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Histology of the Mitsuda Reaction of Healthy Adults with No Known Contacts with Leprosy Patients[1]

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The Mitsuda reaction is a consequence of events that follow the phagocytosis of the heat-killed leprosy bacilli contained in lepromin by the histiocytes (macrophages) of the skin. In spite of its important practical application, microscopic studies of the Mitsuda reaction in healthy persons who are noncontacts of leprosy patients are practically nonexistent in the literature. The histologic analysis of this reaction has been restricted to leprosy patients and their healthy contacts ([1-5, 17, 23, 26-30, 32, 35-37]), while the reported data on healthy noncontacts of leprosy patients is confined to a very small group (11 individuals) ([3]).

The lack of information on the histological meaning of the Mitsuda reaction exhibited by healthy noncontacts of leprosy patients is due, in part, to the difficulties in obtaining candidates, since healthy volunteers often object to being biopsied after inoculation with lepromin. However, it seems that this lack of information chiefly results from the tacit acceptance of the hypothesis that both healthy contacts and noncontacts of leprosy patients behave alike with respect to the late reaction to lepromin injection. Obviously, this hypothesis needs to be tested since, at least in Brazil, noncontacts are less exposed than contacts of leprosy patients, not only to *Mycobacterium leprae* antigens but also to BCG vaccination, both of which may stimulate clinically and histologically positive Mitsuda reactions as well as provoke clinically positive late lepromin reactions with no histological correspondence ([22]). Moreover, it should be recalled that a high proportion of contacts of leprosy patients are consanguineously related to them and, conversely, relatives of leprosy individuals may rarely be classed among those who are noncontacts of leprosy patients.

The frequency of consanguineous relatives of lepromatous patients in population samples tested for Mitsuda reaction may be of great importance, since several indications exist which favor the hypothesis that such individuals would belong to a special genetic strain composed mostly of weak or negative reactors ([7, 12]). These indications are: a) Mitsuda reactivity is a familial trait which may be genetically determined ([6, 7, 10, 13, 24, 31]); b) the probability of manifesting lepromatous leprosy depends upon the consanguinity between the contacts and the lepromatous focus ([10, 11]); c) the proportion of strongly positive Mitsuda reactions elicited in children under three years of age who were administered BCG is significantly higher among those born to healthy parents than among those born to lepromatous couples ([15]); and d) it is well known that both lepromatous leprosy and the susceptibility to this type of disease are associated with a negative Mitsuda reaction.

The present paper reports an investigation on the histology of the Mitsuda reaction exhibited by healthy adult subjects unrelated to leprosy patients and with no known contact with them.

MATERIALS AND METHODS

One hundred adult Brazilian males who lived in São Paulo, SP, at the time of this study, with no consanguineous relationship to leprosy patients, were inoculated intradermally, after informed consent, with 0.1 ml of lepromin on the volar surface of the left arm at about 12 cm from the axillary fossa. This site was chosen for carrying out the skin tests not only to avoid visible scars caused by biopsy, but also because of its larger amount of subcutaneous tissue as

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TABLE 1. *Criteria used for histological classification of the Mitsuda reaction.*

Class	Code	Histological picture
Negative	0	A granulomatous infiltrate without tuberculoid structure, composed of histiocytes full of undigested acid-fast bacilli.
	1	No inflammatory signs in the biopsy, and rare acid-fast bacilli in the intercellular spaces.
	2	A chronic nonspecific inflammatory infiltrate with rare or no acid-fast bacilli.
Positive	3	A small amount of histiocytes exhibiting an epithelioid aspect, which may show a tendency to form nodular granulomas. The intensity of the lymphocytic infiltrate is variable. Giant mulltinucleated cells as well as acid-fast bacilli are rarely seen or absent.
	4	A small amount of epithelioid cells tending to form nodular granulomas. The intensity of the lymphocytic infiltrate is variable. No acid-fast bacilli but some giant multinucleated cells are seen.
	5	A moderate amount of epithelioid cells forming several nodular granulomas. The intensity of the lymphocytic infiltrate is variable. Some giant multinucleated cells but no acid-fast bacilli are seen.
	6	A high concentration of epithelioid cells forming numerous nodular granulomas. The intensity of the lymphocytic infiltrate is variable. Some giant multinucleated cells but no acid-fast bacilli are seen.

compared to the forearm. The inoculated subjects were mostly Caucasoids (15 Negroids and six Mongoloids) on the average 20.9 years of age (S.D. = 3.11 years) and included 34 medical students, 33 soldiers, and 33 other individuals who had had no known contact with leprosy patients. Moreover, none of them had been previously inoculated with lepromin, while only four had been vaccinated with BCG. Three lepromatous patients were also inoculated with lepromin and served as negative Mitsuda controls.

