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Fluorescent Leprosy Antibody Absorption (FLA-ABS) Test for Detecting Subclinical Infection with *Mycobacterium leprae*[1]

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Subclinical infection in leprosy has recently been discussed from an immunological point of view ([3,4,8]). The lepromin test, lymphocyte transformation test, and leukocyte migration inhibition test were used as indicators of cell-mediated immunity (CMI) to *M. leprae*. A disadvantage of these tests is their cross-reactivity to other mycobacteria such as *M. tuberculosis*. It is therefore difficult to evaluate the results of these tests in areas where both leprosy and tuberculosis are endemic. A serological test may be superior in this regard because the test can easily be made specific by the absorption of cross-reacting antibodies in the serum. In fact, Abe, *et al.* ([1]) have reported the fluorescent leprosy antibody absorption (FLA-ABS) test using a smear of *M. leprae* and absorbing the serum with cardiolipin, lecithin, and *M. tuberculosis* polysaccharide. The last antigen was later replaced by a suspension of BCG and *M. vaccae* in order to improve specificity ([2]). Recently, Harboe, *et al.* ([5]) reported encouraging results with a radioimmunoassay using *M. leprae* sonicate as antigen and absorbing the serum with BCG sonicate.

The FLA-ABS test and the other immunological tests were used in a survey for leprosy in Okinawa, southwestern islands of Japan, with a special interest toward detecting subclinical infection with *M. leprae* in household contacts and in schoolchildren. The results obtained to date are described in this paper and discussed from immunological and epidemiological points of view.

MATERIALS AND METHODS

Sera. Sera from leprosy patients were collected at the National Leprosaria, Tama Zensho-en and Okinawa Airaku-en. Leprosy cases were classified according to the Ridley-Jopling classification ([10]). Sera from patients with pulmonary tuberculosis were provided by Dr. Ichiro Toida, Research Institute of Tuberculosis, Kiyose, Tokyo. Sera from laboratory workers were collect-

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ed at the senior author's institute. Sera from patients in general hospitals and from healthy noncontacts were collected at the Kiyose-en Clinical Laboratory, Kiyose, Tokyo. Sera from household contacts and from schoolchildren in Okinawa were either brought on ice or mailed (with 0.1% sodium azide as a preservative) to the senior author's laboratory. The sera were stored at −20°C until tested.

Mycobacterial suspensions. *M. leprae* were obtained from a subcutaneous leproma of an untreated leprosy patient and used for the majority of serological tests. *M. leprae*-infected armadillo liver was obtained through Dr. R. J. W. Rees, National Institute for Medical Research, London, under an IMMLEP Project of WHO, in February 1976. These armadillo-derived *M. leprae* were used in some of the tests, the results being the same as those obtained with human bacilli.

M. leprae were separated from tissues as follows: The tissue was homogenized in a blender with 10 ml of physiological saline per 1 g of tissue while cooling with ice water. The homogenized suspension was centrifuged at 1000 rpm (130 × *g*) for 10 min. The pellet was again homogenized and centrifuged in the same manner. Both supernatants were pooled and centrifuged at 10,000 rpm (9000 × *g*) for 20 min at 0–4°C. After removing the supernatant, pelleted bacilli were suspended in a small volume of saline. Acid-fast bacilli (AFB) were enumerated by Shepard's method ([12]), and the suspension was diluted with saline to contain 1 to 1.5 × 10^8 AFB/ml. The suspension was aliquoted and stored at −20°C. BCG, a marketed product for vaccination manufactured by Japan BCG Laboratory, Kiyose, Tokyo, was cultivated in a Sauton's medium for 4 weeks at 37°C. The bacilli were collected by centrifugation (800 × *g*, 10 min) washed 3 times with physiological saline and lyophilized. One gram of dried bacilli was suspended in 20 ml of phosphate-buffered saline (PBS, NaH_2PO_4 0.45 g, $Na_2HPO_4 \cdot 12H_2O$ 3.227 g and NaCl 8.0 g per liter, pH 7.2) and sonicated with a microtip with minimal power of cell disruptor 185 (Branson Sonic Power Co., Danbury, Connecticut, U.S.A.) for 15 min to obtain a homogeneous suspension. Sodium azide was added to a final concentration of 0.1% and the suspension stored at 0–4°C. *M. vaccae* (ATCC 15483) were cultivated in modified Dubos' medium at 37°C for 1 week, collected by centrifugation as above, and washed 3 times with physiological saline. One volume of pelleted bacilli was suspended in 9 volumes of PBS, sonicated, and stored in the same manner as BCG. The other mycobacteria, *M. tuberculosis* ($H_{37}Rv$), *M. kansasii*, *M. marinum*, *M. smegmatis*, *M. phlei* (Timothy hay), and *M. avium* (Kirchberg) were cultivated on Ogawa's 1% egg medium at 37°C and suspended in saline containing 0.1% sodium azide by light sonication. The bacillary concentration of each suspension was roughly adjusted to 10^8 bacilli/ml by comparing the turbidity.

