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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 2: Close Contacts and Non-contacts of Bacilliferous Leprosy Patients[1]

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This report deals with the results obtained with LRA6 in apparently healthy persons living in the WHO/Burmese government trial area north of Mandalay, as described in Part 1 ([3]). Due to the very high incidence of tuberculoid (TT) and borderline tuberculoid (BT) disease (4% of the population over the ten-year history of the trial at the time of this survey), almost all of the normal population must have been in occasional contact with leprosy patients. In fact one quarter of those skin tested in the healthy population had documentary evidence of having one or more TT, BT or indeterminate (I) leprosy patients as a member of their family. However, since the skin test results from these contacts of non-bacilliferous patients did not differ from those of individuals without household contacts (data not shown), their results have been pooled with the latter and in this paper the two are referred to as the "non-contact" population.

A second especially selected group designated "close contacts" is defined and contains only those individuals living in the same household as a bacilliferous (BL or LL) patient and who beyond reasonable doubt had never themselves suffered a clinically detectable leprosy lesion. The younger the "close contact" the more easily could the latter requirement be fulfilled because of the ten-year surveillance in the trial area.

In this part of the study we have analyzed the skin test results obtained in "non-contacts" and "close contacts" in relation to differences associated with age, sex, and BCG vaccination status. Additionally, because of the great variability between the villages with respect to both leprosy prevalence and incidence, the LRA6 results have been correlated with such variations.

MATERIALS AND METHODS

Skin testing with LRA6 was carried out as described in the first part of this report ([3]). The "non-contacts" have been split into three age groups: a) 6–10, b) 11–18, and c) 19–40 years. The numbers of people tested, male and female, in these three groups and in the "close contact" group are shown in Table 1.

Villages have been clustered according to the incidence of leprosy in BCG unvaccinated individuals. Based on data obtained from the WHO, six village groupings were defined, these being comprised of village clusters having incidences of 0, 1–19, 20–39, 40–59, 60–79, and 80+ cases per 1000 population per ten years.

RESULTS

The overall results obtained in the different groups are shown in Table 1. Although there is no statistically significant difference between LRA6 results for the two sexes among the "non-contact" group, a significant sex difference is seen among the "close contacts" and between "close contacts" and "non-contacts" of the same sex. When the sexes are shown together these

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TABLE 1. *Number and percentage of positive reactors to LRA6 (≥5 mm induration) in the population groups studied. Mean ages of the "close contacts" were 17.7 years for males, 23.1 years for females, and 20.9 years overall.*

Population group	Male		Female		Total	
	No.+/total	(%+)	No.+/total	(%+)	No.+/total	(%+)
Close contacts (of BL and LL)	13/35	(37%)	4/50	(8%)[a]	17/85	(20%)
Non-contacts (normal population)						
Age 6–10	16/159	(10%)	12/147	(8%)	28/306	(9%)
Age 11–18	43/250	(17%)[b]	72/354	(20%)[c]	115/604	(19%)[d]
Age 19–40	38/168	(23%)[b]	71/421	(17%)[c]	109/589	(19%)[d]

[a] Significantly less than male "close contacts," $p < 0.001$, chi-square test.
[b] When "non-contacts" aged 11–40 are combined, significantly less than male "close contacts," $p < 0.01$, chi-square test.
[c] When "non-contacts" aged 11–40 are combined, significantly greater than female "close contacts," $p < 0.05$, chi-square test.
[d] When "non-contacts" aged 11–40 are combined, significantly greater than "non-contacts" aged 6–10, $p < 0.01$, chi-square test.

differences are lost, suggesting that close contact with bacilliferous leprosy has distinctive and apparently opposite effects on the two sexes.

The distributions of responses to LRA6 by diameter of induration in millimeters are shown in Figure 1. The upper histogram (Fig. 1a) shows the distribution in all "close contacts" tested; the lower one (Fig. 1b), the distribution in "non-contacts" aged 11–40. The sexes are shown separately in each histogram. In the "non-contact" group there is no difference in the overall percentage of positives between the sexes, and the mean induration sizes are the same. However, in the "close contact" group the percentage positive and the mean reaction size are greater for males than females. Although the mean induration sizes in the "non-contact" population do not differ between males and females of the same age group, an increase in mean induration size was seen with increasing age. For comparison, the mean induration sizes for the three age groups of the "non-contacts," for the "close contacts" and the tuberculoid patients ([3]) are summarized in Table 2. A separation by sex is shown for the "close contact" group only since the tuberculoid patients, like the "non-contact" population, showed no sex difference.

