdes (TT) (41%), et les plus faibles chez les malades lépromateux (LL) (3%). Dans un sous-groupe de 32 malades TT présentant surtout des lésions neurales, on a observé 56% de positivité à cet extrait LRA6. Dans ce groupe, les réactions positives au LRA6 étaient associées de façon statistiquement significative à la durée de l'atteinte clinique neurologique; 88% des malades qui présentaient des lésions depuis 2 ans ou plus étaient positifs. On a observé que la vaccination par le BCG n'influençait pas les réponses des malades à cet extrait LRA6, encore que chez les patients tuberculoïdes une maladie plus disséminée ait été observée chez les individus non vaccinées. On a également observé une association statistiquement significative entre le développement d'une maladie de type tuberculoïde avec lésions multiples, et un contact antérieur avec des cas index bacillifères; d'autre part, aucune différence n'a pu être décelée dans les réponses au LRA6 entre. Ces malades et les autres malades TT qui présentaient une macule solitaire ou seulement des lésions nerveuses.

La véritable classification des malades de type tuberculoïde "à lésions multiples" (c'est-à-dire présentant des formes TT ou BT graves) suscitait certains doutes, dont on discute dans cet article. Néanmoins, le pourcentage global de réactions positives au LRA6 chez les malades présentant une lèpre indubitablement BT, et dans les autres groupes dimorphes (BB et BL), était de 3%, alors qu'il était de 10% chez les malades atteints de tuberculose.

Dans cette étude, les malades dimorphes ne pouvaient être distingués des malades LL sur la base de leur réponse au LRA6 seulement; toutefois, des données obtenues dans d'autres études suggèrent que l'anergie relative que l'on observe à la fois chez les malades atteints de lèpre dimorphe et chez les patients tuberculeux, peut être attribuée à une suppression généralisée de la réponse à toutes les mycobactéries, alors que l'anergie caractéristique de la lèpre lépromateuse est spécifique pour *M. leprae*.

Le pourcentage de réponses positives noté chez les malades atteints de lèpre du type indéterminé s'élevait à 20%; sur la base de ce seul critère, ces malades ne pouvaient être distingués de la population normale.

Il apparaît dès lors que le LRA6, qui contient une grande proportion d'antigènes cytoplasmiques, peut détecter une forme particulière de réponse à *M. leprae*, qui est responsable de la positivité uniquement dans un groupe sélectionné de malades TT. Cette réaction peut donc être un instrument utile, soit lorsqu'elle est utilisée seule, ou lorsqu'elle est utilisée en combinaison avec d'autre antigènes préparés à partir d'autres mycobactéries pour des épreuves cutanées du type "nouvelle tuberculine," en vue d'explorer les mécanismes immunologiques qui interviennent dans la lèpre, et éventuellement aussi dans d'autres mycobactérioses.

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