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# A Study of Cellular Immunity in Clinically Healthy Children of Parents with Leprosy in Northern Thailand[1]

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Patients with leprosy are well known to have depressed cell-mediated immunologic (CMI) function. The perturbations in CMI function in leprosy patients are most profound in those with lepromatous and borderline leprosy even though patients with untreated active tuberculoid disease also may not have completely normal cellular immune responses ([4, 5, 21]). The ability of the host to respond immunologically to *Mycobacterium leprae* infection has been postulated to be one of the major determinants in the subsequent pathogenesis of clinical leprosy after infection.

Several investigators have studied the immune status of healthy contacts of leprosy patients using skin test ([7, 12, 13, 15, 16, 18]) or *in vitro* methods ([10, 11, 19, 20, 21]). Recent data using either serological ([1, 14]) or *in vitro* lymphocyte responses to *M. leprae* antigens ([11, 12, 19, 20]) suggest that subclinical infection with *M. leprae* is quite common among healthy contacts of persons with clinical leprosy. Whether the pathogenesis of the immunological abnormalities in those persons who subsequently develop clinical leprosy is related to host or organism characteristics or to events in the early preclinical infectious process is not known at present. Neither is it entirely clear whether healthy children whose parents have leprosy have normal immunological responses to mycobacterial antigens. Jamison and Vollum ([15, 16]) observed that Nigerian children from families with leprosy had a substantially lower rate of tuberculin conversion than those whose parents were normal after they were vaccinated with a vole bacillus vaccine for the prevention of tuberculosis. No details were given in their study about the type of leprosy present in the index cases in the families, nor were other studies done of the unresponsive children. The age distribution and prior BCG history of the children in the Nigerian study also were not published. The purpose of our study was to explore further the issue of whether defects in CMI responsiveness to BCG could be detected in healthy children who had a parent with leprosy, and whether variations in this response were related to the clinical type of leprosy in the index case's household.

## MATERIALS AND METHODS

The study population consisted of 302 children, aged 5–18, who attended either the McKean Rehabilitation Institute school (MRI) or a public school in Chiang Mai, Thailand. The children attending the MRI school lived in a common dormitory near the hospital during the school term; 121 of these children were selected for study. Each of these children had at least one parent with clinical leprosy who was being cared for by the staff at MRI. The adult index case(s) in each child's family had been classified clinically by physicians at McKean according to the Ridley-Jopling scale ([22]). Also each child at the MRI school included in our study had been examined at least every 3–6 months by the MRI staff and had been found to be free of clinical leprosy. Children with one or more parents or any other household

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TABLE 1. *Distribution of study population by age.*

| Age (yr) | Normal parents | | TL parents | | LL parents | | Total | |
|---|---|---|---|---|---|---|---|---|
| | No. | % | No. | % | No. | % | No. | % |
| 5–7 | 13 | 7.2 | 4 | 5.4 | 2 | 4.3 | 19 | 6.3 |
| 8–10 | 42 | 23.2 | 7 | 9.5 | 9 | 19.1 | 58 | 19.2 |
| 11–13 | 72 | 39.8 | 40 | 54.1 | 16 | 34.0 | 128 | 42.4 |
| 14–16 | 33 | 18.2 | 17 | 23.0 | 19 | 40.4 | 69 | 22.8 |
| ≥17 | 17 | 9.4 | 3 | 4.0 | 0 | 0 | 20 | 6.6 |
| Unknown | 4 | 2.2 | 3 | 1.0 | 1 | 2.2 | 8 | 2.6 |
| Total | 181 | 100.0 | 74 | 100.0 | 47 | 100.0 | 302 | 99.9 |

contact with leprosy at the tuberculoid end of the spectrum (TT, BT), and no lepromatous cases in the family, were grouped in the tuberculoid leprosy (TL) families. Those children having one or more parents or any other household contacts with leprosy at the lepromatous end of the spectrum (LL, BL, BB) were grouped with the lepromatous leprosy (LL) families.

The control group of 181 children attending a public school in Chiang Mai were selected so that they represented about the same age distribution as the children at the MRI school (Table 1). There were no known cases of leprosy in the households of these control children, and the children themselves were healthy.

