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The Kveim Test in Leprosy [1]

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The classification of leprosy, according to Ridley and Jopling ([12]), is based on the degree of immunity that the host presents when attacked by the bacterial parasite *M. leprae*. The manifestations of clinical leprosy appear to reflect a massive cell-mediated immune response at the tuberculoid end of the spectrum and a complete absence of cell-mediated immunity at the lepromatous end ([12, 19]). The lepromin test elicits a highly positive late response (Mitsuda reaction) in tuberculoid patients and a negative response in lepromatous patients.

Sarcoidosis is a chronic systemic disease of unknown etiology, characterized histologically by noncaseating epithelioid cell granulomata ([14]). The Kveim test, a delayed-type hypersensitivity skin test used in the diagnosis of sarcoidosis, is similar to the lepromin test in many respects. Both are delayed type hypersensitivity skin tests using inactivated particulate antigen and in both cases a positive test is characterized by noncaseating epithelioid cell granulomata ([2]). Studies comparing the lepromin and Kveim tests have been carried out in various parts of the world since 1951 ([9, 11]). The conclusion so far from studies using validated Kveim antigen, with microscopic assessment, has been that false-positive Kveim reactions are rare in all forms of leprosy with the possible exception of Chinese and Japanese patients ([10, 11, 15, 17]). No such study has been done in India so far, using a validated Kveim suspension.

This investigation was, therefore, undertaken to clarify whether there is any cross-reactivity between the mycobacterial antigen of the lepromin test and the unknown antigenic stimulus of the Kveim suspension, and to assess the prevalence of false-positive reactions to the Kveim antigen in all types of South Indian leprosy patients.

MATERIALS AND METHODS

A clinical classification of leprosy in a large number of patients was done first. Skin smears to estimate the Bacteriologic Index and histopathologic examination of skin biopsies of representative lesions were done, to confirm the classification, according to the criteria of Ridley and Jopling ([12]).

Tuberculin sensitivity was tested in all these patients, using two international units of tuberculin with Tween 80. An intradermal inoculum of 0.1 ml of this was given on the volar surface of the forearm. The test was considered positive if a 6 mm or larger area of induration was present 48 hours after the injection, and negative if the area of induration was less than 6 mm. Eighteen tuberculin negative leprosy patients who were willing to undergo the test procedures were thus selected and of these, three were lost to follow-up.

The lepromin test was done using 0.1 ml of antigen, containing 1.6×10^8 bacilli, prepared according to the method of Hayashi-Mitsuda and modified by Wade ([20]). The inoculum was given intradermally on the volar hairless surface of the left forearm at a preselected site. This site was a point obtained at the intersection of a line drawn at right angles to the transverse wrist crease at its midpoint and a line joining the medial epicondyle with the radial styloid process at the wrist. It was usually located about 11 cm from the medial epicondyle and about 14-15 cm from the transverse wrist crease. This point was marked with India ink and its location, with measurements from the anatomical bony landmarks mentioned above, was carefully noted. The late (Mitsuda) lepromin reaction was read after 21 days, measured, biopsied, and five micrometer thick paraffin-embedded sections were examined after staining with hematoxylin and eosin and with the Fite-Faraco acid-fast stain for *M. leprae*.

The Kveim antigen was an internationally validated sarcoidal spleen suspension, pre-

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pared after the methods of Chase ([1]), and of Hurley and Bartholomeusz ([4]), and manufactured by the Commonwealth Serum Laboratories, Melbourne, Batch No. 005-1. Each patient was given a 0.15 ml intradermal inoculum of this antigen. In all cases, the volar surface of the right forearm was used to avoid confusion with the lepromin test which was done on the left forearm. The inoculation was according to the method described by Siltzbach and Ehrlich ([18]), and by Mitchell ([9]). The inoculation site was a point selected as for the lepromin test, marked with India ink, with its distance carefully measured from bony anatomical landmarks. It was checked to be clinically free of leprosy lesions.

The injection site was examined on the 40th day after inoculation and, whether macroscopically positive or not, was biopsied in every case. That the site of biopsy was, in fact, the site of inoculum was ensured by rechecking the measurements from the known anatomical landmarks. The biopsied specimens were formalin-Zenker fixed, and five micrometer thick paraffin-embedded sections were cut, stained with hematoxylin-eosin stain, and the modified Fite-Faraco stain for acid-fast bacilli. Histologic evaluation was graded as positive, equivocal, or negative, according to the criteria of Siltzbach and Ehrlich ([18]), and of Mitchell ([9]).