The relationship between age and skin test response to LRA6 is shown in Figure 2. It should be noted that the points on this graph lie between the years, i.e., at 6½, 7½ years, etc., and not at 6, 7, 8 years, etc. This is because the graph was constructed by sequentially amalgamating the results for successive years, both to increase the numbers of persons representing each point on the graph and to reduce chance fluctuations due to small numbers. The first point represents the combined percentage positive for ages 6 and 7, the second point for 7 and 8, the third for the ages 8 and 9, and so on.

The effect of leprosy incidence in the village of domicile on LRA6 positivity in "non-

TABLE 2. *Comparison of mean skin test induration sizes among individuals showing a positive skin test (≥5 mm induration) to LRA6 in three different population groups.*

Population group	Mean induration size in millimeters
Non-contacts	
Age 6–10	6.3
Age 11–18	7.5
Age 19–40	9.0
Close contacts	
Male	10.0[a]
Female	7.5
Tuberculoid leprosy patients	9.4

[a] Significantly greater than the mean induration size in "non-contacts" aged 11–40, $p < 0.01$, Student's t test.

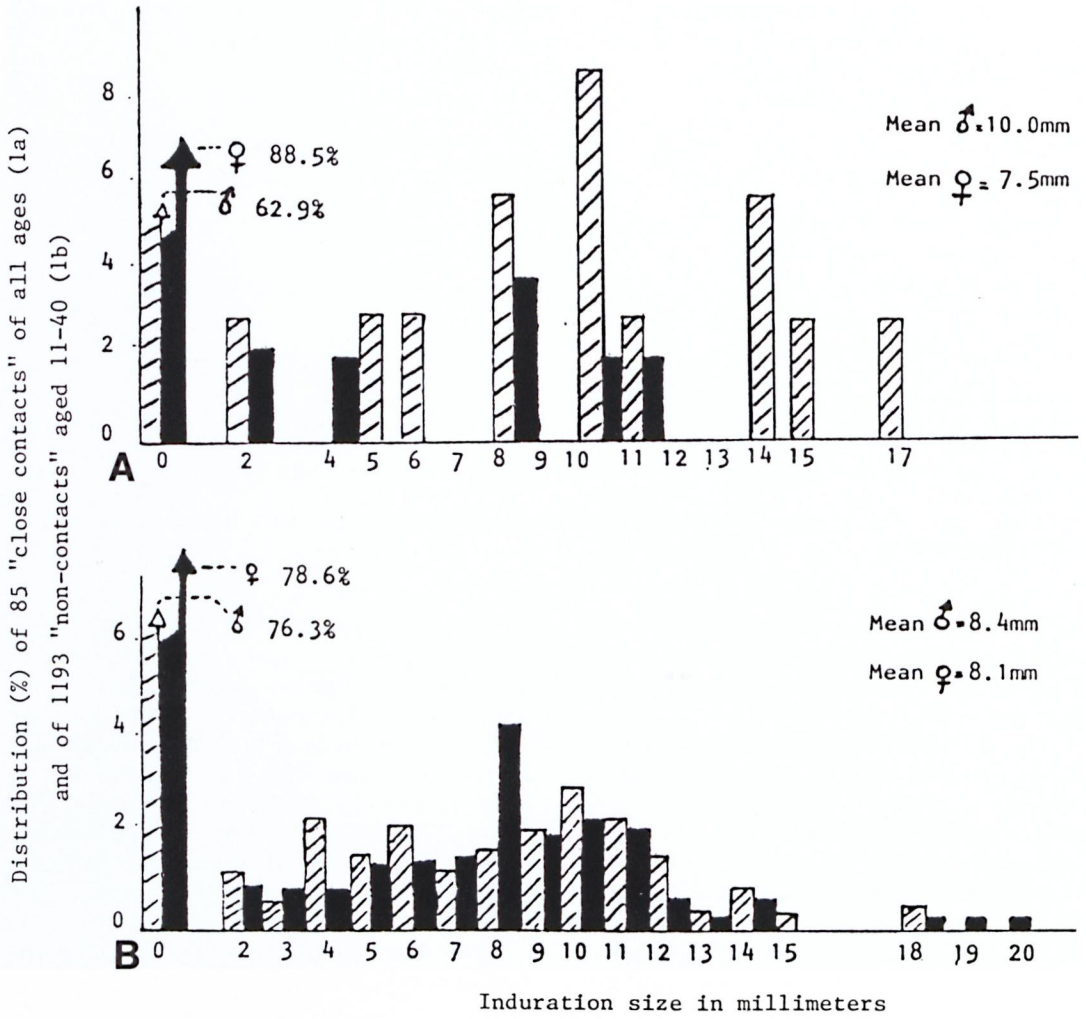

FIG. 1. Histograms showing the results obtained with LRA6 when used in "close contacts" in all ages (Fig. 1a) and "non-contacts" aged 11–40 (Fig. 1b). The two percentage figures beside the vertical arrows on each histogram in the 0 mm column indicate the respective percentages of male (♂) and female (♀) non-responders in each population studied. Male (▨), Female (■).

contacts" of two age groups, 6–10 and 11–40, is shown in Figure 3. A phenomenon common to both age groups is demonstrated. The percentage positivity to LRA6 in the normal population rises with increasing leprosy contact. Having reached a peak at a certain level of contact the LRA6 positivity then falls as the leprosy contact, in terms of leprosy incidence, rises still further. Of particular note is the fact that the peak LRA6 response in the 11–40 year olds occurs with less leprosy contact (i.e., in a lower leprosy incidence grouping) than it does in the younger age group.

Within these same village groupings there was virtually no difference in positivity to LRA6 between the BCG vaccinated and unvaccinated "non-contact" populations. The data relating to this is therefore not shown. The percentage of protection, however, afforded by BCG against leprosy in each of these village groupings varied dramatically. These findings will be reported separately.

DISCUSSION

Skin testing with LRA6 appears to detect increasing exposure to *M. leprae* as age in-