The lepromin used was from *M. lepraе*-infected armadillo tissue prepared according to WHO standards ([16]). The site of the lepromin inoculation was previously marked with the help of a small ruler containing three identical orifices, the centers of which were on the same straight line. Each of the extreme orifices served to guide the sites where puntiform tattoos were made by injecting India ink, while the central orifice guided the site of the intradermal injection of lepromin.

The biopsies of the Mitsuda reactions were taken with a 3 mm punch from the clinically positive reactors, while those of the clinically negative and doubtful reactors were obtained with a 5 mm punch after determining with the small ruler mentioned above the site where lepromin was inoculated. In all cases, the biopsies were taken immediately after the clinical reading of the Mitsuda reaction.

The skin biopsies were fixed soon after removal in 10% Formalin. Each fixed biopsy was split into two fragments with a razor blade applied perpendicularly to its external surface, and prepared for inclusion in paraffin. The sections of each specimen (usually 4 to 12) were deparaffinized and rehydrated as usual, the cells being stained with hematoxylin and eosin (H&E), and the bacilli stained according to the Ziehl-Neelsen-Faraco method. The slides were numbered and analyzed blind.

The clinical responses to the lepromin injection were graded according to the recommendations of the VI International Leprosy Congress (Madrid, 1953) ([33]). The criteria used for the histological classification of the late lepromin reaction are given in Table 1.

RESULTS

The three lepromatous patients who served as controls each exhibited a clinically and histologically negative Mitsuda reaction, the latter being a granulomatous infiltrate composed of histiocytes full of undigested acid-fast bacilli (AFB) (code 0). As can be seen in Table 2, this type of histological reaction was not found among the healthy subjects. The three histologically negative reactions which were observed among these individuals included two biopsies showing no inflammatory signs and rare AFB in the intercellular spaces (code 1), and

TABLE 2. *Distribution of 100 healthy adults, with no known contact with leprosy patients, according to the results of both the clinical and histological examinations of the Mitsuda reaction.*

Histological classification		Clinical classification					Total
Class	Code	−	±	+	++	+++	
Negative	0	—	—	—	—	—	—
	1	2	—	—	—	—	2
	2	1	—	—	—	—	1
Positive	3	—	1	3	2	3	9
	4	1	9	12	12	4	38
	5	—	7	18	12	2	39
	6	—	2	6	3	—	11
Totals		4	19	39	29	9	100

one biopsy disclosing a chronic inflammatory infiltrate with rare AFB (code 2).

Table 2 also shows that a histological positivity of the Mitsuda reaction was observed not only in all 77 clinically positive Mitsuda reactors (39 +, 29 ++, and 9 +++) but also in all 19 subjects who were doubtful reactors (±), as well as in 1 of 4 clinically negative Mitsuda reactors. BCG vaccination seems not to have had any significance, since the four individuals previously vaccinated were not concentrated in a special class (one individual ±, code 3; one +, code 3; one +, code 4; and one ++, code 4).

A significant association ($p = 0.000025$) between the clinical and histological readings of the Mitsuda reactions could be demonstrated when these results were grouped in two classes (negative and non-negative). In contrast, when an independence test was applied to the different degree of histological and clinical positive reactions to lepromin injections (codes 3, 4, 5, and 6 vs +, ++, and +++), it was seen that the intensity of both types of positive responses were not significantly associated [$\chi^2 = 6.890$, 6 degrees of freedom (DF), $0.30 < p < 0.50$]. The same random distribution of the different degrees of the histologically positive reaction was seen among the 96 infiltrations detectable clinically ($\chi^2 = 5.924$, 9 DF, $0.80 < p < 0.90$).

