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Immunologic Skin Titration in Leprosy Patients and Contacts [1, 2]

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It is well known that leprosy mainly damages the tissues derived from ectoderm such as skin and nerves. Its pathogenic mechanism seems to be a consequence of the interaction between *Mycobacterium leprae* and the host, the latter being the most important since from its variations in capacity to react arises the varied expression of the different clinical types found in this disease.

An effective immunologic response in leprosy is expressed through cell-mediated immunity, which does not however exclude the participation of the humoral response in the broad spectrum which characterizes leprosy. This spectrum comprises the tuberculoid (T) pole of high resistance to the lepromatous (L) low resistance, including the intermediate dimorphous or borderline (D or B), and the initial indeterminate (I) form.

To measure the cellular immune reactivity, several *in vivo* and *in vitro* tests have been used. The *in vivo* tests that have been used include: a) hypersensitivity tests with lepromin; b) hypersensitivity tests with tuberculin, mumps, candidin, histoplasmin and dinitrochlorobenzene (DNCB); and c) allograft rejections.

The *in vitro* tests most commonly used are the lymphocyte transformation test (LTT) to phytohemagglutinin (PHA) and lepromin and the leukocyte migration inhibition test (LMIT) to lepromin. Of the *in vivo* tests, we have performed the lepromin reaction to evaluate the immune response to *M. leprae* antigens.

We have used standardized bacillary lepromin which replaces the Mitsuda-Hayashi lepromin. Standardization was accomplished by means of microscopic counting and the results were expressed as the number of acid-fast *M. leprae* per milliliter. The concentration was 160×10^6 *M. leprae*/ml which yields reliable and comparable results and at the same time when used at lower concentrations, helps to reduce false positive results. The reaction to lepromin was evaluated by means of the Fernandez reaction (FR) and the Mitsuda reaction (MR).

Various concentrations of lepromin were applied simultaneously in different skin sites in the same individual. This leads to an immunologic skin titration which appears to be more sensitive for evaluating the immunologic status than the method currently in general use in which a single dose of 40×10^6 *M. leprae*/ml is utilized.

MATERIALS AND METHODS

Patients and contacts. The behavior of adults of both sexes belonging to the T and I leprosy groups and contacts of L and I patients were studied. Controls were healthy volunteer adults of both sexes. Table 1 shows the number of patient contacts and controls, the individuals tested and their age ranges.

Standardized bacillary lepromin. The lepromin was standardized according to the recommendations of WHO ([4]) and lyophilized to allow its storage for up to five years. Appropriate dilutions were performed with a 0.5% phenol solution for use.

Skin tests. At least three injections of lepromin were applied simultaneously to different sites of the scapular region in each individual. The concentrations used were 1.25, 2.5, 5, 10, 20, 40, and 80×10^6 *M. leprae*/ml. The FR ([2]) was read after 48 hours and expressed as square millimeters, which value was obtained by multiplying the major and minor diameters of the reaction. The MR ([5]) was read at the 21st day and its value calculated as the sum of the major and minor diameters divided by two.

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TABLE 1. *Patients, contacts and controls tested with lepromin.*

	Fernandez Reaction					Mitsuda Reaction				
	Patients		Contacts		Controls	Patients		Contacts		Controls
	T	I	L	I		T	I	L	I	
No. of individuals	27	16	84	19	87	25	13	73	19	139
Age range	11-73	25-80	6-80	12-49	18-37	11-73	14-80	6-80	12-49	18-37

Statistical analysis. A dose versus response plot was obtained for each patient and comparisons among the groups were performed by covariance analysis or Student's t test.

RESULTS

The results are shown in Figures 1, 2 and 3. For the FR (Fig. 1), positive and significant linear regressions were obtained in all the groups studied ($p < 0.001$). The largest responses were given by the T patients and the smallest by the I patients. The control group was tested with three doses only; 10, 20, and 40 × 10^6 *M. leprae*/ml. The dose versus response plot was also positive and significant ($p < 0.001$).

Tuberculoid patients showed a higher response when compared with the other groups ($p < 0.001$). Furthermore, T patients had a response four times (75%) higher than those from L contacts. In the MR (Fig. 2), L contacts showed in the whole range studied a lower response than the other groups. This

