The Kveim Test in Lepromatous and Tuberculoid Leprosy[1, 2]

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The results of the Kveim test in human leprosy have been variable. Negative (macroscopic) findings were reported in 10 patients with lepromatous leprosy ([15]), and in a group of 20 that included tuberculoid as well as lepromatous forms ([4]). On the other hand, the test was considered positive (again macroscopically) in about one-half of 14 Bantu patients with tuberculoid leprosy, but "only occasionally" in 12 with lepromatous disease; the average diameter of the nodules in the former group substantially exceeded that in the latter ([7]).

In an international study of Kveim tests in leprosy ([13]) 59 cases were included, from the following sources: Turkey 27 (15 lepromatous, 1 tuberculoid, 11 indeterminate) [3]; Japan 13 (10 lepromatous, 3 tuberculoid); Israel 11 (5 lepromatous, 5 tuberculoid, 1 indeterminate); Finland 5 (2 lepromatous, 5 tuberculoid), and Italy 3 (all lepromatous). The histologic reactions, as read at the reference laboratory, were regarded as weak-positive in two and equivocal in a further five of the 10 lepromatous cases in Japan, and as equivocal in one of the three Japanese tuberculoid cases. However, among the 25 lepromatous, nine tuberculoid and 12 indeterminate cases in the other contributing countries, the Kveim reactions were negative.

The evidence on Kveim reactions in leprosy is thus unclear; if present at all, its occurrence would seem to be much lower than in sarcoidosis.

In the present investigation 39 leprosy patients, predominantly from the same ethnic group (Chinese) and area (Malaysia), were tested and assessed histologically, using one source of Kveim material which had been well validated. The patients fell into three groups: 21 with lepromatous leprosy; nine with tuberculoid leprosy; and a third group of nine patients (8 lepromatous, 1 tuberculoid) who showed poor "conversion" to tuberculin sensitivity after BCG vaccination. All the patients had received antileprosy treatment, varying from one month to 26 years, and in most cases for two or more years; only seven could be considered to have active disease. Two patients had been treated also for pulmonary tuberculosis, which had responded and was inactive at the time of the Kveim tests.

All the patients had been tuberculin tested in the past, most on several occasions, and the test was repeated just before the Kveim test if it had not been done within the previous few months. In lepromatous leprosy there is a reduced sensitivity to tuberculin ([1, 8, 17]) and also impaired ability to develop delayed hypersensitivity to 2,4-dinitrochlorobenzene ([16]). This, together with the constant finding of a negative lepromin test in lepromatous leprosy, suggests that in this disease, as in sarcoidosis, there might be an association between anergy and Kveim reactivity. Particular attention was therefore paid to Kveim reactivity in relation to tuberculin sensitivity in the lepromatous group.

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3 These 27 cases are included in a detailed report ([2]) being prepared on Kveim test results among 38 patients with leprosy studied in Turkey.

TABLE 1. *Race and age of patients.*

Group	Race	Age (years)		
		Under 30	30 & over	Total
Lepromatous leprosy	Chinese	14	3	17
	Malay	3	0	3
	Aborigine	0	1	1
	Total	17[a]	4	21
Tuberculoid leprosy	Chinese	6[a]	3	9
Poor responders to tuberculin after BCG (8 lepromatous, 1 tuberculoid leprosy)	Chinese	5	1	6
	Malay	1	0	1
	Aborigine	2	0	2
	Total	8[b]	1	9

[a] Youngest age 17.
[b] Youngest age 16.

SELECTION OF PATIENTS

Lepromatous leprosy (21 patients). These were selected (by J.M.H.P.) from hospital patients, most of whom had been tuberculin tested at least twice. All were male, 17 of them Chinese. Their ages ranged from 17 to 39 years, 17 being under 30 (Table 1). None gave a history of previous BCG vaccination, and none showed a BCG scar. Classification as to type of leprosy was based on clinical examination and the results of skin smears for leprosy bacilli; in a few cases there was histologic confirmation (9).