DISCUSSION

The demonstration that the intensity of the clinically read Mitsuda reaction is not associated with the intensity of the histological response to the lepromin injection was indeed surprising, since it is universally accepted that the size of the induration at the site of the lepromin inoculation is an indicator of the capacity of the host to destroy the injected *M. leprae*. Therefore, our data support the hypothesis that the conclusions based on studies of the Mitsuda reaction exhibited by leprosy patients and their healthy contacts cannot always be extended to healthy noncontacts. Thus, among the latter the intensity of the clinically read Mitsuda reaction has no value for indicating the degree of lysogenic capacity of the macrophages for the phagocytized *M. leprae*.

According to the results presented in Table 2, it may be said that the infiltration with less than 3 mm diameter at the site of a lepromin inoculation should no longer be considered as a doubtful or as a negative reaction if exhibited by healthy noncontacts of leprosy individuals. Among these persons, these infiltrations should be regarded as a positive reaction since all 19 examined subjects showing the so-called doubtful reaction had a positive response on histological grounds. Obviously, the same recommendations do not apply to doubtful Mitsuda reactions exhibited by leprosy patients and their healthy contacts ([3–5]).

The data presented in Table 2 also support the suggestion that the ulceration observed on healthy Mitsuda reactors may be disregarded when the clinical positiveness of this reaction is being evaluated ([25]), since a nodular granuloma was not seen at the lepromin injection site in most of the +++ reactors. Moreover, none of them revealed a microscopic response of the highest degree, i.e., code 6.

With regard to the three clinically nega-

tive Mitsuda responses with no signs of histological positivity exhibited by the healthy subjects, it is curious that none has shown the microscopic picture seen in the biopsies of the Mitsuda reactions of the three lepromatous patients who served as controls. It is not easy to explain the histological pictures observed in the biopsies of these healthy individuals, since a negative reaction is expected to show the phagocytized bacilli resting unchanged within the macrophages ([8–10, 18, 19, 21]). These findings, which have also been reported by other authors ([1, 3–5, 28, 32, 36]), have been attributed to technical difficulties in determining the central area of the late response to lepromin injection ([20]), to the inoculation of an abacillary or paucibacillary lepromin (a technical error that may be committed if one uses refrigerated and unshaken lepromin[12]), or to the autolysis of the histiocytes full of undigested heat-killed *M. leprae* ([10]).

The difficulties in determining the central area of the clinically negative Mitsuda reaction have been carefully circumvented in the present work, and the possibility of injections of a paucibacillary or abacillary lepromin can be ruled out. Therefore, it seems plausible to suppose that the histological picture found in the clinically and microscopically Mitsuda-negative healthy subjects was due to the autolysis of the histiocytes previously full of undigested leprosy bacilli. The reasons for the short life span of the histiocytes full of phagocytized heat-killed *M. leprae* in some Mitsuda-negative reactors are unknown, but speculations on the origin of this cellular self destruction have been presented elsewhere ([10]).

With respect to the microscopically positive response found in one clinically negative Mitsuda reactor, it should be stated that this positive reaction without clinical expression cannot be attributed to a technical error. In fact, no histological evidence could be found to support the hypothesis that the lepromin injection was performed at a deeper level than usual. So, this case favors the hypothesis that among the healthy, clinically negative Mitsuda reactors there are individuals whose macrophages are able to digest phagocytized *M. leprae,* transforming themselves into epithelioid cells. Such persons would fail to manifest this capacity clinically because they are not sensitized by mycobacterial antigens ([10, 11]). These subjects are most probably the well-known Mitsuda-negative reactors who become able to express clinically their macrophages' capacity to lyse leprosy bacilli simply after repeated lepromin injections ([14]) or BCG vaccination ([15, 34]).

Finally, it should be stressed that the observed frequency of the histologically negative late lepromin reactors (3%) is significantly lower than that seen among healthy contacts of leprosy patients. For instance, among 62 of these contacts, Bechelli, *et al.* ([5]) found 22.6% to be histologically negative Mitsuda reactors (corrected $\chi^2 = 13.607$, 1 DF, $p < 0.001$). At present, we do not know whether this result can be attributed solely to a high frequency of consanguineous relatives of lepromatous patients among the healthy contacts of leprosy cases, since other methodological differences exist between the present study and that of Bechelli, *et al.* ([5]), e.g., the type of lepromin employed. Nevertheless, it seems clear that such data prompt the investigation of this hypothesis by analyzing histologically the Mitsuda reaction of healthy contacts of lepromatous patients separated according to their consanguinity to the index case.