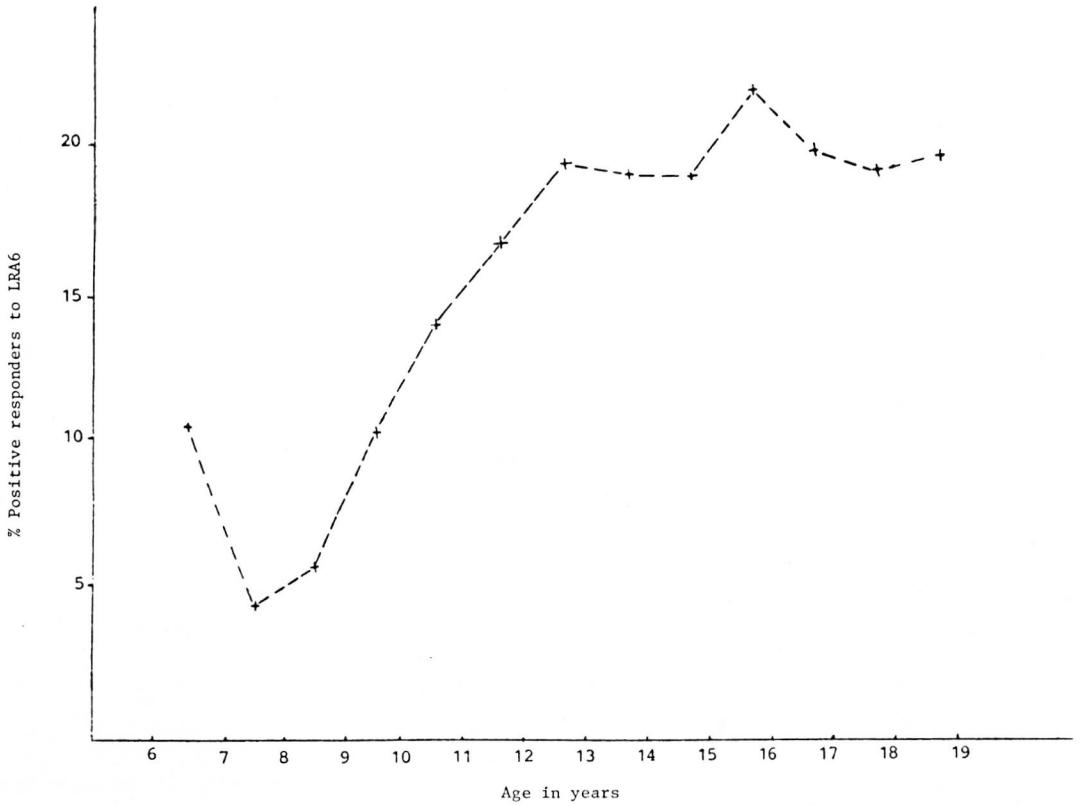

FIG. 2. The correlation between LRA6 positivity (≥5 mm) and age (in years) in the "non-contact" population.

creases, as shown by the statistically significant increase in positivity to LRA6 between the 6–10 and 11–40 (combined 11–18 and 19–40) year-old age groups in the "non-contact" population (Table 1). This was a systematic stepwise increase in positivity, reaching a peak around the age of 16 years (Fig. 2). This was similar to that found when detecting increased contact with environmental mycobacteria with increasing age when skin testing with other "new tuberculins" ([5]). It will be noted, though, that in this figure an anomalous small peak of positivity occurred in the youngest persons tested. By way of explanation of this anomaly, the 11 positive responders to LRA6 who constitute this first point on the graph (Fig. 2) were derived from testing 98 children from 16 villages, yet 5 of these 11 positives came from only one village. Thus, in this instance, LRA6 may have been detecting subclinical infection in these children. This is supported by the fact that they lived and played close to where a borderline (BB) leprosy patient was living.

Further indication that increasing exposure to *M. leprae* is detected by skin testing with LRA6 is given by the increase in mean induration size of LRA6 responses observed with increasing age (Table 2); the trend being similar in both males and females.

However, increasing contact with *M. leprae* was associated not only with an increase in the percentage of positive responders to LRA6 but also with a decrease in positivity as contact with *M. leprae* became excessive. This is demonstrated by the results shown in Figure 3. In both age groups, 6–10 and 11–40, of the "non-contact" population, positivity to LRA6 increases with increasing leprosy incidence to a peak in village groupings of relatively low leprosy incidence. Thereafter, as the leprosy incidence becomes excessive, suppression is invoked which results in pro-