These patients were selected to represent three categories on the bases of tuberculin tests: (1) tuberculin negative, i.e., those who had shown negative reactions to one tuberculin unit (TU) of PPD, Lot RT23 with Tween (*vide infra*), during 1964-1966 (7 patients, 4 of whom had also been negative, and 1 only weakly positive, to 20 TU); (2) tuberculin positive, i.e., those who had shown consistently positive reactions to one TU (9 patients, whose readings ranged from 9 to 27 mm.); and (3) tuberculin reverters, i.e., those who had shown positive reactions to one TU in 1964 but had since then been negative to this dose (5 patients, who were also negative or only weakly positive to 20 TU).

Four of the group (all in the tuberculin negative category) had been treated for less than one year and were suffering from clinically active leprosy; the rest had been treated for 18 months or more.

The Kveim testing of this group took place early in 1966.

Tuberculoid leprosy (nine patients). These also were selected (by J.M.H.P.) from the hospital patients. All were male Chinese; their ages ranged from 17 to 46 years, six being under 30 (Table 1). The diagnosis of tuberculoid leprosy was made on clinical examination together with results of skin smears; all were smear-negative for leprosy bacilli on all occasions.

Two patients were negative to 1 TU, one of them on three occasions in 1964-66. The other seven were positive to 1 TU during this period (4 on three occasions) with readings from five to 28 mm.

None of this group gave a history of BCG vaccination, and none had a BCG scar. The patient who had been treated for pulmonary tuberculosis had responded well, and his radiograph, after a year of treatment, showed only scarring at the right apex, considered to be inactive.

Three patients had been treated for less than six months and had clinically active leprosy; the rest had been treated for two or more years.

The Kveim testing of this group was carried out in early 1967.

Tuberculin negative lepromatous leprosy with poor response to tuberculin after BCG vaccination (9 patients). A high prevalence of Kveim positivity was reported in a small group of apparently healthy young adults in Britain who had failed to be "converted" to tuberculin sensitivity after two BCG vaccinations ([5]). Therefore it was decided to carry out Kveim tests in patients who were "nonconverters" to tuberculin sensitivity after BCG vaccination.

The patients were selected (by J.M.H.P. or J.H.S.P.) from a group of about 850 who had been tuberculin tested during a hospital survey (Table 1). All had been tested with 1 TU followed, in those giving negative results, by 20 TU. Patients whose tests with the larger dose of tuberculin were also negative, i.e., less than five millimeters in duration, were vaccinated with BCG (Glaxo freeze-dried vaccine; BCG 1). The vaccination sites were inspected after two weeks to check for a papule with or without ulceration, and again at 12 weeks for the presence or absence of a scar. At this time the patients were re-tested using 20 TU, and those with readings to the larger dose of tuberculin of less than 10 mm. induration were considered to be nonconverters and eligible for the Kveim tests. Only nine of the original entry qualified: one of them had tuberculoid, but the other eight had lepromatous leprosy. The first Kveim test (Kveim 1) was made three and one-half to four and one-half months after BCG 1. It had been intended to follow this (first) Kveim test by the usual biopsy, but because no nodules were present after six weeks, and only small evanescent nodules had been observed (in three cases) at earlier periods, this biopsy was omitted except in one patient (done at 6 weeks).

A second vaccination (BCG 2) was given to eight of the nine patients five to six months after BCG 1. (The ninth patient was dropped from the study at this stage because of severe erythema nodosum leprosum.) Tuberculin tests were made 12 weeks later, in a similar way to those following BCG 1. Most patients now gave larger reactions to 20 TU; only two were now less than 10 mm. (in fact no reaction at all), the rest being between 10 and 20 mm. Nevertheless they were all given a second Kveim test (Kveim 2) four and one-half months after BCG 2 in one case and three and one-half months after in the other eight. In five patients biopsies were made at four or six weeks; nodules had developed and were usually visible at the time of biopsy though never more than 4 mm. in diameter. The other three were not biopsied; they had either no nodules or in one case a very small transient response. The three patients who were not biopsied after Kveim 2 all volunteered for a third Kveim test (Kveim 3) plus a biopsy even if no nodule was seen, and this was given seven and one-half months after BCG 2. Very small nodules were observed in each case and each was biopsied at four weeks.