A positive Kveim reaction was diagnosed if the test site, on biopsy, showed one or more characteristic noncaseating epithelioid cell granulomata, with a dense lymphocytic cuff. An equivocal reaction showed chronic inflammatory cells and/or occasional epithelioid cells without definite granuloma formation. A negative reaction showed no epithelioid cells.

The entire tissue in all the equivocal and negative blocks was serially sectioned and each section was examined with the light microscope.

RESULTS

The lepromatous and borderline lepromatous patients were grouped as "lepromatous," and the tuberculoid and borderline tuberculoid patients were grouped as "tuberculoid" patients. There were 11 patients in the lepromatous group and 4 in the tuberculoid group. The patients' ages ranged from 9 to 45 years. All the patients except two were male. A summary of the classification of the 15 patients is given in Table 1. All the patients were active cases of leprosy, ranging from a reported duration of one month to about eight years. Eight of the fifteen cases had not had any previous treatment and seven had various types of therapy, including sulfones and indigenous treatment.

Lepromin test results. All those in the lepromatous group showed a negative 21 day lepromin reaction and all those in the tuberculoid group were lepromin positive, on both gross and microscopic examination. The results are tabulated in Table 2.

Kveim test results. All the cases were macroscopically negative for an early reaction (at 24 and 48 hours) to the antigen.

TABLE 1. *Classification of cases.*

Classification of leprosy	No. patients	Total	Group assigned
Polar lepromatous (LL)	3		lepromatous
Borderline lepromatous (BL)	1	4	spectrum
Borderline tuberculoid (BT)	8		tuberculoid
Polar tuberculoid (TT)	3	11	spectrum

TABLE 2. *Results of lepromin test (Mitsuda—21 day reaction).*

Group	No. patients	Lepromin (Mitsuda)		
		Negative	Mild positive	Positive
Lepromatous spectrum	4	4	—	—
Tuberculoid spectrum	11	—	1	10

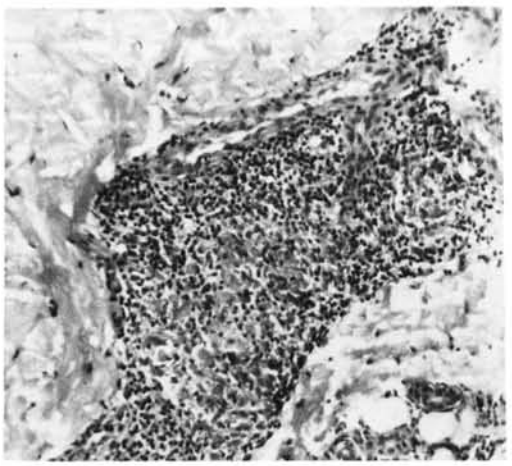

FIG. 1. Positive Kveim test. Well formed granuloma composed of epithelioid cells and a dense cuff of lymphocytes. H & E stain, ×100.

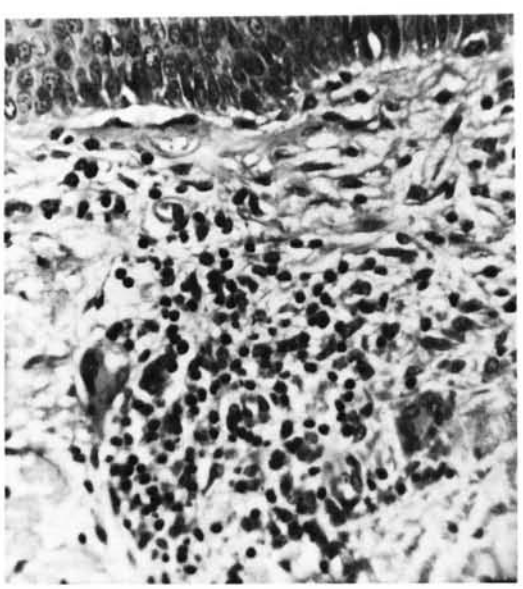

FIG. 2. Equivocal Kveim test. An ill formed granuloma composed of a few histiocytes, lymphocytes and a poorly-formed giant cell. H & E stain, ×250.