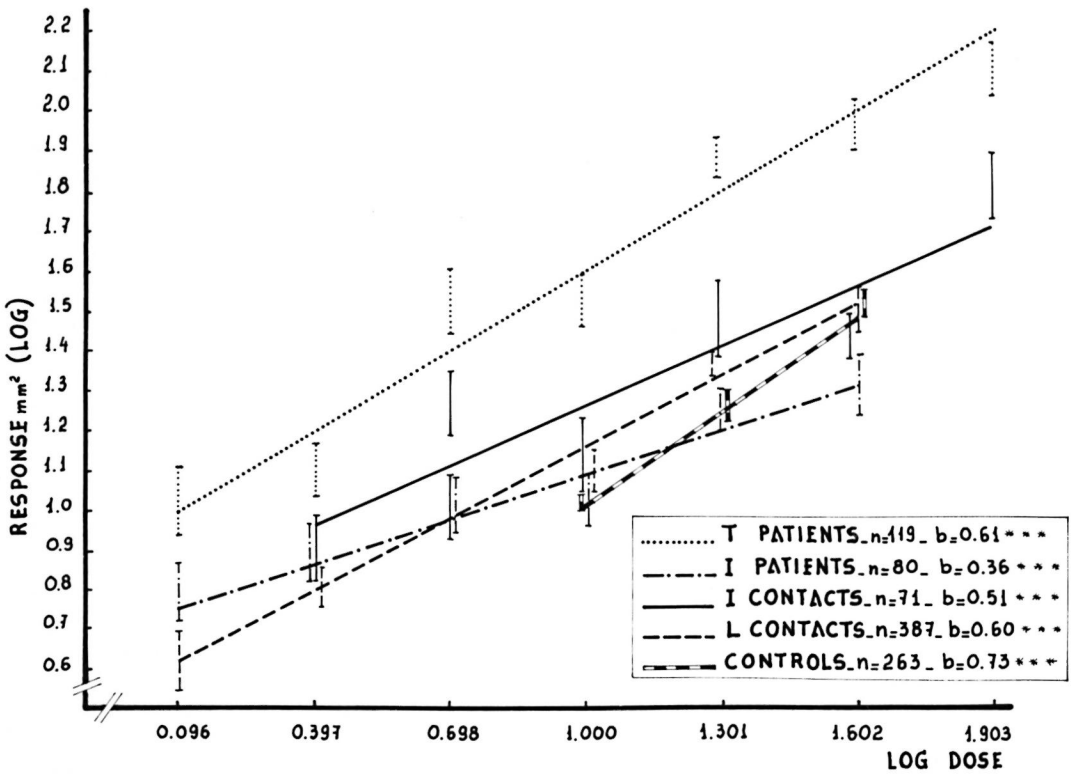

FIG. 1. Fernandez reaction. Dose-response plot of patients and contacts. Regression lines calculated with the method of least squares. The vertical lines represent the standard error of the mean. ***$p < 0.001$.

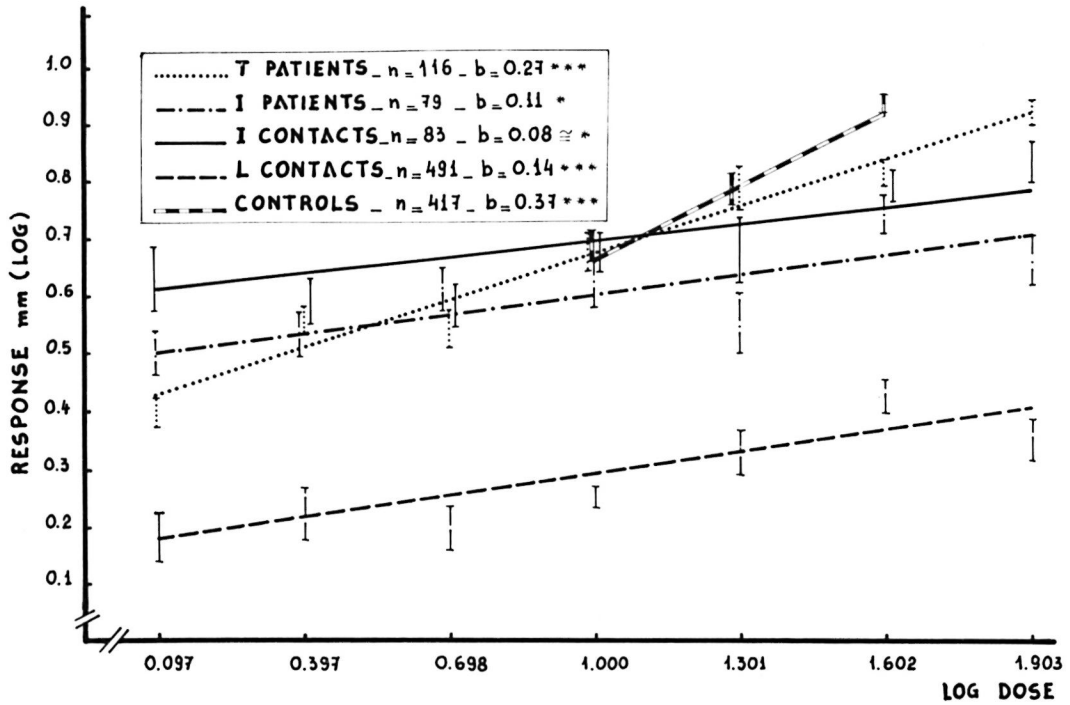

FIG. 2. Mitsuda reaction. Dose-response plot of patients and contacts. Regression lines calculated with the method of least squares. The vertical lines represent the standard error of the mean. *$p < 0.05$; ***$p < 0.001$.