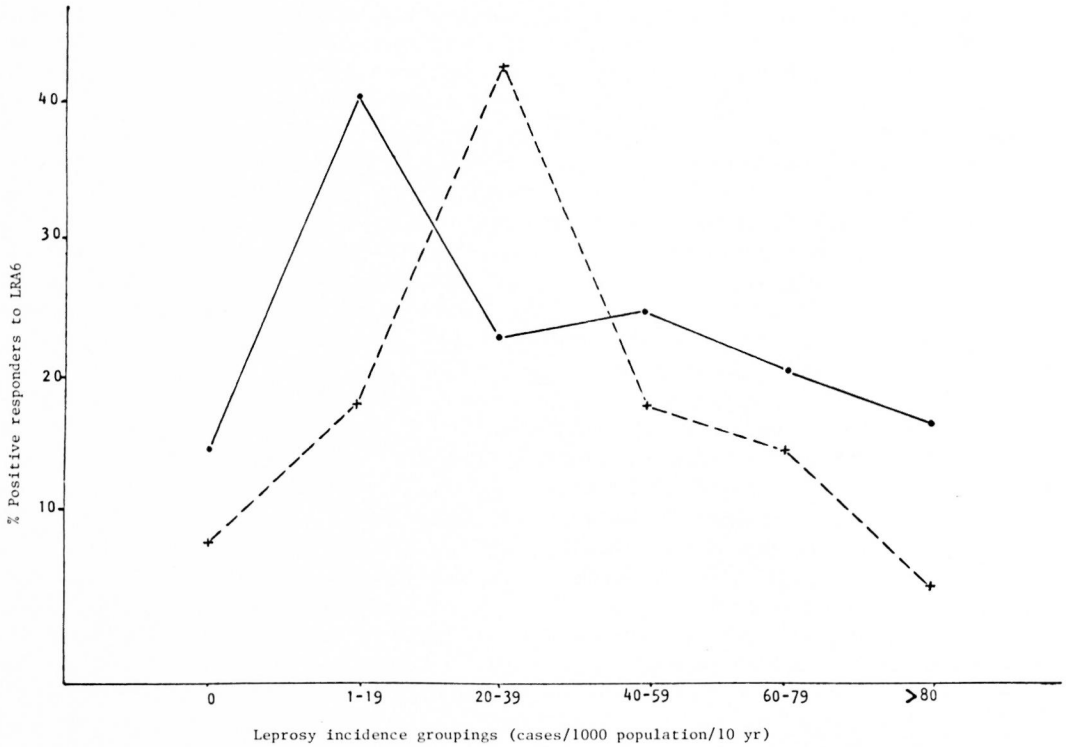

FIG. 3. The percentage of positive responders (≥5 mm) to LRA6 in village groupings of varying leprosy incidence (see text for explanation) for two age groups of the "non-contact" population. 6–10 yr = +– – –+; 11–40 yr = ———.

gressive loss of LRA6 positivity. It is significant that the peak positivity occurs in the 11–40 age group at a lower level of leprosy incidence than in the 6–10 year age group. This can be explained by the older age group having greater contact with leprosy due to longer exposure. This would explain the equivalent (approximately 40%) positivity to LRA6 between 11–40 year olds in the 1–19 cases per 100 population per ten years incidence grouping to that of the 6–10 year olds in the 20–39 incidence grouping. Thus the total degree of contact with *M. leprae* is a function of both the amount and the duration of exposure. In other diseases such as tuberculosis this would normally lead to a steadily increasing level of positivity. This indeed was observed in relation to leprosy in Iran ([4]), a country of low endemicity. In Burma, though, the situation is modified by this factor of suppression that comes into play even among those without household contact with bacilliferous leprosy patients in villages of very high leprosy incidence. LRA6 positivity is thus as low in villages with an incidence >80 cases per 1000 per ten years as it is in villages with zero incidence (Fig. 3).

This phenomenon of differing amounts of leprosy contact affecting skin test responsiveness is further demonstrated by the striking difference between male and female "close contacts" with regard to LRA6 positivity (Table 1). As pointed out in Part 1 ([3]), adult Burmese men and women differ markedly with respect to their social behavior. Female members of a family who tend to stay at home are in exceedingly close contact with leprosy in those families in which there is a bacilliferous case, since these patients are liable to stay at home also, whatever their sex. By contrast, the male members of the family are only subject to intermittent contact with the bacilliferous index case due to their differing work commitments. It is suggested that in male "close contacts" this leads to the acquisition of positivity to LRA6 to a level significantly greater than that found in the adult "non-contact" population (Table 1). For those