Thus, biopsies were made from eight patients who received two vaccinations and two or three Kveim tests; and an additional case was biopsied after only one vaccination and subsequent Kveim test. No patient was biopsied more than once.

The Kveim testing of this group was carried out in 1964-1965.

METHODS

Technic for Kveim. The tests were carried out ([14]) using Lots 8 and 10, Type I (Chase-Siltzbach) preparation, both derived from the sarcoidosis-involved spleen of patient "J" (2.7 and 3.0 mgm./ml, respectively) and prepared as previously described ([3, 11]). To exclude foreign material the syringe and needle were autoclaved wrapped in aluminum foil, and were rinsed with sterile saline solution before the test material was drawn up. After the intracutaneous injection of 0.15 ml. (405 μgm. for Lot 8, and 450 μgm. for Lot 10), the injection site was marked at the edge of the wheal with a tattoo spot using sterile "Pelikan" ink. The ink was drawn into another syringe and the needle placed vertically on the skin at the edge of the wheal and gently rotated under slight pressure so that the ink was deposited in the skin. As a

TABLE 2. *Macroscopic and microscopic results of Kveim tests.*

Group	Tuberculin test category	Total biopsied	Mean maximum diameter of nodule (mm.)	Mean diameter of nodule at biopsy (mm.)	Kveim-positive	Kveim-equivocal	Kveim-negative
Lepromatous leprosy	Negative	7	0.9	0.9	1 (weak)	1	5
	Positive	9	1.3	0.8	0	1	8
	Reverted	5	1.2	0.8	0	0	5
	Total	21	1.1	0.8	1	2	18
Tuberculoid leprosy	Negative	2	2.5	0	0	1	1
	Positive	7	2.4	1.7	0	3	4
	Total	9	2.4	1.3	0	4	5
Poor responders to tuberculin after BCG (8 lepromatous and 1 tuberculoid leprosy)	Negative	9	2.2	1.3	0	2	7