A history was taken from each child and the school health records were examined to determine whether previous BCG vaccination had been given. Additionally each child was examined for the presence of a BCG scar. Since BCG is generally administered intracutaneously in Thailand, most recipients of BCG develop a small scar at the site of vaccination. Those with a definite history of BCG but without a scar were considered to have received previous BCG vaccination.

All children were skin tested on the volar aspect of the forearm with 0.1 ml of PPD tuberculin (5 tuberculin units, Tween 80 stabilized PPD-tuberculin, Aplisol®, Parke-Davis Company, Detroit, Michigan, U.S.A.) and 0.1 ml of *Candida albicans* antigen (1:1000 dilution, Hollister-Steir Laboratories, Spokane, Washington, U.S.A.). Both skin-testing antigens were shipped from the United States and stored at 4°C. The same lot of each antigen was used throughout these studies. The skin tests were read 24 hr and 48 hr after application by two readers. They were examined in a good light, and the area of induration perpendicular to the long axis of the forearm was palpated and measured to the nearest millimeter. The results obtained by the two readers were averaged. Readings were done at MRI without knowledge of the clinical leprosy classification of the parents. Lepromin testing was not done in this study.

Those children with candida and tuberculin reactions of less than 5 mm were considered to be skin-test negative. Children with a tuberculin reaction of less than 7 mm were given a dose of 0.1 ml of BCG by intradermal injection. The tuberculin-negative children were re-tested six weeks after BCG had been given. A conversion to tuberculin positivity was considered to have occurred if there was at least a 4 mm or greater increase in induration to a diameter of at least 10 mm on the post-BCG tuberculin skin test. The BCG was a lyophilized vaccine which had been prepared by Glaxo Laboratories Ltd., Greenford, Middlesex, England. The vaccine had been stored at 4°C and was reconstituted immediately prior to use. Vaccine from the same source had been routinely used in the Pediatrics Department at the Faculty of Medicine of Chiang Mai University. Follow-up studies done by one of the authors (SS) demonstrated an 85% tuberculin conversion rate among healthy infants and children 6–8 weeks after they received the Glaxo BCG vaccine.

Statistically significant differences between the skin test results on the various groups of students were evaluated using the Chi-square test or Fisher's exact probability test, depending on the sample size of the groups being compared.