who wish rather more than this circumstantial evidence to support this viewpoint of the importance of innoculum effect, the evidence will only be forthcoming once means of environmental sampling can be applied to *M. leprae* as they can to other mycobacterial species.

Another observation that may have some significance is that among individuals showing a positive response to LRA6, the male "close contacts" exhibit an LRA6 response of larger mean induration size than the adult "non-contacts." When compared specifically with the 11–18-year-old "non-contacts," the difference attains statistical significance (Table 2). It could be questioned as to how meaningful this difference is since the ages are not identically matched. However, only four of the male "close contacts" were over the age of 23, the remainder being between the ages of 7 and 23 (mean age 17.7 years) (Table 1). If any comparison were to be made outside of the 11–18 age group it should be made towards the 6–10 year olds rather than the 19–40 year olds. If this were done then the difference between the "close contacts" and "non-contacts" would be even greater. In female "close contacts" the excessive exposure to leprosy bacilli that probably occurs results in the failure to develop, or the loss of, positivity to such a degree that not only is the response rate significantly lower than in male "close contacts," but it is also distinctly lower than that in the female "non-contact" population.

From the WHO data it is known that in the "non-contact" population the incidence of leprosy male : female is 2:1 ([1]), while in "close contact" families, the ratio is 1:1 (Dr. Gallego, Team Leader, WHO trial, personal communication). This "catching-up" of leprosy incidence among "close contact" females, together with their skin test results, suggests that few female "close contacts" possess a cell-mediated response to the leprosy bacillus. This lack of sensitization, or active suppression through antigen overload, is associated with an increased susceptibility to leprosy compared with the "non-contact" female population.

