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Cell-Mediated Immunologic Status of Healthy Members of Families with a History of Leprosy [1, 2]

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In lepromatous leprosy the causal organism, *Mycobacterium leprae*, proliferates in a host incapable of mounting the cellular immune response required to control the organism. This lack of specific cell-mediated immunity (CMI) to *M. leprae* antigens is demonstrated *in vivo* by a negative lepromin skin test and *in vitro* by a failure of lymphocytes to transform or to elaborate macrophage or leucocyte migration inhibitory factors when cultured in the presence of *M. leprae* antigens ([6, 11, 17, 19]). In addition, patients appear to have a partial depression of nonspecific CMI ([4, 5, 6, 8, 15, 24, 25]). The nonspecific immune responses improve following therapy but the anergy to *M. leprae* remains ([5, 8, 12, 18]).

It is not known whether the inability to respond to *M. leprae* antedates and predisposes to the development of lepromatous leprosy, or results from infection with the organism. The factor N hypothesis of Rotberg, cited by Newell ([20]), proposes that a certain proportion of the population is constitutionally incapable of reacting to *M. leprae*, and that from this group lepromatous leprosy cases are derived. This hypothesis is supported by the data of Dharmendra and Chatterjee ([7]) which indicated that lepromin negative individuals were much more likely to develop the lepromatous form of leprosy than were positive reactors. Newell ([20]) noted that lepromatous leprosy seems not to occur as a fixed proportion of leprosy infections, but reaches a maximum prevalence rate of some 5 to 10 per 1,000 regardless of the total leprosy prevalence rate. He suggested that the development of lepromatous leprosy in an infected person is a host-determined characteristic possessed by a fixed proportion of all populations.

Jamison and Vollum ([16]) observed that in Nigeria children from families with leprosy (type not specified) showed a much lower rate (18%) of conversion to tuberculin positivity after vaccination with a vole tuberculosis vaccine than children from families with no history of leprosy (90% conversion). This suggested that leprosy occurs in families in which there is a readily detected natural weakness in the ability to mount a cellular immune response.

In this paper we report the results of a study in which several parameters of CMI have been measured in carefully matched pairs of healthy children from an endemic leprosy area in the Central District of Papua New Guinea. One child in each pair came from a family in which there was leprosy, and the other from a family with no history of leprosy.

MATERIALS AND METHODS

Subjects. The subjects studied came from two adjacent coastal villages near Port Moresby where the prevalence of leprosy was high. Five hundred and fifteen people, from a total population of 545, were clinically examined and 17 cases of leprosy found. On clinical grounds, ten of these were lepromatous and seven tuberculoid. Detailed genealogies of all families were taken and used as the basis for selection of subjects. Twenty children without leprosy, aged from 5 to 18 years, were chosen from families in which cases of leprosy had been identified. These children all had at least one sibling or one parent with leprosy, some had both, and some also had a grandparent with leprosy. The index leprosy cases in these families were all classified clinically as lepromatous. On subsequent histopathologic examination,

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kindly carried out by Dr. D. S. Ridley, two were classified as polar lepromatous (LL), four as indefinite lepromatous (LI) and two as indeterminate ([22, 23]). Twenty age- and sex-matched control ehildren were selected from families in which no leprosy cases had been identified. The control children had no sibling, parent, parent's sibling or grandparent with leprosy.

Antigens and mitogen. Purified phytohemagglutinin (PHA) was obtained from Wellcome Research Laboratories, England; RT23 tuberculin purified protein derivative (PPD) was obtained from Statens Seruminstitut, Copenhagen, Denmark; standard Mitsuda lepromin (1.6×10^8 bacilli/ml) was kindly provided by Drs. G. P. Walsh and C. V. Reich of the Leonard Wood Memorial, Philippines, and by Dr. R. J. W. Rees of the National Institute for Medical Research, Mill Hill, England; *Candida albicans* intradermal testing solution was obtained from Bencard, Brentford, England.

Lymphocyte transformation tests. A microtechnic was used in which leukocytes were cultured in a total volume of 0.1 ml. Ten milliliters peripheral blood were collected in a syringe containing 200 U preservative-free heparin. After sedimentation of the red cells in the inverted syringe at 37° C, the plasma layer containing the leukocytes was expressed. The leukocytes were collected by centrifugation, washed once with medium 199 containing 50% normal human AB serum and resuspended finally in serum at final concentration of 5×10^6 leukocytes/ml. Fifty microliter volumes were dispensed into Falcon tubes.