Careful examination of the Kveim antigen injection sites 40 days after inoculation showed no visible or palpable papule or nodule in any of the cases. Histologic examination of the injection site at this time showed 4 of the 11 tuberculoid cases to be positive (Fig. 1). One was equivocal (Fig. 2) and the remaining six were negative.

One of the four cases in the lepromatous group showed a definite epithelioid cell granuloma localized by lymphocytes with no central caseation or necrosis, and no acid-fast bacilli. Foamy macrophages containing acid-fast bacilli were present elsewhere in the section, suggesting features of lepromatous infiltration of the skin. Serial sections of the block did not show any neural involvement in the epithelioid granuloma, nor were any borderline features noted in the patient on both clinical and histopathologic examination. Therefore, this epithelioid cell granulomatous reaction was interpreted as a positive Kveim reaction with concomitant lepromatous infiltration of the skin. The other three cases in this group were negative. Results are summarized in Table 3.

DISCUSSION

"Delayed-type hypersensitivity reactions" refer to allergic reactions which develop many hours after the test antigen is placed in or on the tissues. This type of reaction is a specifically provoked, slowly evolving, mixed cellular response and is histologically characterized by an accumulation of lymphocytes, mononuclear cells, epithelioid cells, and macrophages ([3]).

In leprosy, this reaction is observed as evidence of an intact cell-mediated immune response to the antigen of the leprosy bacillus. The Mitsuda 21 day lepromin reaction is a delayed-type hypersensitivity reaction by a sensitized cell-mediated immune apparatus to the antigenic stimulus of dead *M. leprae*. The gross characteristics of the positive

TABLE 3. *Kveim test results.*

Group	No. patients	Kveim reaction		
		Positive	Equivocal	Negative
Lepromatous spectrum	4	1	—	3
Tuberculoid spectrum	11	4	1	6

Mitsuda or "late" reaction are well known. Biopsy 21 days after inoculation shows noncaseating epithelioid cell granulomata in the dermis, resembling those of tuberculoid leprosy. It is not a diagnostic test, as tuberculin is, since it is found positive in a proportion of normal healthy people with no past or present clinical evidence of leprosy or contact with the disease ([2]). The lepromin reaction is histologically identical to the delayed-type hypersensitivity reaction.

Much literature has accumulated over the last 20 years on comparisons between the positive Kveim and lepromin (Mitsuda) reactions, and between tuberculoid leprosy and sarcoidosis ([5, 7, 8, 10, 11, 16, 21, 22]). Most of the older literature claimed that the positive Kveim test was more common in tuberculoid than in lepromatous cases. These older studies, however, did not utilize a validated antigen and these results, which were variable, were based only on macroscopic readings of the Kveim test ([11]).

More recently, Siltzbach's International Kveim Test study, using validated antigen with histologic assessment of the Kveim tests, showed a "false positive" Kveim reaction in only 1.2% of 173 subjects with nonsarcoid diseases. All these "false positive" Kveim results were in leprosy cases. Kveim reactions in a total of 70 leprosy patients from Finland, Israel, Italy, Turkey and Japan were studied ([11]). The results from all countries except Japan were negative. The positive Japanese reactors were mostly among lepromatous patients (Table 4). A similar study, conducted in Malaysia ([10]), showed equivocal Kveim reactivity among tuberculoid patients, and positive and equivocal reactions among lepromatous patients. It is noteworthy that the positive Kveim reactors were of ethnically similar Mongoloid stock, i.e., patients who were Japanese and Malaysian Chinese.

The present study found 4 of the 11 tuberculoid patients to be Kveim positive, 1 of the 11 to be equivocal, 1 of the 4 lepromatous patients was Kveim positive, and all the remainder were negative. The antigen was an internationally validated one, prepared after the Chase-Siltzbach type I method ([4]), obtained from one source and used in a group of patients from an area endemic for leprosy. The prevalence of Kveim positive reactions appears to be higher in South Indian patients than in the Japanese or Chinese (Table 4).

Pearson ([10]) and Rees ([11]) have postulated that Kveim positive leprosy patients may have skin changes which predispose to granuloma formation following the injection of certain materials, and suggested that such granulomata would represent an isopathic type of reaction as described by Sagher ([13]). However, this will not explain the epithelioid cell granuloma following a Kveim test in a lepromatous patient. Furthermore, Sagher's isopathic reaction is not confirmed in South India ([6]). The thesis that there might be a relationship between the positive lepromin and Kveim reactions is invalidated by the lone positive reaction among the lepromatous cases, and by the larger number of lepromatous rather than tuberculoid patients who have reacted to the Kveim antigen among the Japanese patients.