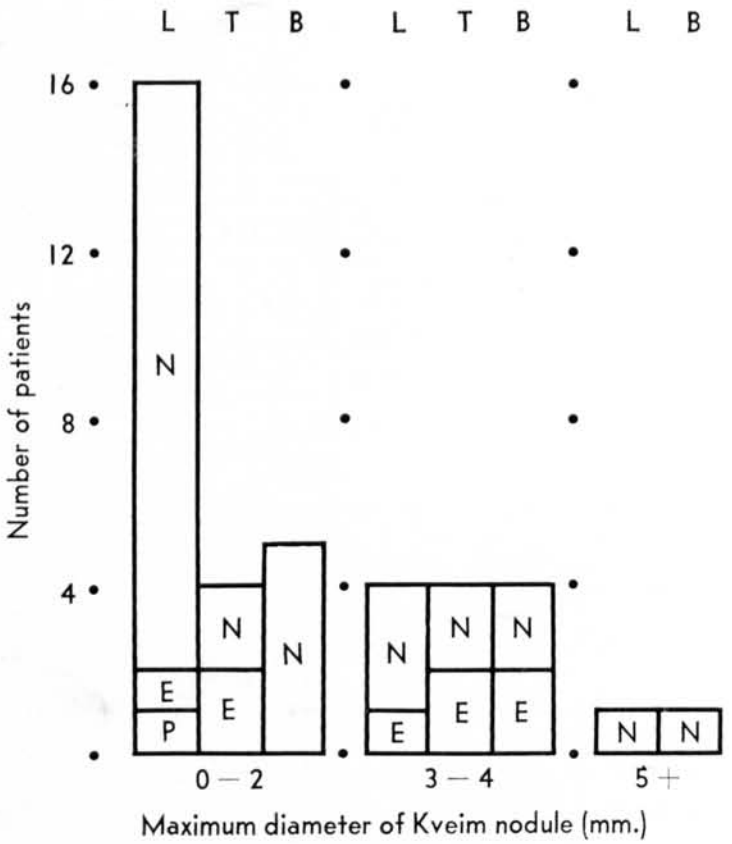

FIG. 1. Distribution of positive (P), equivocal (E) and negative (N) Kveim tests according to maximum diameters of nodules, in lepromatous (L) tuberculoid (T) and BCG (B) groups of patients.

further safeguard the position of the tattoo was recorded from the nearest convenient landmark. Most tests were made on the mesial aspect of the upper arm just above the elbow.

The test sites were examined weekly, and any nodules were measured then and again just before biopsy. For the lepromatous group the biopsy was made at three weeks in six patients (who had small nodules that were already fading), and at four weeks in 15 (one of whom had a small persistent nodule, the others showing only minimal transient nodules). For the tuberculoid group all biopsies were made at four weeks (when all lesions were diminishing and two had disappeared).

The biopsies were taken under local anesthesia, the full thickness of the skin down to subcutaneous tissue being excised so as to include any nodules. The specimen was placed in Lowy's fixative and sections prepared and stained with hematoxylin and eosin, and with Ziehl-Neelsen stain. The slides were assessed (by D.S.R.) for histologic changes suggesting leprosy and for acid-fast bacilli, and (by L.E.S.) for Kveim reactions.

Technic for tuberculin tests. The tests were made intracutaneously on the forearm with 0.1 ml. of PPD, Lot RT 23 with Tween (WHO), containing one or 20 tuberculin units (TU), this corresponding to approximately five and 100 TU respectively of PPD without Tween. The test was read at 48 hours. Induration of less than five millimeters was regarded as a negative reaction, of five millimeters or more as positive.