Interestingly, when there is little difference in the social behavior pattern between the sexes and their chance for contact with leprosy outside the household is the same, as is the case up to the age of about 14 years in the "non-contact" population, then the leprosy incidence in the two sexes is the same ([1]). The small (6%) sex difference with regard to LRA6 positivity in the "non-contact" population in adults is probably explicable on the basis of their exposure being similar enough not to allow the gross difference seen between male and female "close contacts" but sufficient to allow a greater incidence of leprosy in males than females.

It is generally accepted that data gathered with Dharmendra and lepromin testing imply that skin test reactivity to *M. leprae*-derived reagents does not only reflect prior exposure to *M. leprae*. This same "non-specific" reactivity may also apply to LRA6. However, a) with the low concentration of reagent used in Burma (2.0 μg/ml), b) with the high response rate in certain categories of tuberculoid leprosy patients and low rate in tuberculosis patients ([3]), and c) with the absence of difference in LRA6 positivity between BCG vaccinated and unvaccinated normal populations, it is possible, in this study at least, that reactivity to LRA6 is probably influenced to the greatest extent by immune recognition of *M. leprae*. On this basis and taking into account those anergic to *M. leprae* among the "non-contact" population, the seemingly low overall figure of 19% positivity to LRA6 in the adult "non-contacts" would in effect be substantial if considered as indicating that almost one in five had developed a subclinical infection with *M. leprae* to which they had subsequently mounted a cell-mediated response. Additionally, in a previously published study concerning Iran ([4]), the percentage of positive responders was higher. Although the dose (10 μg/ml) and method of preparation of the cell-free extract from *M. leprae*-A used in Iran were different, the degree of positivity to LRA6 in the apparently healthy population in both studies (even accepting that some of these will be non-specific reactors) suggests that infection with *M. leprae* is very much more common than the expression of clinical disease.

If, though, as pointed out in Part 1 ([3]), LRA6 detects a particular form of response to *M. leprae*, there may be other forms of

response that have gone undetected. However, the evidence from this survey and from the study carried out in Iran ([4]) indicates that acquisition of LRA6 positivity correlates to some degree with protective immunity. Not only is skin test positivity to these cell-free extracts of *M. leprae*-A found in healthy persons but, in certain circumstances, it is associated with long term freedom from development of disease. This was the case in the BCG vaccinated children of bacilliferous leprosy patients in the Iran study, in whom skin test positivity to LRA6 was 76%. The Burmese results additionally suggest that such positivity may become unregulated (male "close contacts") and eventually suppressed under certain conditions if exposure to *M. leprae* is excessive (female "close contacts").

Although the increased reaction sizes to LRA6 among male "close contacts" may indicate recurrent boosting due to greater contact with *M. leprae*, it could also denote loss of, or failure in, the regulatory control involved in this form of response. Whether such heightened responses then lead to loss of protective immunity and consequently tuberculoid disease (presumably of the "neural" type as seen in Part 1) ([3]) is not as yet known. Whatever the case, we reiterate the suggestion made in Part 1 ([3]), that in leprosy the amount of contact with the organism rather than its pathogenicity is of paramount importance both in determining the type of lesions that develop in patients and the overall response to LRA6 that can be detected in the community.

In this context the recent work of Challacombe and Tomasi ([2]) in demonstrating that oral intake of particulate antigen, in the form of *Streptococcus mutans*, can induce 'negative' responses, i.e., suppressor regulation of cell-mediated immunity to the same antigen, or others administered concurrently when given parenterally at a later date, points to how such suppressive mechanisms to other particulate antigens, e.g., *M. leprae* could be of importance *in vivo*.

SUMMARY

Healthy persons living in the leprosy endemic Irrawaddy Valley region north of Mandalay, Burma, were skin tested with a cell-free extract derived from *M. leprae* isolated from armadillos (LRA6). They were divided into two groups: 1499 "non-contacts" aged 6–40 and 85 "close contacts." The former group included persons who had no leprosy contact other than by virtue of living in an endemic area, or only non-bacilliferous leprosy contacts within their families. The "close contact" group contained only healthy adults who had a bacilliferous, BL or LL, contact within their immediate family.