TABLE 4. *Reported Kveim test studies in leprosy summarized.*

Area of study	No. patients	Lepromatous			Tuberculoid		
		Number	% Positive or weak positive	% Equivocal	Number	% Positive or weak positive	% Equivocal
Japan	13	10	20	50	3	0	33
Malaysia (Chinese)	30	21	5	10	9	0	44
Other countries (Turkey, Israel, Finland, Italy)	57	28	0	0	13	0	0
Present study (South India)	15	4	25	0	11	36	9

Totals do not tally where "indeterminate" group is omitted.

It is concluded, therefore, from this investigation that the prevalence of a positive or equivocal Kveim reaction among leprosy patients in South India is higher than that in Japanese and Chinese patients as reported by other workers. This may be due to ethnic factors. Lepromatous patients exhibit anergy to the antigenic stimulus of *M. leprae*, which is probably specific as suggested by the positive response in one lepromatous patient to the Kveim antigen. This response might indicate that leprosy patients react nonspecifically to the Kveim antigen and that, in fact, a definite relationship between the Kveim antigen and the lepromin antigen does not exist. It is also suggested that further studies of Kveim reactivity in Indian leprosy patients need to be done using a larger number of cases.

SUMMARY

The response to lepromin and Kveim antigens was compared and studied in 15 leprosy patients who were tuberculin negative. Of the 11 lepromin positive tuberculoid patients, 4 were Kveim positive, 1 was equivocal, and the rest were negative. Of the four lepromin negative lepromatous patients, one gave a positive Kveim test while the other three were negative. It has been shown that false-positive Kveim reactions are found in a higher percentage of South Indian leprosy patients than in those of other backgrounds, such as Japanese and Malaysian Chinese patients. It is also suggested that no definite relationship exists between the reaction of leprosy patients to lepromin and Kveim antigens. We further suggest that the anergy exhibited by lepromatous patients to the antigen of *M. leprae* is specific, as evidenced by the positive Kveim response in one lepromatous patient.

RESUMEN

Se estudió y comparó la respuesta a la lepromina y al antígeno de Kweim en 15 pacientes con lepra que eran tuberculino-negativos. De los 11 pacientes tuberculoides lepromino-positivos, 4 fueron Kweim positivos, 1 fué dudoso y el resto fueron negativos. De los 4 pacientes lepromatosos lepromino-negativos, uno dió una prueba de Kweim positiva y los otros 3 fueron negativos. Se ha demostrado que las reacciones de Kweim falso-positivas se encuentran en un porcentaje mayor en los pacientes con lepra del Sur de la India que en los de otros antecedentes étnicos, tales como Japoneses y Chinos Malayos. También se sugiere que no existe una relación definida entre la reacción de los pacientes con lepra al antígeno de Kweim y a lepromina. Además, sugerimos que la anergia que presentan los pacientes lepromatosos al antígeno de *M. leprae* es específica, como lo evidencia la respuesta positiva al antígeno de Kweim en un paciente lepromatoso.

RÉSUMÉ

La réponse à la lépromine et à l'antigène de Kveim a été comparée et étudiée chez 15 malades de la lèpre qui étaient négatifs à la tuberculine. Parmi 11 malades tuberculoïdes positifs à la lépromine, 4 étaient positifs pour le Kveim, la réponse d'un était ambiguë et les autres étaient négatifs. Parmi les 4 lépromateux négatifs à lépromine, 1 donnait une réponse positive pour le Kveim, les 3 autres étant négatifs à cette épreuve. On a montré que des réactions de Kveim faussement positives pouvaient être observées dans un pourcentage plus élevé chez les malades de la lèpre du Sud de l'Inde, que chez les lépreux appartenant à d'autres groupes ethniques, tels que les japonais, ou les sino-malaisiens. On suggère également qu'aucune relation définitive n'existe entre les réactions présentées par les malades de la lèpre à la lépromine et à l'antigène de Kveim. On suggère en outre que l'allergie démontrée par les malades lépromateux à l'égard de l'antigène de *M. leprae* est spécifique ainsi qu'en témoigne une réponse positive au Kveim chez un malade lépromateux.

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