RESULTS

A summary of the findings is given in Table 2. It will be seen that one Kveim

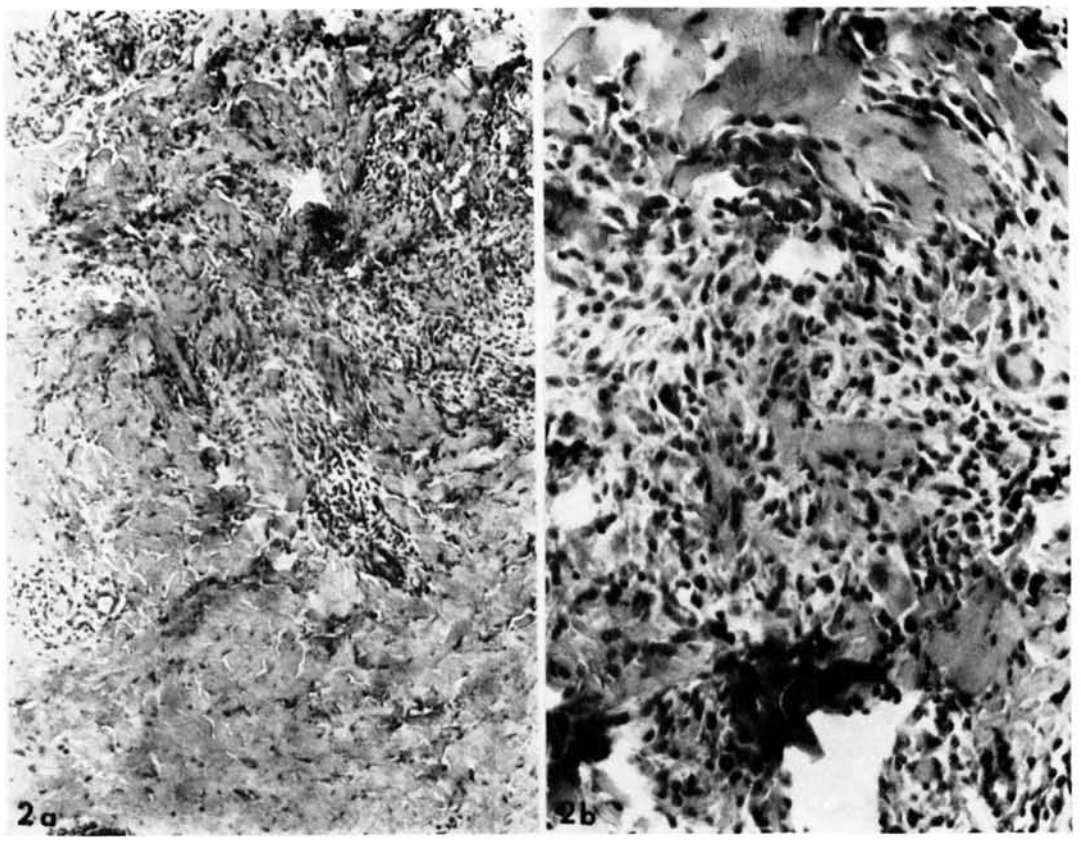

FIG. 2. Histology of Kveim test, patient with lepromatous leprosy. (a) Diffuse cellular infiltration of the mid-corium consisting mainly of lymphocytes and scattered histiocytes with a rudimentary follicular arrangement of histiocytes in the upper right corner (X140). (b) Focal collection of epithelioid cells from a rudimentary follicle in the corium with a giant cell on the right (X250). Weak positive Kveim reaction.

positive (histologic) result and two equivocal readings were obtained in the 21 lepromatous cases, and four equivocals in the nine tuberculoid cases. The granulomas in the BCG group were equivocal in two of the 11 cases. All the remaining tests were negative. One patient in the lepromatous group with an equivocal Kveim test was not x-rayed. The chest radiographs of all other patients with positive or equivocal results were normal.

There was no clear association of granulomas with any particular tuberculin test result, but the numbers in the subgroups were too small to be definite; nor was there any apparent association with the activity of the disease, the single Kveim positive and five of the seven equivocal results being in patients who had received more than two years of treatment.

The mean maximum diameter of the tuberculoid group nodules was 2.4 mm. (range 0 to 5 mm.), whereas that of the lepromatous group was 1.1 mm. (range 0 to 5 mm.). In Figure 1 the distribution of these diameters is given, and it will be seen that only two of the total of 39 cases of leprosy had nodules of five millimeters or more diameter and both these were Kveim negative.

In the present investigation care was taken to distinguish histologic changes of leprosy from those typical of, or approaching, Kveim positive appearances. In the tuberculoid group no acid-fast bacilli and no leprosy changes were seen in the Kveim biopsies. In the lepromatous group acid-fast bacilli and leprosy changes were absent also in the three patients who were either Kveim positive (1 patient) or equiv-