Reagents. Carbon tetrachloride of special reagent grade (Wako Pure Chemical Industries, Ltd., Doshucho, Higashi-ku, Osaka) was put in a staining dish and was renewed after being used for several pretreatments of the specimens. A 1% solution of trypsin was prepared by dissolving Difco's trypsin (1:250) in 0.2 M Tris-HCl buffered saline (pH 8.0). The solution was aliquoted and stored at −20°C until use. At the time of use, this stock solution was diluted 1:10 with the same Tris-buffered saline. Cardiolipin-lecithin solution (each 0.4% in absolute alcohol) was obtained from Sumitomo Chemical Co., Ltd., Doshucho, Higashi-ku, Osaka. One volume of this solution was mixed rapidly with 19 volumes of PBS and referred to as diluent A, which was used for the absorption of the serum in the FLA-ABS test. Nine volumes of diluent A were mixed with 1 volume of a 1% bovine serum albumin (fraction V) solution (w/v in PBS) and referred to as diluent B, which was used for the dilution of absorbed serum. Anti-human IgG fluorescent antibody (FA) prepared from rabbit antiserum with activity against both heavy and light chains was the lyophilized product of Eiken Chemical Co., Ltd., Hongo, Bunkyo-ku, Tokyo. This reagent was reconstituted with 0.5 ml of distilled water per vial at the time of use and absorbed by adding an equal volume of 5% (w/v) BCG suspension. After incubating at 37°C for 30 min, the mixture was centrifuged at 2000 rpm (500 × *g*) for 15 min, and the supernatant was again centrifuged at 10,000 rpm (5500 ×

g) for 5 min using a microcentrifuge (M-15, Sakuma Seisakusho, Ltd., Ohta-ku, Tokyo). The clear supernatant was carefully removed from the pelleted bacilli, diluted with PBS to the dilution indicated (usually 1:40), and filtered through a membrane filter (0.22 micron pore size). The solution was used immediately after preparation as a secondary antibody in the FLA-ABS test.

FLA-ABS test. The principal techniques of this test are similar to those of the FTA-ABS test ([6]). Since the description of the technique in a previous paper ([1]) was not detailed and the method of absorption was later improved ([2]), the present procedures are described in detail as follows:

1) Smear of *M. leprae*: One platinum loopful of *M. leprae* suspension was smeared in each circle of a glass slide used for immunofluorescence tests (S4314 No. 2, Matsunami Glass, Ltd., Kishiwada, Osaka). The smear was air-dried at room temperature or dried with warm air (a hair-dryer) in a humid climate.
2) Pretreatment of the smear: This procedure was essential to enhance the intensity of the immunofluorescence of the bacilli in crude suspension, probably because pretreatment eliminates tissue-lipids and tissue-proteins that might interfere with the antigen-antibody reaction. The smears were soaked in carbon tetrachloride at room temperature for 10 min and then dried in air. Each smear was covered with 2 or 3 drops of 0.1% trypsin solution and incubated in a moist chamber at 37°C for 1 hr. The slides were then washed with PBS for 5 min, with shaking. This washing was repeated 3 times. After drying the slides, vertical lines were drawn with nail polish between the circles.
3) Absorption and dilution of test serum: The serum (0.05 ml) was mixed with equal volumes of BCG and *M. vaccae* suspension and diluted to a final 1:10 by adding 0.35 ml of diluent A (0.05 ml of serum to a final volume of 0.50 ml). The mixture was incubated at 37°C for 30 min and centrifuged at 2000 rpm (500 × *g*) for 15 min to pellet the majority of the bacteria. The supernatant was again centrifuged at 10,000 rpm (5500 × *g*) for 5 min with a microcentrifuge (M-15, Sakuma Seisakusho, Ltd., Ohta-ku, Tokyo). The clear supernatant was removed and further diluted with diluent B to prepare serial four-fold dilutions, i.e., 1:40, 1:160, 1:640, 1:2560, etc.
4) Primary reaction: The serum dilutions were placed on the antigen (AFB) smears and incubated in a moist chamber at 37°C for 1 hr. After washing with PBS in the same manner as above, the vertical lines of nail polish were removed, and the slides were dried with cool air.
5) Secondary reaction: The smear was covered with 1 or 2 drops of secondary antibody solution and incubated in a moist chamber at 37°C for 1 hr or at 4°C overnight.
6) Washing and mounting: After washing with PBS again, the smear was allowed to dry and mounted with 0.05 M Na carbonate-buffered glycerol (pH 9.5) and a coverslip.
7) Reading: This was always conducted with coded sera so that the reader did not know the origin of the individual serum. A fluorescent microscope, model FM 200 (Tiyoda Optical Co., Ltd., Nihonbashi, Chuo-ko, Tokyo) was used throughout the experiment. Magnifications from 400× to 650× were sufficient for observing the immunofluorescence of *M. leprae*. The intensity of fluorescence was recorded on a scale of ± to 4+, which is comparable to that used in the FTA-ABS test ([6]). Although both UV and BV filter systems were available, the latter was found to be more useful in combination with the interference filter system, AIF and FIF (Tiyoda Optical Co., Ltd., Nihonbashi, Chuo-ko, Tokyo). In the BV filter system the criteria for the reading were as follows: 4+, very strong fluorescence of almost all bacilli; 3+, strong fluorescence of the majority of bacilli; 2+, many bacilli showed definite fluorescence, but weakly fluorescent bacilli were also seen; 1+, definite fluorescence in the minority of bacilli, but weak in the majority; ±, weak fluo-

TABLE 1. *FLA-ABS test in leprosy patients.*

Classification of leprosy	No. of cases	Positive		Distribution of antibody titer (10×4^x)								
				x =							Mean	
		No.	%	1	2	3	4	5	6	7	of x	S.D.
LL	129	128	99.3	15	25	30	40	12	6	0	3.21	1.32
LL with ENL	7	7	100	1	1	4	0	1	0	0	2.86	1.22
BL	8	8	100	1	1	2	3	1	0	0	3.25	1.28
BB	12	12	100	1	2	4	3	1	0	1	3.42	1.56
BT	8	7	87.5	2	2	2	1	0	0	0	2.29	1.11
TT	17	13	76.5	2	3	3	5	0	0	0	2.85	1.14
Suspected	2	2	100	0	0	1	1	0	0	0	3.50	0.50
Total	183	177	96.7	22	34	46	53	15	6	1	3.15	1.30

rescence in a few bacilli; −, bacilli were recognized without observable specific fluorescence. The specific green color of fluorescein isothiocyanate (FITC) was always confirmed by using the interference filter. Clustered bacilli or bacilli in tissue-fragments were not considered in the readings because these bacilli often emitted either exceptionally strong or nonspecific fluorescence. Two plus or more fluorescence of isolated bacilli caused by the 1:40 or higher dilution of serum was considered to be positive, but 1+ or less fluorescence at any dilution and 2+ or more at the 1:10 dilution were considered as negative because such fluorescence was sometimes seen in the slides with the sera from non-endemic areas. The antibody titer was expressed by x in 10×4^x, the maximum dilution of serum giving a positive reaction.