The overall positivity to LRA6 among adults in the two groups was 19% and 20%, respectively, but there was considerable variability in different subpopulations. The acquisition of LRA6 positivity was shown to be a function not only of the concentration of leprosy to which persons were exposed but also the duration of exposure. First, there was a statistically significant increase in LRA6 positivity with age among "non-contacts" rising from 9% in the 6–10 year olds to 19% in the 11–40 year olds. Secondly, although both 6–10- and 11–40-year-old "non-contacts" showed a peak of about 40% positivity to LRA6 in villages of relatively low leprosy incidence, the 11–40 year olds attained this peak in village groups of even lower incidence than did the children. Excessive exposure to *M. leprae* resulted in loss of this LRA6 positivity as shown by the progressive decline in the percentage of positive LRA6 responders as leprosy incidence increased, the maximal effect being seen in village groupings of 80+ cases per 1000 population per ten years.

Among "close contacts" a gross sex difference not apparent among the "non-contacts" was found. Female "close contacts" who had demonstrably greater exposure to the bacilliferous index case than did their male counterparts gave only 8% positivity to LRA6 compared with 37% in males. Male "close contacts" also demonstrated LRA6 responses of significantly greater induration size.

These observations for the "non-contact" and "close contact" populations suggest that the degree of contact with *M. leprae* affects the level of positivity and the strength of response to LRA6. They further suggest that anergy to the leprosy bacillus may occur even in the clinically unaffected person.

RESUMEN

Se determinó la reactividad en piel a un extracto libre de células de *M. leprae* aislado de armadillos, en personas sanas del valle de Irrawaddy, una región donde la lepra es endémica, al norte de Mandalay, Birmania. Las personas se dividieron en 2 grupos: 1499 "no contactos" entre 6 y 40 años de edad, y 85 "contactos íntimos." El primer grupo incluyó a personas cuya única relación con lepra fue la de ser habitantes de un área endémica, y a personas en contacto con casos familiares no bacilíferos. El grupo de "contactos íntimos" estaba formado sólo por adultos sanos que tenían contacto con algun caso bacilífero familiar BL o LL.

La positividad general al extracto antigénico (LRA6) entre los adultos de los 2 grupos fue del 19% y del 20%, respectivamente, pero hubo una variabilidad considerable en diferentes subpoblaciones. La adquisición de positividad al LRA6 estuvo en función de la intensidad del contacto con la lepra y de la duración de la exposición. Primero, hubo un incremento estadísticamente significativo en la positividad al LRA6 que fue proporcional a la edad; entre los "no contactos" aumentó del 9% (grupo de 6–10 años) al 19% (grupo de 11–40 años). Segundo, aunque tanto los "no contactos" de 6–10 años como los de 11–40 años mostraron un pico de positividad al LRA6 de aproximadamente el 40% en villas de incidencia de lepra relativamente baja, el grupo de 11–40 años alcanzó este pico en villas aún de menor incidencia. La exposición excesiva al *M. leprae* dió como resultado la pérdida de la positividad al LRA6, como se deduce por la progresiva disminución en el porcentaje de reactores positivos al LRA6 conforme aumentó la incidencia de lepra. El máximo efecto se observó en villas de 80 casos por 1000 habitantes por 10 años.

Entre los "contactos íntimos" se encontró una cierta diferencia sexual, no aparente entre los "no contactos." Dentro del primer grupo, las mujeres, que tenían una demostrable mayor exposición al caso bacilífero que sus contrapartes masculinos, diéron sólo un 8% de positividad al LRA6, en comparación con el 37% en los hombres. Los hombres del grupo de "contactos íntimos" también mostraron respuestas al LRA6 de un mayor tamaño de induración.

Estas observaciones para las poblaciones de "no contactos" y de "contactos íntimos," sugieren que el grado de contácto con el *M. leprae* afecta el nivel de positividad y la intensidad de la respuesta al LRA6. Sugieren además, que la anérgia al bacilo de la lepra puede ocurrir aún en personas clínicamente nò afectadas.