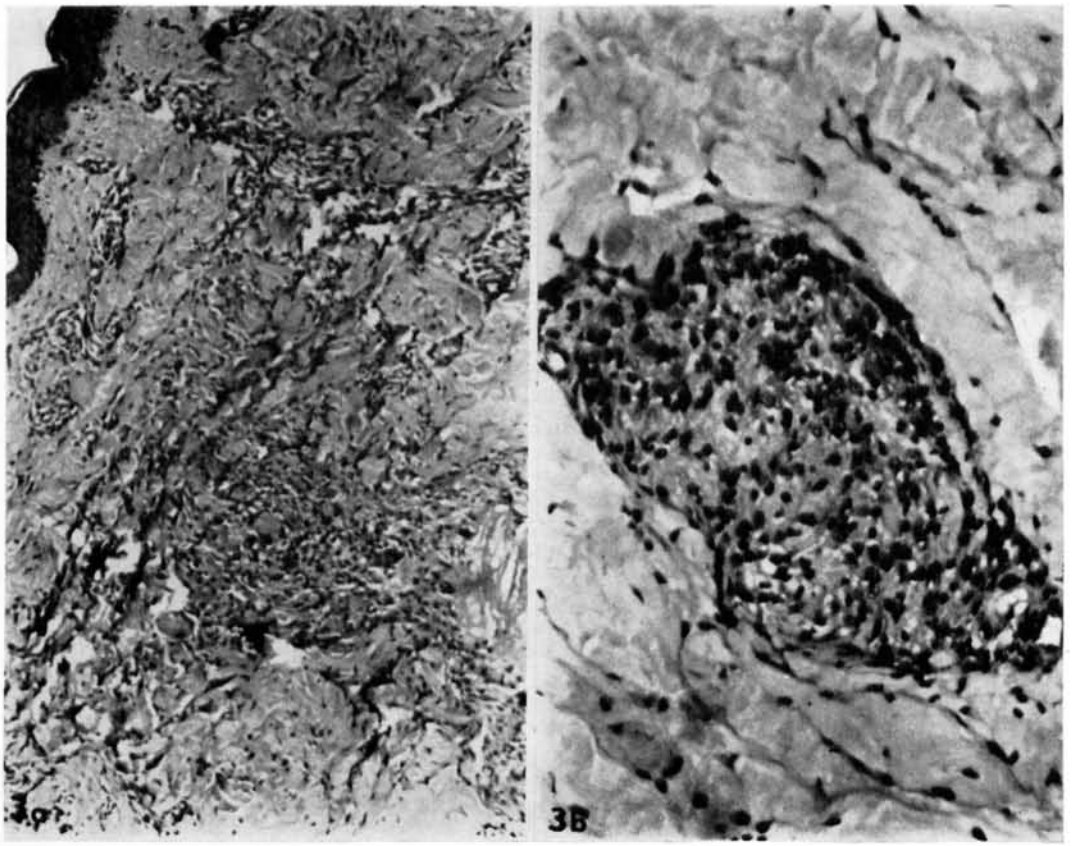

FIG. 3. Histology of Kveim test, patient with lepromatous leprosy. (a) Poorly circumscribed aggregate of histiocytes is present in the mid-cutis (X140). (b) A single circumscribed focus of epithelioid cells lies in a deeper layer of the skin (X400). Equivocal Kveim reaction.

ocal (2 patients); in the remaining 18 there were small foamy cell infiltrations in 12, acid-fast bacilli in eight and five were negative. (In one patient there were acid-fast bacilli present in nerve bundles, though histologically there was no obvious leprous granuloma.) Microscopic appearances of the single positive and of representative equivocal Kveim test results in the lepromatous and tuberculoid groups of patients are illustrated in Figures 2-4. All show the presence of granulomas. However, the proportion of epithelioid cells and the follicular arrangement of these and other cells within the granulomas was variable, and only in one case (Fig. 2) were these features sufficiently characteristic to be regarded as Kveim positive.

In the BCG group one Kveim test on each patient was biopsied and of these two gave equivocal results and none of the macroscopic readings exceeded 4 mm. Thus there was no evidence that in this special group BCG increased Kveim reactivity in patients with lepromatous leprosy.

DISCUSSION

An advantage of the present study is that the Kveim material was a product which had been validated by use in the International Kveim Test Study ([12]). It may be noted in this connection that a hospital patient suffering from lepromatous leprosy who developed sarcoidosis (diagnosed clinically and confirmed by lymph node biospy) gave a positive Kveim test (macro- and microscopic) with the same Kveim material.