BCG-fluorescent antibody (FA) test. This test was performed together with the FLA-ABS test with the sera of schoolchildren because it was thought that BCG vaccination was likely to cause the production of cross-reacting antibodies which might interfere with the specificity of the FLA-ABS test. Smears of the BCG suspension (10^8 bacilli/ml) were treated with carbon tetrachloride. Treatment with trypsin was omitted because it did not influence the intensity of the immunofluorescence obtained with leprosy sera. The smear was reacted with serial four-fold dilutions of unabsorbed serum. PBS containing 0.1% (w/v) BSA was used for this dilution. Further procedures were the same as those used in the FLA-ABS test.

Rubella virus hemagglutination inhibition (R-HI) test. A commercially available kit (Toshiba Chemicals Co., Ltd., Chiyoda-ku, Tokyo), was employed for this test. This test was conducted as a primary reason for collecting sera from schoolchildren because rubella was epidemic in Okinawa a few years ago. A R-HI titer of 1:64 or higher was considered to be positive.

Lepromin test. Household contacts were tested with a standard lepromin (160 million bacilli/ml) immediately after the blood collection. The size of Mitsuda's reaction was measured after 4 weeks according to the reading criteria recommended at the 8th International Congress of Leprosy ([9]).

RESULTS

The sensitivity and specificity of the FLA-ABS test. The results of FLA-ABS tests with the sera of leprosy patients are shown in Table 1. Virtually all of the LL, BL, and BB cases were positive. The percentage of positive reactions decreased from BT to TT but was still high in TT cases. The distribution of the antibody titers showed a gradual decrease from the lepromatous to the tuberculoid end of the spectrum. However, there were striking variations in the antibody titers of individual sera within each group of patients, and the mean antibody titer in each group did not show the expected relationship to the spectrum of leprosy. This may be due to the presence of relatively more active cases in the BB and TT groups.

The results of this test in non-leprosy cases are summarized in Table 2. In house-

hold contacts, 91.9% were positive. Among nearly 15,000 schoolchildren in Okinawa, 173 children were tested because they had a palpable auricular nerve or suspicious skin eruption. A positive reaction was found in 109 (63%) of them. Among 19 laboratory workers in the senior author's institute, 5 persons were found to be positive. Among them, 4 were persons who have been working in leprosy for more than 15 years. These positive reactions may well be caused by subclinical infection with *M. leprae* since the test was entirely negative in patients with pulmonary tuberculosis and in healthy non-contacts. Only 2 out of 138 cases from general hospitals located in leprosy non-endemic areas showed a positive reaction. The specificity of the test was checked by determining cross-reactivity with the other mycobacteria as shown in Table 8. Two sera (K50 and K70) cross-reacted with *M. smegmatis*. Absorbing the serum with a 20% suspension of *M. smegmatis* abolished the reactivity to *M. smegmatis* but did not affect the antibody titer to *M. leprae*. Moreover, the reaction to *M. leprae* was completely absorbed with the addition of 6×10^8 *M. leprae* obtained from an infected armadillo's liver. Therefore, these two cases probably represent subclinical infections with *M. leprae* although it was difficult to confirm a history of leprosy contact.

TABLE 2. *FLA-ABS test in non-leprosy cases.*

Cases	No. of cases	Positive	
		No.	%
Household contacts	62	57	91.9
Schoolchildren in endemic area[a]	173	109	63.0
Laboratory workers	19	5	26.3
Patients with pulmonary tuberculosis	18	0	0
Cases from general hospitals in non-endemic area	138	2	1.5
Healthy non-contacts	50	0	0

[a] Tested because of a palpable auricular nerve or a suspicious skin eruption.