RÉSUMÉ

On a procédé à des épreuves cutanées avec un extrait sans cellules dérivé de *M. leprae* isolés à partir d'armadillos (LRA6), chez les personnes saines vivant dans la vallée de l'Irrawaddy, une région endémique pour la lèpre au Nord de Mandalay, en Birmanie. Ces personnes ont été divisées en deux groupes: 1499 "non contacts" âgés de 6 à 40 ans, et 85 "contacts proches." Le premier groupe comprenait des personnes qui n'avaient eu comme contact avec la lèpre que le fait de vivre dans une région endémique, ou bien qui appartenaient à des familles comprenant des malades de lèpre non bacillifères. Le groupe "contact proche" ne comprenait que des adultes sains qui avaient été dans leur famille immédiate en contact avec un malade BL ou LL.

Le taux de positivité au LRA6 parmi les adultes des deux groupes était respectivement de 19 et 20%, mais on a constaté des écarts considérables dans les différentes sous-populations. On a démontré que l'acquisition d'une positivité au LRA6 dépendait non seulement du nombre de cas de lèpre auxquels les personnes étaient exposées, mais aussi de la durée d'exposition. En premier lieu, on a observé une augmentation statistiquement significative de la positivité au LRA6 avec l'âge parmi les non-contacts; le taux s'élevait de 9% dans le groupe d'âge 6–10 ans, à 19% chez les personnes âgées de 11 à 40 ans. Ensuite, malgré le fait que ces deux groupes de non-contacts âgés de 6 à 10 ans, et de 11 à 40 ans, montraient l'un et l'autre un pic d'environ 40% de réaction positive au LRA6 dans les villages ayant une incidence de lèpre relativement faible, le groupe de 11 à 40 ans atteignait ce pic dans des groupes de villages qui présentaient une incidence encore plus faible dans le groupe d'age 6–10 ans. Une exposition excessive à *M. leprae* entraîne une perte de la positivité à cette épreuve LRA6, ainsi qu'en témoigne de déclin progressif du pourcentage de personnes répondant positivement au LRA6 à mesure que l'incidence de la lèpre augmente. L'effet maximal étant constaté dans les villages groupant plus de 80 cas pour 1000 pendant dix ans.

Parmi les "contacts proches," une très forte différence a été notée entre les sexes, et une telle différence n'étant pas observée chez les non-contacts. Les "contacts proches" de sexe féminin, dont on avait pu démontré qu'ils avaient été davantage exposés au cas index bacillifère que ne l'avait été leur contrepart masculin, fournissaient seulement un taux de 8% de positivité au LRA6, alors que ce taux était de 37% chez les hommes. Les "contacts proches" de sexe masculin témoignaient également présentant d'une dimension d'induration significativement plus large dans la réponse au LRA6.

Ces observations pour les populations de "non-contacts" et "contacts proches" suggèrent que le degré de contact avec *M. leprae* affecte le niveau de positivité et l'intensité de la réponse au LRA6. Ces résultats suggèrent de plus que l'anergie au bacille de la lèpre peut survenir également chez des personnes qui ne présentent pas de signes cliniques.

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area who so willingly came forward to allow themselves to be skin tested.

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REFERENCES

1. Bechelli, L. M., Garbajosa, P. G., Mg Mg Gyi, Uemura, K., Sundaresan, T., Tamondong, C., Dominguez, V. M. and Walter, J. Some epidemiological data on leprosy collected in a mass survey in Burma. Bull. WHO **48** (1973) 335–344.
2. Challacombe, S. J. and Tomasi, T. B. Systemic tolerance and secretory immunity after oral immunisation. J. Exp. Med. **152** (1980) 1459–1472.
3. Shield, M. J., Stanford, J. L., Garbajosa, P. G., Draper, P. and Rees, R. J. W. The epidemiological evaluation, in Burma, of the skin test reagent LRA6; a cell-free extract from armadillo-derived *M. leprae*. Part 1: Leprosy patients. Int. J. Lepr. **50** (1982) 436–445.
4. Stanford, J. L., Rook, G. A. W., Samuel, N., Madlener, F., Khamenei, A. A., Nemati, T., Modabber, F. and Rees, R. J. W. Preliminary immunological studies in search of correlates of protective immunity carried out on some Iranian leprosy patients and their families. Lepr. Rev. **51** (1980) 303–314.
5. Stanford, J. L., Shield, M. J. and Rook, G. A. Récherches sur une série de nouvelles tuberculines. Première partie: les éffets de l'âge, du domicile, de la vaccination par le BCG et de la tuberculose. Bull. Int. Union Tuberc. **56**(1–2) (1981) 31–37.