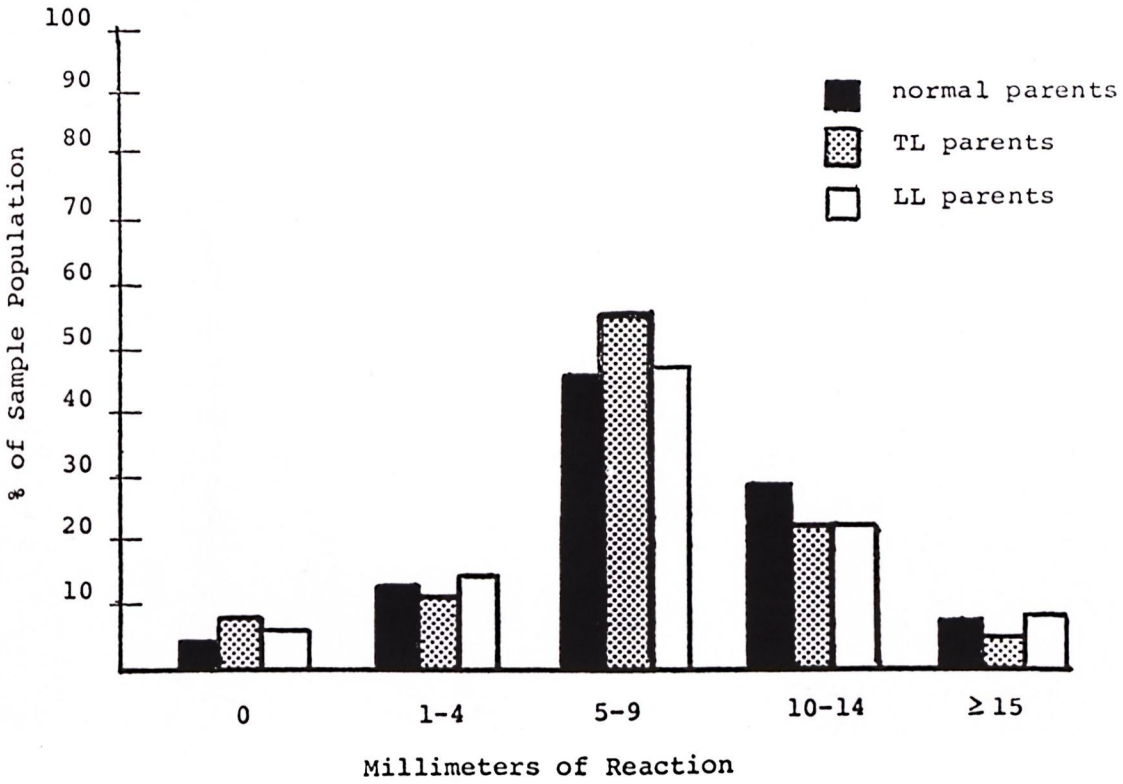


FIG. 1. Initial candida skin test results.

## RESULTS

The age distribution of children in the three groups was similar (Table 1). Most (about 80%) of the children in the three groups had received BCG vaccine in the past but this had usually been given in the first few years of life and several years prior to our study in most children. The frequency and timing of prior BCG vaccination was similar in the three groups of children.

Skin test responses to candida antigen are shown in Figure 1. Neither the prevalence of a positive reaction nor the distribution of students by the diameter of their induration responses were significantly different in the three groups. The prevalence of a positive reaction to candida was 150 (83%) of 181 children in those with normal parents, 60 (81%) of 74 children in the tuberculoid parent group, and 30 (64%) of 47 children in the lepromatous parent group.

The distribution of tuberculin reactions by the diameter of induration was similar in the three groups of children in those who had a history of prior BCG vaccination (Fig. 2). In the children with a history of a prior BCG vaccination, the percentages of positive tuberculin reactions were: children from normal families, 92 (63%) of 145; 30 (50.8%) of 59 children from TL families; and 22 (61%) of 36 children from LL families. None of these rates were significantly different from each other. In the fewer children without a history of previous BCG immunization, the tuberculin reactor rate for the children in the various types of families varied between 18.5% in those from normal families, 46% for those from TL families, and 30% of those from LL families (Fig. 3); these differences were not statistically significant.

When tuberculin-negative children were given BCG, conversion to a positive tuberculin skin test occurred in 57 (64%) of 89 children from normal families and 28 (77%) of 36 children from TL families, but in only 7 (38.9%) of 18 children from LL families (Table 2). The difference in conversion rates