PHA, PPD and lepromin were diluted in medium 199 containing penicillin (100 U/ml) and streptomycin (100 μg/ml), and 50 μl aliquots were added to the leukocytes. PHA was used at a final concentration of 0.25 μg/tube, PPD at 0.4 μg/tube and in the case of lepromin, standard Mitsuda lepromin which had been dialysed against medium 199 containing antibiotics was diluted a further twofold in this medium and 50 μl aliquots used (approximately 4×10^6 bacilli/tube). These concentrations were shown to be optimal in dose response experiments. Control tubes received 50 μl medium 199 containing antibiotics. All cultures were set up in triplicate, and the leukocytes from each pair of age- and sex-matched subjects were tested on the same day. Cultures were incubated at 37°C in an atmosphere of 5% CO_2 in air saturated with water vapor.

In PHA stimulated cultures and controls, 0.5 μCi (methyl-^{3}H) thymidine (Radiochemical Centre, Amersham, England; specific activity 2 Ci/mmole) was added to each tube after 48 hours incubation, and cells were harvested after a further 16 hours. For PPD- and lepromin-stimulated cultures and controls, label was added after 72 hours and cells harvested 24 hours later. The cells were cooled on ice, centrifuged and washed once with saline. They were then incubated for two hours at room temperature in the presence of 0.5 ml 2M KOH and 0.5 ml 0.4% albumin, and the nucleoprotein precipitated with 2 ml 40% trichloracetic acid (TCA) for two hours at 4°C. The precipitate was washed once with 10% TCA, dried, and dissolved in 0.5 ml Soluene (Packard Instrument Co., Illinois, U.S.A.) before adding scintillant.

For PPD- and lepromin-stimulated cultures, results were expressed as a stimulatory index, which is the ratio of the mean incorporation of ^{3}H-thymidine in stimulated cultures to that in control tubes. A ratio of two or greater was considered positive transformation. For PHA-stimulated cultures, results were expressed as mean c.p.m. incorporated.

The microtechnic was validated by testing the response to lepromin of clinically classified tuberculoid and lepromatous leprosy patients using this method (Fig. 1). No lepromatous patient responded to lepromin *in vitro*, while tuberculoid patients varied markedly in their response, ranging from no response up to a stimulatory index of 20. The difference in proportion of responders in the two groups was statistically significant ($p = 0.007$). PHA responses were significantly lower in the lepromatous patients ($p = 0.001$), but the difference in response to PPD was not significant.

Skin tests. Skin tests were carried out after blood had been taken for lymphocyte transformation studies. Lepromin testing was done first, with PPD and candida being tested after the late lepromin (Mitsuda) reaction had been read. One tenth milliliter volumes of lepromin (containing 1.6×10^7 bacilli), PPD (containing 0.02 μg, 1 T.U.) and candida antigen (containing 100 μg)

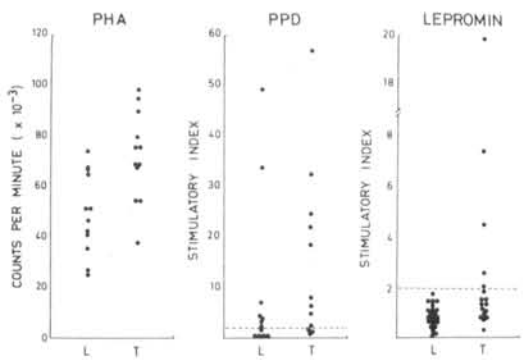

FIG. 1. Lymphocyte transformation responses of leprosy patients to PHA, PPD and lepromin. L, lepromatous; T, tuberculoid.

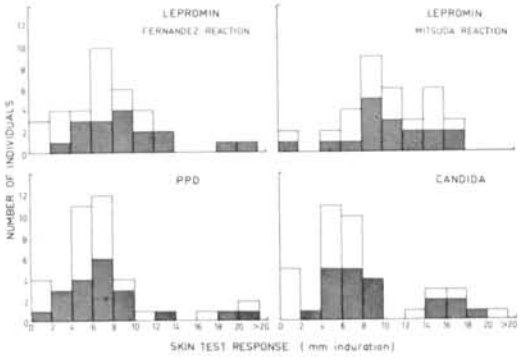

FIG. 2. Skin test responses to lepromin, PPD and candida. Shaded areas represent children from families with leprosy.

were injected intradermally into the volar surface of the forearm. Reactions were read as millimeters of induration by two persons independently, and a mean value adopted where necessary. For lepromin, the early (Fernandez) reaction was read at 48 hours and the Mitsuda reaction at 22 days. PPD and candida skin tests were read at 48 hours. Grading of lepromin reactions was recorded according to WHO (1953) recommendations ([26]).