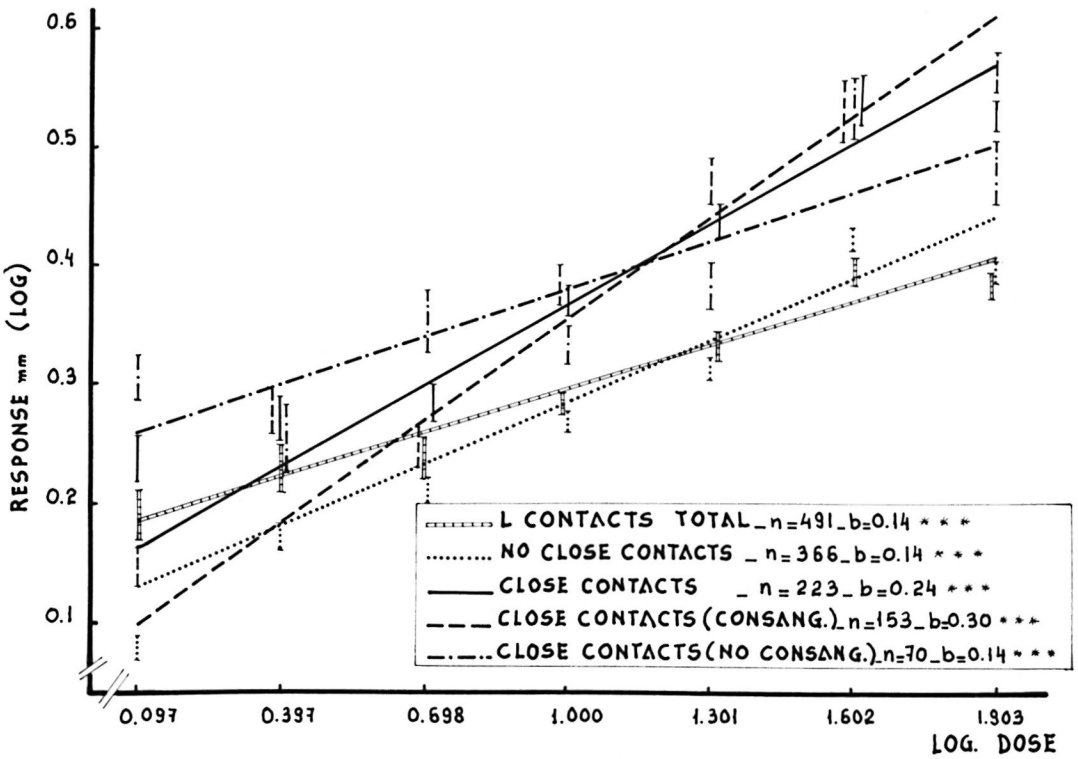

FIG. 3. Mitsuda reaction. Dose-response plot of L contacts. Regression lines calculated with the method of least squares. The vertical lines represent the standard error of the mean. ***$p < 0.001$.

poorer response was highly significant ($p < 0.001$) and shows an impairment in the response of this group when challenged with the antigen.

To study the possibility of a genetic or environmental factor in this response, the L group was divided into close contacts and non-close contacts. The close contacts were subdivided into two groups: consanguineous and non-consanguineous (Fig. 3). The four groups showed very low response, similar to the original L contacts.

DISCUSSION

The dose versus response plot allows the exploration of the immunologic phenomena in a better way than using a single dose. The results obtained in this experiment show that the immunologic reaction is quantitative and highly sensitive for studying the immunologic behavior of leprosy patients and their contacts.

In the FR, the immune titration curves have similar values in all the groups studied except in the case of T patients. This larger response in T patients can be related to a greater capacity of this form of high resistance to develop an efficient, although not wholly adequate immune reaction.

This measurement of immune response allows us to evaluate the magnitude of the reaction of one group in relation to another. Thus, it was found that the response of the T patients is four times stronger than that of the L contacts.

The average values of the MR response in all cases were at a level similar to that of the controls, except in the case of L contacts which showed a significantly lower response, indicating an important immune depression of this group.