SUMMARY

Mitsuda tests were performed on 100 healthy adult Brazilian males unrelated consanguineously to leprosy patients and with no known contacts with this disease. Three lepromatous patients served as negative Mitsuda controls. The lepromin employed was from *Mycobacterium leprae*-infected armadillo tissue prepared according to WHO standards. Clinical response to the lepromin inoculation was graded according to the recommendations of the VI International Leprosy Congress held in Madrid (1953). The histological reaction was graded according to the authors' criteria based on the intensity of the granulomatous response.

The clinically positive (77), the doubtful (19), and 1 out of 4 clinically negative Mitsuda reactors disclosed histologically positive reactions of different degrees of intensity. A significant association between the clinical and histological readings of the Mitsuda reaction was demonstrated when the

results of both readings were grouped in two classes: negative and non-negative. However, the different degrees of the histologically positive lepromin reactions were distributed at random among the clinically positive Mitsuda reactors.

The frequency of histologically negative Mitsuda reactors observed among the healthy subjects who had no known contacts with leprosy patients (3%) was significantly low when compared to the frequencies reported in the pertinent literature concerning healthy contacts of patients with Hansen's disease.

RESUMEN

Se hizo la prueba de Mitsuda en 100 hombres brasileños adultos sanos, algunos, contactos sin relación sanguínea de pacientes con lepra y otros, sin contacto conocido con la enfermedad. Como controles Mitsuda negativos se incluyeron 3 pacientes lepromatosos. La lepromina utilizada se preparó de tejido de armadillo infectado con *M. leprae* de acuerdo a los estándares de la WHO. La respuesta clínica a la lepromina se graduó de acuerdo a las recomendaciones del VI Congreso Internacional de la Lepra en Madrid (1953). La reacción histológica se graduó de acuerdo al criterio de los autores según la intensidad de la respuesta granulomatosa.

Los individuos clínicamente positivos (77), los dudosos (19), y uno de 4 individuos Mitsuda negativos, dieron reacciones histológicamente positivas de diferente grado de intensidad. Se demostró un significante grado de asociación entre las lecturas clínicas e histológicas cuando los resultados de ambas lecturas se agruparon en 2 clases: negativos y no negativos. Sin embargo, los diferentes grados de positividad histológica se encontraron distribuídos al azar en los reactores clínicamente positivos. La frecuencia de reactores histológicamente negativos entre los sujetos sanos que no tuvieron contacto conocido con la enfermedad (3%) resultó significativamente baja en comparación con las frecuencias reportadas en la literatura.

RÉSUMÉ

On a pratiqué des épreuves de Mitsuda chez 100 adultes brésiliens en bonne santé, sans consanguinité avec des malades de la lèpre, et n'ayant jamais, pour autant qu'on le sache, été exposés à la maladie. Trois malades lépromateux ont servi de témoin négatif pour l'épreuve de Mitsuda. La lépromine utilisée avait été récoltée à partir de tissu de tatous infectés par *Mycobacterium leprae*, et préparée selon les normes de l'OMS. La réponse clinique à l'inoculation de lépromine a été mesurée d'après les recommandations du VIème Congrès International de la Lèpre, Madrid, 1953. La réaction histologique a été graduée d'après des critères définis par les auteurs, sur la base de l'intensité de la réponse granulomateuse.

Des réactions histologiques positives, avec des degrés d'intensité variables, ont été relevées chez 77 sujets avec réactions cliniquement positives, 19 avec réactions douteuses, de même que chez un des 4 sujets ne réagissant pas cliniquement à la lépromine de Mitsuda. Si l'on groupe les résultats des lectures en deux classes, les négatifs et ceux qui ne sont pas négatifs, on constate une association significative entre les résultats cliniques et histologiques de la réaction de Mitsuda. Néanmoins, tous les degrés de réactions histologiquement positives à la lépromine étaient distribués au hasard parmi les réacteurs cliniquement positifs au Mitsuda.

La fréquence des réacteurs histologiquement négatifs à l'épreuve de Mitsuda (3%) parmi ces individus en bonne santé, n'ayant jamais eu aucun contact connu avec des malades de la lèpre, était significativement basse lorsqu'on la comparait aux fréquences des réactions observées chez des contacts en bonne santé de malades de la lèpre, telles qu'elles sont relatées dans la littérature.

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