Statistical methods. Correlations were determined by the Spearman rank correlation coefficient, r_s. The significance of difference in proportions was determined by Fisher's exact test. Wilcoxon's matched pair signed rank test was used to test the significance of the difference between test and control groups for each parameter of CMI measured. For the data in Figure 1, the Mann-Whitney U test was used to test the significance of the difference between lepromatous and tuberculoid patients in their response to PHA and to PPD. Because none of the lepromatous patients responded to lepromin, this test was not suitable for the lepromin data, and Fisher's exact test was used.

RESULTS

Skin tests. The results of skin tests are shown in Figure 2. Of the 35 children lepromin tested (five children were not done), 33 (94%) reacted at 22 days with induration greater than 4 mm (Mitsuda positive). The two nonreactors gave no induration, and one came from each group. There was a lower incidence of reactivity at 48 hours with eight (23%) having induration greater than 10 mm (Fernandez positive). Most individuals reacted to PPD and candida with induration greater than 4 mm.

A comparison of lepromin reactivity was possible in 17 matched pairs. Twenty matched pairs were available for comparison of reactivity to PPD and to candida. There was no significant difference between the children from families with leprosy and the control group in their reaction to any of the antigens, with the exception that the Fernandez lepromin reaction was greater amongst the children from families with leprosy ($0.02 < p < 0.05$).

Lymphocyte transformation tests. In Figure 3 the *in vitro* lymphocyte responses to PHA, PPD and lepromin are shown for each of the two groups of children. For each test, 19 paired comparisons were possible. There was no significant difference in the *in vitro* responses of the two groups for any of the tests.

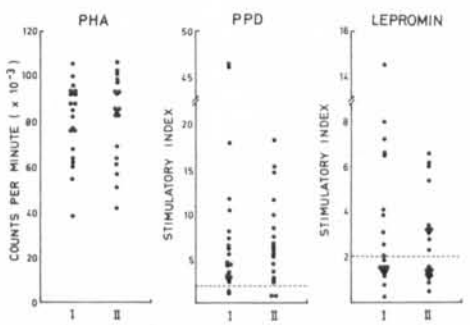

FIG. 3. Lymphocyte transformation responses to PHA, PPD and lepromin of (I) children from families with leprosy, and (II) children from families with no history of leprosy.

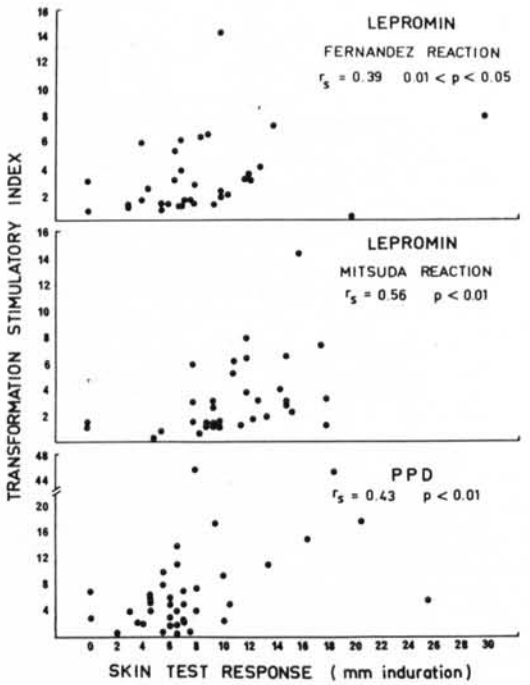

FIG. 4. Correlation of lymphocyte transformation with skin test response to lepromin and to PPD.

There was a significant correlation between the skin test response and *in vitro* response to both lepromin and PPD (Fig. 4). Lymphocyte transformation to lepromin correlated better with the Mitsuda reaction than with the Fernandez reaction. Nevertheless, one third of the subjects had a positive Mitsuda reaction (>4 mm) and a negative *in vivo* response to lepromin.

The degree of lymphocyte transformation to PHA showed a correlation with age which was highly significant (Table 1). Each of the other parameters tested also showed a correlation with age to a greater or lesser degree.