In accord with our results, Cochrane *et al* [1] found in children an important decrease in positive MR lepromin reactions when the degree of contact with open cases increased. On the other hand, recently Godal *et al* [3] using LTT and LMIT, which have a sufficient degree of specificity for *M. leprae,* found a lower proportion of response among contacts of lepromatous patients compared with contacts of the tuberculoid patients, and the lowest proportion was found among contacts of untreated lepromatous patients who are obviously the most infectious. In our results it was not possible to explain the particular behavior of the L contact group, either consanguineous or non-consanguineous whose responses are similar, through the influence of a genetic factor.

Thus, it is thought that a possible environmental factor, such as an altered presentation of the *M. leprae* to the host, perhaps by bonding to certain substances, e.g. Ig, which might in some way mask the antigen determinants of the bacillus, interfere with its recognition and produce an inadequate weak immune response.

Finally, the advantage of using the immunologic titration method is related not only to the physiopathologic study of the disease but also to the prognostic evaluation of patients under treatment, and as a measure of the risk of healthy contacts to develop "open" forms of leprosy.

SUMMARY

A method of studying delayed-type hypersensitivity was developed with specific antigen in leprosy patients and contacts, measuring the dose-response curve at different lepromin concentrations. This "immunologic titration" is highly efficient for discriminating the degree of hypersensitivity reactions among the groups tested.

With respect to the Fernandez reaction, the results obtained showed that there was a similar behavior in all groups studied, except in the tuberculoid group which had a more intense response, four times higher than that yielded by contacts of lepromatous patients.

In the Mitsuda reaction, a similar behavior was also found among the different groups, except with respect to the reactivity intensity of contacts of lepromatous patients. Here it was demonstrated that this group had a significant depression in response to *M. leprae* antigen when compared with that from the other groups, independent of the degree of consanguinity or closeness to bacilliferous cases.

In order to explain this immunosuppression in contacts of lepromatous patients, a hypothesis is proposed. It is suggested that changes could occur in *M. leprae* derived from lepromatous patients, diminishing their capacity to produce an adequate immune response.

RESUMEN

Se presenta un método immunológico a nivel cutáneo para estudiar en enfermos de lepra y

convivientes, la respuesta precoz (reacción de Fernandez) y la tardía (reacción de Mitsuda) de cada individuo sometido a un estímulo simultáneo y creciente con lepromina bacilar normalizada (concentraciones entre 1.25×10^6 y 80×10^6 bac/ml). Los datos se ajustaron con líneas de regresión dosis-respuesta para cada grupo experimental.

Con respecto a la reacción de Fernandez, todos los grupos en estudio, pacientes, convivientes y testigos sanos dieron correlaciones dosis-respuesta positivas y altamente significativas, destacándose la máxima respuesta del grupo de enfermos T.

En relación con la reacción de Mitsuda, también se obtuvieron pendientes positivas y significativas en todos los grupos estudiados. No obstante, el grupo de convivientes L desarrolló una respuesta significativamente menor a la de los restantes grupos experimentales, que fue independiente tanto del grado de consanguinidad, como del tipo de contacto con el paciente.

Para explicar esta inmunodepresión en los convivientes L, se postula que el bacilo de Hansen proveniente de los enfermos bacilíferos abiertos sería poco antigénico, porque probablemente saldría con sus sitios antigénicos cubiertos o enmascarados (Ig?), lo que condicionaría esa respuesta inmunológica débil.

RÉSUMÉ

On a mis au point une méthode permettant d'étudier l'hypersensibilité de type retardé avec un antigène spécifique chez les malades de la lèpre et chez des contacts, en mesurant la courbe de réponse à des concentrations différentes de lépromine. Cette "titration immunologique" est très efficace pour différencier le degré des réactions d'hypersensibilité dans les groupes étudiés.

En ce qui concerne la réaction de Fernandez, les résultats obtenus ont montré qu'il existe un comportement similaire dans tous les groupes étudiés, à l'exception du groupe tuberculoïde que présentait une réponse plus intense, en fait quatre fois plus forte que celle manifestée par les contacts de malades lépromateux.

En ce qui regarde la réaction de Mitsuda, un comportement analogue a également été trouvé dans les différents groupes, si ce n'est pour ce qui concerne l'intensité de la réaction des contacts de malades lépromateux. Chez ces sujets, on a démontré une diminution significative de la réponse à l'antigène de *M. leprae,* lorsque l'on compare celle-ci avec celle obtenue dans d'autres groupes, indépendamment du degré de consanguinité ou de la proximité avec les cas bacilliferès.

On a proposé une hypothèse qui devrait expliquer cette immunosuppression chez les contacts de malades lépromateux. On suggère qu'il pourrait se produire des modifications dans *M. leprae* obtenu à partir de malades lépromateux; il en résulterait une diminution dans leur capacité de développer une réponse immunitaire adéquate.

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