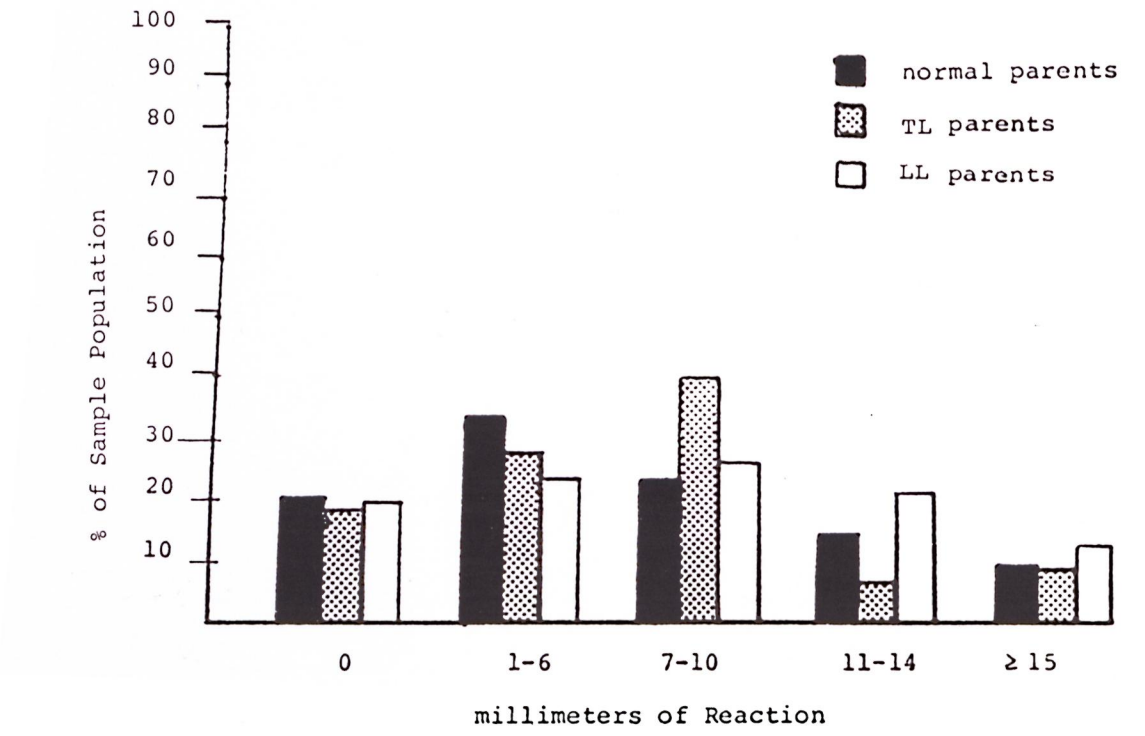


FIG. 2. Initial tuberculin skin test reactions in children with prior BCG vaccination.

between children in normal and TL families was not significant. However, the tuberculin conversion rate after BCG in children from LL families was significantly lower than in children from TL families ($\chi^2 = 10.3$, $p < 0.01$) and from normal families ($\chi^2 = 3.85$, $p < 0.05$).

Among children who were initially negative to both tuberculin and candida skin tests and who were subsequently given BCG vaccination 12 of 14 (85.7%) children of normal families, 4 of 6 (66.7%) children of tuberculoid families, and 0 of 3 (0%) children of lepromatous families converted their tuberculin tests. Although the number of children in this group who were negative when tested with both antigens is quite small, the difference between the tuberculin conversion rate in the children from lepromatous families and the rate in those from normal families is significant (Fisher's exact test, $p = 0.015$). The ages of the three children (10, 12, and 14 years) from lepromatous families who were initially tuberculoid and candida negative and unresponsive to BCG were similar to the other children in our study.

## DISCUSSION

Our study indicates that children from households of lepromatous leprosy patients are less responsive to BCG than those from households with only tuberculoid leprosy patients or normal persons. Our data are similar to those reported by Jamison [15, 16], but a direct comparison between the two studies is not entirely possible since the type of leprosy in the children's households was not reported in the Nigerian study. The type of vaccine utilized and the method of tuberculin skin testing were also different in the two studies.

The relatively poor tuberculin responses to BCG among children in households where one or more parent had lepromatous leprosy might help explain the discrepant results obtained in different populations in studies of the efficacy of BCG in the prevention of leprosy. In the trials reported from Uganda [17], where tuberculoid leprosy is the predominant form of the disease encountered, the efficacy of BCG was much higher than that found in Burma [2], where lepromatous disease was more frequent.