Of the leprosy groups here included, the lepromatous group, aside from the one

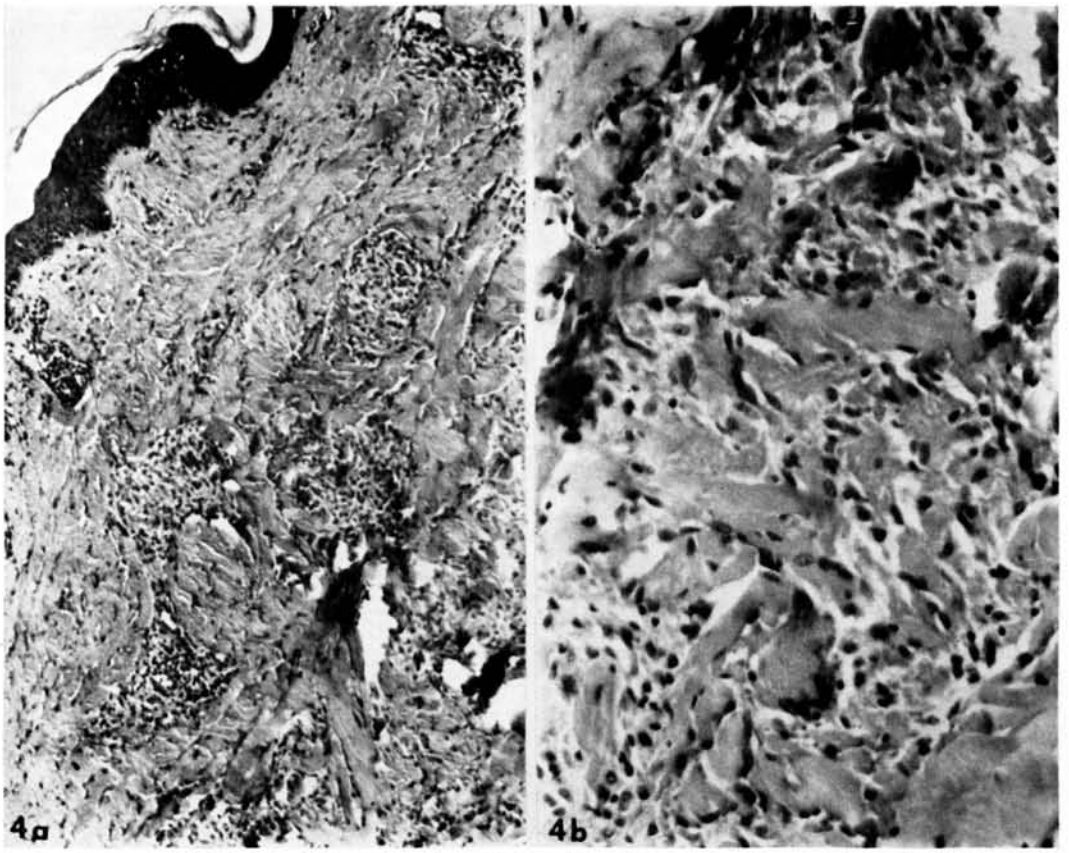

FIG. 4. Histology of Kveim test, patient with tuberculoid leprosy. (a) Small focal collection of cells in the mid-corium (X140). (b) An admixture of a few epithelioid cells, some giant cells and nonspecific inflammatory cells lying mainly between the collagen bundles (X400). Equivocal Kveim reaction.

weak positive, had a proportion of equivocal Kveim test results little, if at all, higher than might be found in a normal population. On the other hand, the figure of four equivocal results among the nine tuberculoid cases is higher than one might expect. This correlates with the larger mean diameter of their nodules, though only one (Kveim negative) was 5 mm. or more. In the BCG group the two equivocal granulomatous responses to the Kveim suspension were no more typical in appearance than those in the other groups, and it is likely that in lepromatous leprosy, as in sarcoidosis ([6]), BCG fails to augment Kveim reactivity. Certainly the BCG group did not show the high prevalence of Kveim positivity found in apparently healthy persons who had been BCG vaccinated but failed to be converted to tuberculin sensitivity ([5]).