Clinical follow-up. In the time since this study was carried out, two of the children have presented at Port Moresby General Hospital with leprosy which clinically was classified as lepromatous. On histopathologic examination, one was reported to be LI, the other BL. These were the two children who had failed to give a positive Mitsuda lepromin reaction. They had also failed to respond to lepromin *in vitro*. One, a girl aged 13, came from a family with leprosy; while the other, a girl aged 8, came from the control group and was the first known case of leprosy in her family. These children presented with disease 16 months and 10 months, respectively, after the initial study was performed.

TABLE 1. *Correlation of different parameters of cell-mediated immunity with age (Spearman rank test).*

Parameter	r_s	p
Lymphocyte transformation		
PHA	0.56	$p < 0.01$
PPD	0.65	$p < 0.01$
Lepromin	0.36	$0.01 < p < 0.05$
Skin tests		
Lepromin (Fernandez)	0.53	$p < 0.01$
Lepromin (Mitsuda)	0.43	$p < 0.01$
PPD	0.42	$p < 0.01$
Candida	0.40	$p < 0.01$

Recently, a repeat clinical examination has been carried out on all the other children included in the study. No further cases of leprosy were found.

DISCUSSION

Twenty apparently healthy children from families with a history of leprosy have been assessed by a number of parameters of cellular immune competence, and compared with age- and sex-matched controls from families without leprosy living in the same environment. No evidence was obtained for a depression of CMI in the children closely related to cases of leprosy. There was no significant difference between the responses of the two groups of children for any of the parameters measured, except the Fernandez skin reaction which was greater in the group from families with leprosy. This may be due to greater exposure of these children to *M. leprae*. However, if this is so, it is not reflected in the lymphocyte responses to lepromin *in vitro*.

The fact that 33 of 35 children tested had a positive Mitsuda reaction indicates that there is no widespread anergy to lepromin amongst this population in which leprosy is highly endemic. Approximately one third of the children with a positive Mitsuda reaction, however, failed to respond to lepro-

min *in vitro*. Since skin testing was carried out after the *in vitro* tests, it is possible that, in these individuals, the positive skin test resulted from sensitization by the test dose of lepromin itself ([2, 9]) and that the lymphocyte transformation results give a better indication of cellular immunity to leprosy that has developed naturally. Alternatively, the difference may reflect a lack of sensitivity in the *in vitro* test, or a true dissociation of blastogenic and delayed hypersensitivity responses. In spite of this discrepancy, the degree of lymphocyte transformation to lepromin correlated better with the Mitsuda than with the Fernandez skin reaction.

The age dependency of the acquisition of lepromin positivity in endemic areas has been documented ([14]). In the present study, also, there was a significant tendency for the large (3+) Mitsuda reactions and the positive *in vitro* responses to lepromin to occur in the older groups (>12 years; $p = 0.002$, $p = 0.02$, respectively). While this probably reflects a greater exposure to *M. leprae* with increasing age, the marked age dependency of the PHA response indicates that maturation of the cellular immune system is also an important factor. Pisciotta *et al* ([21]) also reported increase in PHA responsiveness with age up to the age of 12 years.

When the present study was organized, leprosy families were chosen in which the index case(s) were clinically classified by two of us to be lepromatous, rather than tuberculoid, in the hope of increasing the probability of detecting a deficiency in the CMI response of close relatives. As reported in METHODS, however, histopathologic examination showed most of the index cases not to be polar lepromatous. Our results, nevertheless, are in direct contrast with those of Jamison and Vollum ([16]), who found that children from families with leprosy (type not specified) had a markedly depressed rate of conversion to tuberculin positivity after vaccination with a vole tuberculosis vaccine. Tuberculosis, as well as leprosy, is endemic in the villages in the present study, and all of the children had received BCG vaccination some years previously. The two groups showed equal reactivity to PPD both *in vivo* and *in vitro*.

The possibility remains that certain healthy people have a pre-existing cellular immune defect which predisposes them to lepromatous leprosy, and the work of Dharmendra and Chatterjee ([7]) would suggest that such individuals are to be found amongst those who are lepromin negative. Only two lepromin negative individuals were detected in the present study, and over the succeeding 16 months, both of these children developed leprosy. Clinically, both cases were classified as lepromatous, and subsequent histopathologic examination classified one as LI and the other as BL. Whether or not these children had a pre-existing cellular immune defect is not known since, although appearing clinically normal, they were probably already infected with *M. leprae* at the time of the study. One of these children, an eight year old girl, belonged to the control group of children. If the pairs involving her and her siblings are excluded from the analysis, no alteration in the significance of differences between the two groups occurs, except that the difference in Fernandez reactivity becomes no longer significant. It is unlikely, therefore, that the inclusion of these children in the control group has masked a depressed response in the group from families with leprosy.