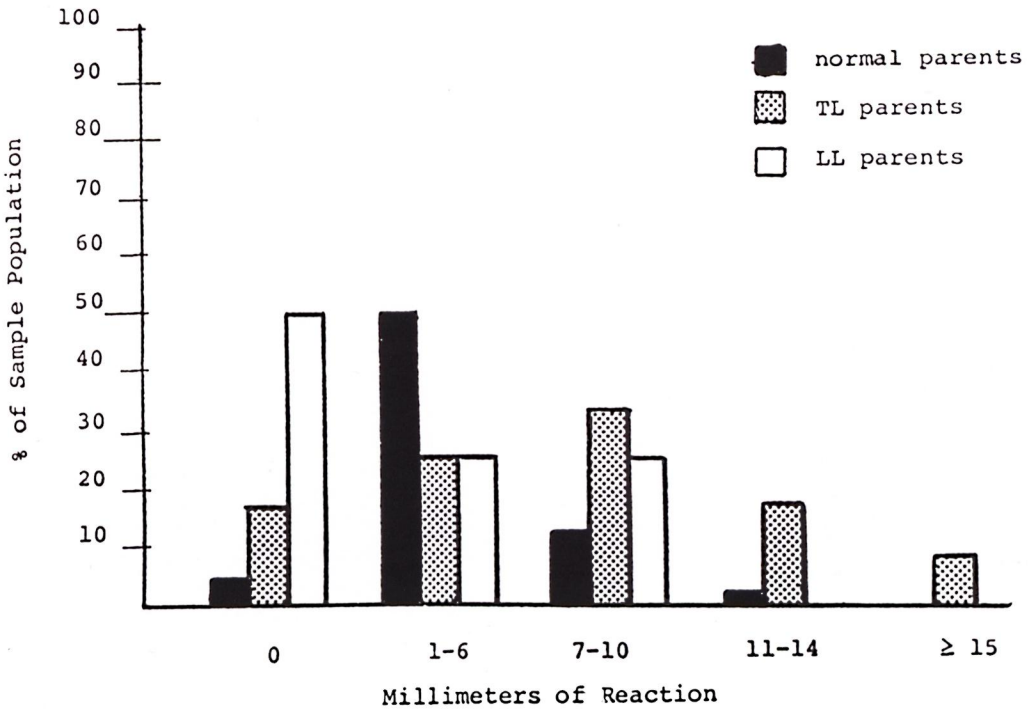


FIG. 3. Initial tuberculin skin test reactions in children without prior BCG vaccination.

It is not clear whether the decreased responsiveness to BCG among healthy children of lepromatous parents was primarily genetically determined or related to an inapparent mycobacterial infection in these children. A number of investigators using serological methods ([1, 14]) or *in vitro* lymphocyte transformation tests ([3, 11, 12, 19, 20, 21]) have obtained data suggesting that inapparent infections may be common among healthy contacts of leprosy patients. Studies of Bjune ([3]) suggest that active immune suppression of responses to mycobacterial antigens may occur in clinically normal contacts of lepromatous patients.

In studies reported by Menzel, *et al.* ([19, 20]), responses to *M. leprae* antigens in the lymphocyte transformation tests (LTT) of normal contacts were primarily dependent upon the type and degree of infectiousness of the index case in the household and not the degree of consanguinity with the index case.

Recently efforts have been directed at developing an effective vaccine for the prevention of leprosy ([8, 9, 10]). Shepard, *et al.* ([23]) have reported live BCG to be the most effective cultivable mycobacteria for sensitizing mice to foot pad challenge with *M. leprae* and in protecting them against an infective challenge with *M. leprae.* The variable results obtained with BCG vaccination of humans for the prevention of lep-

TABLE 2. *Post BCG PPD-tuberculin skin test conversions in children who were tuberculin negative on first testing.*[a]

| Post BCG tuberculin reaction | Normal parents | | TL parents | | LL parents | |
|---|---|---|---|---|---|---|
| | No. | % | No. | % | No. | % |
| Nonconversion | 32 | 36.0 | 8 | 22.2 | 11 | 61.1 |
| Conversion | 57 | 64.0 | 28 | 77.7 | 7 | 38.9 |
| Total | 89 | 100.0 | 36 | 99.9 | 18 | 100.0 |

[a] TL vs normal parents, $\chi^2 = 2.54$, $p > 0.10$. LL vs normal parents, $\chi^2 = 3.85$, $p < 0.05$. LL vs TL parents, $\chi^2 = 10.3$, $p < 0.01$.

TABLE 3. *Post BCG PPD-tuberculin skin test reaction in children who were negative to both PPD-tuberculin and candida on testing.*

| Tuberculin reaction | Normal parents | | TL parents | | LL parents[a] | |
|---|---|---|---|---|---|---|
| | No. | % | No. | % | No. | % |
| Nonconversion | 2 | 14.3 | 2 | 33.3 | 3 | 100.0 |
| Conversion | 12 | 85.7 | 4 | 66.7 | 0 | 0 |
| Total | 14 | 100.0 | 6 | 100.0 | 3 | 100.0 |

[a] Fisher's exact test: LL vs normal parents, p = 0.015. LL vs TL parents, p = 0.119.

rosy suggest that other antigens are needed for an effective vaccine.

However, the possible unresponsiveness to an antileprosy vaccine of persons who are already infected with *M. leprae* is of considerable concern to investigators who are developing a vaccine ([8, 9, 10]). Convit, *et al.* ([6]) have studied skin test conversion and *in vitro* LTT responses to lepromin among Mitsuda negative contacts and patients with indeterminate and treated lepromatous leprosy. Evidence of LTT responsiveness to lepromin was obtained after immunization with a mixture of heat-killed *M. leprae* and live BCG, but not by the injection of either component alone. Therefore, these authors have advocated this mixture as an antileprosy vaccine. Shepard, *et al.* ([24]) have demonstrated that whether sensitization or tolerance to mycobacterial antigens results depends upon the route of original challenge. These intriguing studies indicate the need for additional information concerning the immune responses to mycobacterial vaccines among healthy contacts of lepromatous patients.