It remains uncertain why some patients with leprosy, particularly the tuberculoid group, develop granulomas which are not typical of a positive Kveim test result. One hypothesis is that these subjects have skin changes which predispose to granulomas following the injection of certain materials: such granulomas would represent an isopathic type of reaction ([10]), though this reaction was reported for lepromatous leprosy.

It is also interesting that the great majority of positive and equivocally positive reactions in leprosy came from Chinese or Japanese patients, who have a similar ethnic origin. A practical corollary is that in an endemic leprosy area, confusion could sometimes arise in distinguishing between sarcoidosis and leprosy, although clinical manifestations, e.g., hilar adenopathy and

pulmonary mottling on radiography, should assist the diagnosis or lead to further investigation. The difficulty would be greatest in the skin lesions of patients with tuberculoid leprosy in whom there might be no acid-fast bacilli, although their skin lesions, unlike sarcoidosis, show anesthesia.

Bearing in mind that the frequency of Kveim positive results decreases as the duration of sarcoidosis lengthens, a limitation of the present study is that all the patients had been treated for leprosy, many of them for a long period. It would not be ethical to delay treatment until a Kveim test had been completed, but it would be reasonable to test immediately on admission, while the disease is undoubtedly in an active stage. A further study on these lines is planned.

SUMMARY

Kveim tests using a validated material have been undertaken in Malaysia on 39 patients (32 Chinese; 4 Malay and 3 Aboriginal) with lepromatous or tuberculoid leprosy. All the patients had been treated for leprosy, most for two or more years. The tests were read microscopically.

Of the 21 lepromatous patients one gave a weak positive and two an equivocal Kveim test whereas four of the nine tuberculoid patients gave equivocal or weak Kveim positivity. Only the tuberculoid form elicits a higher proportion of granulomas than might be expected in a comparable normal population.

Of nine patients (8 lepromatous; 1 tuberculoid) who failed to sensitize well to tuberculin following two BCG vaccinations, two gave equivocal Kveim tests similar in appearance to those in the other groups.

RESUMEN

En Malasia se practicaron pruebas de Kveim. usando antígeno válido, en 39 enfermos (32 chinos. 4 malayos y 3 aborígenes) con lepra lepromatosa o tuberculoide. Todos los enfermos habían sido tratados por su enfermedad, la mayoría por más de 2 años. La prueba fué leído micropicamente. De 21 enfermos lepromatosos, 1 dió una respuesta débilmente positiva y 2 una respuesta, equívoca a la prueba de Kveim, en tanto que 4 de los 9 tuberculoides dieron respuestas equívocas o débilmente positivas. Solamente la forma tuberculoide produjo una pro porción de granulomas mayor que le se puede esperar de una población normal comparable. De 9 enfermos (8 lepromatosos y 1 tuberculoide) que no se sensibilizaron bien a la tuberculina después de 2 vacunaciones con BCG, 2 dieron pruebas de Kveim equívocas, similares en apariencia al de los otros grupos.

RÉSUMÉ

Des tests de Kveim utilisant un matériel valable ont été realísés en Malaysie chez 39 malades (32 chinois, 4 Malais, 3 Aborigenes) atteints de lèpre lépromateuse ou tuberculoïde. Tous ces malades avaient été traités contre la lèpre la plupart depuis 2 ans ou plus. Les tests on été lus au microscope.

Sur les 21 malades des lépromateux, un a présenté un test faiblement positif et 2 un test douteux, alors que 4 des 9 malades atteints de lèpre tuberculoïde montraient un test de Kveim faiblement positif ou douteux. Seule la forme tuberculoïde déclenche une proportion de granulomes plus élevée que celle normalement rencontré dans une population normale comparable.

Sur 9 malades (8 lépromateux, 1 tuberculoïde) qui aprés deux vaccinations par le BCG ne presentaient pas la sensibilité a la tuberculine, 2 ont donné des tests de Kveim douleux et semblables en apparence á ceux des autres groupes.

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