Beiguelman ([3]) reported that in children a negative lepromin reaction is more frequent among families in which both parents are lepromin negative. Balina *et al* ([1]) reported that lepromin negative offspring of lepromatous leprosy cases had, like their affected parent, a diminished response to PHA in lymphocyte transformation tests as well as the expected lack of response to lepromin, while lepromin positive offspring and lepromin negative unrelated controls had PHA responses in the normal range. These studies are suggestive of hereditary factors being involved in cellular immune competence and, by inference, in susceptibility to lepromatous leprosy.

Godal and coworkers ([10, 13]) found that in healthy (adult) contacts, positive lymphocyte transformation to lepromin depended on the degree and duration of exposure to leprosy patients. They found immunologic evidence of exposure to *M. leprae* in approximately 50% of subjects with household or occupational contact with leprosy for at least one year. Interestingly, household contacts of active lepromatous leprosy cases who had been on treatment for less than six months were less reactive than contacts of cases who

had been treated for longer than six months. Although the numbers involved were not statistically significant, the authors speculated that "superexposure" to *M. leprae* may have a suppressive effect on the immune response of contacts. This interpretation favors environmental rather than hereditary factors being of importance in susceptibility to lepromatous leprosy.

If a specific pre-existing cellular immune defect predisposes to the development of lepromatous leprosy, final proof may be difficult to establish; since in any study of lepromin negative individuals in an endemic leprosy area, doubt always exists as to whether such persons are already infected with *M. leprae*. Even so, the fact that potential lepromatous cases in an endemic leprosy area can be detected by a negative lepromin skin test is of great practical significance, since chemoprophylaxis or immunologic reconstitution of such persons should greatly reduce the future transmission of leprosy in that population.

SUMMARY

The cell-mediated immune status of 20 apparently healthy children from families with a history of leprosy has been studied. They have been compared with 20 age- and sex-matched controls from families with no history of leprosy. Lymphocyte transformation tests using PHA, PPD and lepromin and skin tests to lepromin, PPD and candida were carried out. No evidence of a depression of cell-mediated immunity in the children from families with leprosy was obtained.

The only two children giving a negative Mitsuda lepromin skin test both subsequently developed leprosy in the succeeding 16 months. One was classified histologically as indefinite lepromatous and the other as borderline lepromatous. This emphasizes the practical significance of a negative lepromin skin test in an endemic leprosy area as a prognosis of clinical lepromatous leprosy.

RESUMEN

Se estudió el estado de las reacciones de inmunidad celular de 20 niños aparentemente sanos provenientes de familias con antecedentes de lepra. Se compararon con 20 controles similares en cuanto a edad y sexo, provenientes de familias sin antecedentes de lepra. Se hicieron pruebas de transformación de linfocitos con PHA, PPD y lepromina y pruebas intradérmicas con lepromina, PPD y cándida. No se obtuvo evidencia de una depresión de la inmunidad celular en los niños provenientes de familias con antecedentes de lepra.

Los únicos dos niños que dieron una prueba intradérmica de Mitsuda negativa desarrollaron lepra posteriormente, dentro de los 16 meses subsiguientes. Uno fué clasificado histológicamente como lepromatoso indefinido y el otro como LB. Esto enfatiza el significado práctico de una prueba intradérmica de lepromina negativa en un área endémica de lepra como prognosis de lepra lepromatosa clinica.

RÉSUMÉ

On a étudié l'état de l'immunité cellulaire chez 20 enfants apparemment normaux, appartenant à des familles avec antécédents de lèpre. Ces enfants ont été comparés avec 20 témoins de même âge et de même sexe, apartenant à des familles sans antécédents de lèpre. On a mené des épreuves de transformation lymphocytaire avec PHA, PPD et lépromine, de même que des épreuves cutanées à la lépromine, au PPD et à Candida. Aucun signe d'une diminution de l'immunité cellulaire chez les enfants des familles avec antécédents de lèpre n'a été observé.

Les deux seuls enfants qui avaient montré une reaction de Mitsuda négative à l'épreuve cutanée à la lépromine, ont ensuite développé la maladie dans les 16 mois qui ont suivi l'étude. L'un a été classifié histologiquement comme un lépromateux de forme non définie, et l'autre comme un borderline lépromateux. Ceci souligne la signification pratique d'un résultat négatif à l'épreuve cutanée à la lépromine en zones endémiques pour la lèpre en tant que signe pronostique d'une lèpre lépromateuse clinique.

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