## SUMMARY

A study was done of the response to PPD-tuberculin, candida and the tuberculin conversion rate after BCG vaccination among 302 healthy children in northern Thailand. The children were grouped according to whether their parent(s) or other household contact(s) had tuberculoid leprosy (74 children), lepromatous leprosy (47 children), or all family members were healthy (181 children). No significant differences were detected in the responses to candida or PPD-tuberculin on initial skin testing or in the history of a previous BCG vaccination in the three groups of children. However, among the children who were initially tuberculin negative, significantly decreased PPD conversion rates occurred in children from lepromatous families in comparison to those from tuberculoid families ($p < 0.01$) or normal families ($p < 0.05$). In the children from lepromatous families who were initially PPD and candida negative, 0 of 3 developed PPD-tuberculin conversions after BCG in comparison to 12 of 14 (85.7%) from normal families ($p = 0.015$).

These data indicate that some children from lepromatous families were relatively unresponsive to stimulation with BCG and possibly other mycobacterial vaccines. The immunopathogenesis of this relative unresponsiveness should be further defined, since it might have important implications for the prevention of leprosy with a vaccine.

## RESUMEN

Se estudió la respuesta a PPD-tuberculina y a Candida, así como el grado de conversión a la tuberculina después de la vacunación con BCG, en 302 niños sanos del norte de Tailandida. Los niños se agruparon según si alguno de sus padres (o ambos) u otro contacto familiar tenía lepra tuberculoide (74 niños), lepra lepromatosa (47 niños), o si toda la familia era sana (181 niños). No se encontraron diferencias significativas en la respuesta a Candida o a PPD-tuberculina cuando se hicieron las pruebas dérmicas iniciales ni cuando se tomó en cuenta la historia previa de vacunación con BCG en los tres grupos estudiados. Sin embargo, entre los niños que fueron inicialmente tuberculino negativos, el grado de conversión a positivos fue significativamente menor en los niños de familias lepromatosas que en los niños de familias tuberculoides ($p < 0.05$). Ninguno de 3 niños de familias lepromatosas que inicialmente fueron negativos a PPD y Candida se tornaron positivos al PPD después de la vacunación con BCG pero ésto si sucedió en 12 de 14 niños (85.7%) de familias sanas ($p = 0.015$).

Estos datos indican que algunos niños de familias lepromatosas fueron relativamente refractarios a la estimulación con BCG y posiblemente a otras vacunas microbacterianas. La inmunopatogénesis de esta relativa falta de respuesta debe estudiarse con más detalle puesto que puede tener implicaciones importantes en la prevención de la lepra por el uso de vacunas.

## RÉSUMÉ

Chez 302 enfants sains du Nord de la Thailande, on a procédé à une étude concernant la réponse à la tuberculine-PPD, et le taux de virage de la tuberculine

après vaccination par le BCG. Les enfants ont été groupés selon que leurs parents ou d'autres contacts domiciliares souffraient de lèpre tuberculoide (74 enfants), de lèpre lépromateuse (47 enfants), ou étaient sains (181 enfants). Aucune différence significative n'a été décelée dans les réponses à Candida ou à la tuberculine PPD, lors d'une épreuve cutanée initiale, ou selon les antécédents d'une vaccination BCG préalable, dans ces trois groupes d'enfants. Néanmoins, lorsque les enfants étaient initialement négatifs à la tuberculine, on a constaté une diminution significative des taux de virage au PPD chez les enfants provenant de familles lépromateuses, en comparaison à ceux qui appartenaient à des familles tuberculoides ($p < 0.01$) ou à des familles normales ($p < 0.05$). Chez 3 enfants de familles lépromateuses, initialement négatifs pour le PPD et le Candida, aucun n'a développé de virage à la tuberculine PPD après BCG, alors que 12 sur 14 (85.7%) des familles normales ont présenté un tel virage ($p = 0.015$).

Ces données indiquent que certains enfants de familles lépromateuses sont relativement insensibles à une stimulation par le BCG, et peut-être à d'autres vaccins mycobactériens. L'immunopathogénèse de cette absence relative de réponses devrait être davantage précisée, car elle pourrait avoir des conséquences importantes pour la prévention de la lèpre par un vaccin.

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