FLA-ABS test in household contacts. Household leprosy contacts were divided into several subgroups according to their age, sex, blood-relationships to the leprosy patient, classification of the disease in the leprosy patient with whom they had contact, and lepromin reaction. The percentage of positive reactions and their mean antibody titers were compared among these subgroups. The results are shown in Table 3. There were no significant differences in the percentages of positives among age groups, but the mean antibody titer was highest in infants less than 4 years old and tended to decrease with increasing age. This may indicate that leprosy infection is most frequent in infancy. There were no significant differences in the percentages of positives and the mean antibody titers when analyzed according to sex. Mean antibody titers were higher in children and grandchildren of leprosy patients than in brothers and sisters of patients. Household contacts of lepromatous patients showed a higher mean antibody titer than contacts of borderline or tuberculoid patients. The antibody titer may therefore reflect the intensity of exposure to *M. leprae*. Three contacts of tuberculoid patients were positive; two were children, and one was a sister of the patient. However, it was not clear whether these tuberculoid patients were themselves infectious or whether there was another source of infection common to both the contact and the patient. Thirty-nine contacts were lepromin skin-tested. Mitsuda's reaction was doubtful (4 mm or less) in 7 of these contacts, 6 of whom were children from 5 to 9 years old.

Humoral immune responses in school children. An annual dermatologic examination of schoolchildren was carried out from May to June 1977 in one city and in two villages in a central area of the main island of Okinawa and in three villages in northern areas of this island. Among nearly 15,000 children examined, 173 children were tested serologically because they had either a palpable auricular nerve or a suspicious skin eruption. The results are shown in Table 4. Overall, the FLA-ABS test was positive in 63% while the BCG-FA and the R-HI test were positive in 73.4% and 17.9%, respectively. Results varied according to the localities of the schools. The FLA-ABS test showed a slightly higher percentage of positive reaction in the northern villages than in the central area, reflecting a higher incidence of leprosy in the former ([11]).

TABLE 3. *FLA-ABS test in household contacts.*

Subgroups		No. of cases	Positive		Antibody titer	
			No.	%	Mean	S.D.
Grand total		62	57	91.9	2.65	1.11
Age	0–4	8	7	87.5	3.14	0.90
	5–9	27	25	92.6	2.64	1.15
	10–14	7	7	100	2.57	1.51
	15–19	6	5	83.3	2.40	1.52
	20–	11	10	90.9	2.78	0.63
	Unknown	3	3	100	2.33	0.47
Sex	Male	23	22	95.7	2.81	1.10
	Female	39	35	89.7	1.91	2.03
Blood relationship with the patient						
	Child	40	36	90.0	2.67	1.04
	Brother or sister	7	7	100	1.57	1.13
	Grandchild	10	10	100	3.60	0.52
	Others	5	4	80.0	2.00	0.71
Classification of the disease of the patient	L	52	48	92.3	2.85	1.05
	B	5	5	100	1.40	0.55
	T	3	3	100	1.33	0.58
	Unknown	2	1	50.0	3.00	—
Lepromin reaction (mm)	≧10	2	1	50.0	4.00	—
	5–9	30	29	96.7	2.86	1.07
	≦4	7	7	100	2.71	1.38
	0	0	0	0	—	—
	Not tested	23	—	—	—	—

There were no differences in the BCG-FA and R-HI tests in this respect. The BCG-FA test was more frequently positive in girls than in boys, but the difference was not statistically significant. The FLA-ABS test showed no significant differences in the percentages of positive reactions according to school year, but the BCG-FA test was more frequently positive in the middle school years. This may be due to the effect of BCG vaccination. The R-HI test was far more frequently positive in middle school children than in primary school children, which reflects the fact that rubella was epidemic mainly in the middle school children. As shown in the lower half of Table 4, the results of tuberculin skin tests, a history of BCG vaccination, and the findings on auricular nerves and/or skin eruptions showed no significant correlation with any of these serological tests.

The χ^2 test was used to test for correlations among the 3 test results as shown in Tables 5, 6, and 7. The FLA-ABS test did not correlate with the BCG-FA test or with the R-HI test. On the other hand, as shown in Table 7, there was a significant correlation between the BCG-FA test results and the R-HI test results. It is not clear whether this correlation is due to a high positivity of both tests in middle school children or whether it suggests some immunological relationship between the rubella virus and BCG. FLA-ABS and BCG-FA tests were both positive in 83 children, as shown in Table 5. Sera from 58 of these children were sampled at random, and the specificity of the FLA-ABS test was checked by testing for cross-reactivity with other mycobacteria. As shown in Table 8, 57 of these 58 sera did not react with any of the 6 species of mycobacteria other than *M. leprae*. One serum gave positive reactions with all of these mycobacterial species. This serum (S28) showed identical antibody titers against *M. leprae*, *M. smegmatis*, and *M. avium*. The antibody titer to *M. leprae* was not influenced by additional absorption with *M. smegmatis* or *M. avium* whereas the reaction was completely absorbed with the addition of 6×10^8 *M. leprae*. This case, a middle school girl with

TABLE 4. *Serological tests in schoolchildren.*

Subgroups	FLA-ABS		BCG-FA		R-HI	
	No. of cases positive/ total	%	No. of cases positive/ total	%	No. of cases positive/ total	%
Grand total	109/173	63.0	127/173	73.4	30/168	17.9
Locality						
Central area	45/79	57.0	53/79	67.1	12/78	15.4
Northern villages	62/88	70.5	68/88	77.3	15/84	17.9
Sex Male	73/119	61.3	83/119	69.7	23/115	20.0
Female	36/54	66.7	44/54	81.5	7/53	13.2
School year						
Lower classes in primary school	28/41	68.3	26/41	63.4	7/95	7.4
Upper classes in primary school	36/58	62.1	43/58	74.1		
Middle school	44/72	61.1	57/72	79.2	23/71	32.4
Tuberculin test						
Positive & doubtful	18/32	56.3	27/32	84.4	5/32	15.6
Negative	10/21	47.6	17/21	81.0	4/21	19.1
BCG vaccination						
Experienced	43/64	67.2	47/64	73.4	10/63	15.9
Not experienced	13/25	52.0	18/25	72.0	7/25	28.0
Palpable auricular nerve	95/155	61.3	113/155	72.9	26/150	17.4
Skin eruption and others	14/18	77.8	14/18	77.8	4/18	22.2
Palpable auricular nerve						
Bilateral	36/55	65.5	38/55	69.1	6/53	11.3
Unilateral	59/100	59.0	75/100	75.0	20/97	20.6

suspicious skin eruptions on the back, was later diagnosed as having leprosy.

DISCUSSION

The fluorescent leprosy antibody absorption (FLA-ABS) test proved to be highly sensitive and specific in detecting the antibody response against *M. leprae*. The sensitivity of the test, expressed as the percentage of positive reactions in leprosy sera, was high throughout the spectrum of leprosy. These findings coincide with results described in a previous paper ([1]). Therefore, it is suggested that the absorption of serum with BCG and *M. vaccae* does not influence antibodies directed against the specific antigen(s) of *M. leprae*. Harboe, *et al.* ([5]) have reported positive results with their radioimmunoassay in 61 of 62 lepromatous sera, all of 12 borderline sera, and 20 of 48 tuberculoid sera after absorption with BCG sonicate. The sensitivity of the FLA-ABS test, therefore, seems to be comparable with, or higher than, that of the radioimmunoassay. This suggests that the FLA-ABS test may be a useful parameter for long-term studies in leprosy and may be comparable to the radioimmunoassay in this regard ([13]).

As emphasized in the present communication, another use of the FLA-ABS test is the detection of subclinical infection with *M. leprae* in healthy contacts. Studies of experimental infections in nude mice ([7]) have shown that the production of anti-*M. leprae* antibodies could be detected by immunofluorescence techniques at an early stage after the infection. In order for the

TABLE 5. *Correlation between FLA-ABS and BCG-FA tests.*

BCG-FA test	FLA-ABS test		Total
	Positive	Negative	
Positive	83	44	127
Negative	26	20	46
Total	109	64	173

$\chi^2 = 1.13$.

TABLE 6. *Correlation between FLA-ABS and R-HI tests.*

R-HI test	FLA-ABS test		
	Positive	Negative	Total
Positive	17	13	30
Negative	89	49	138
Total	106	62	168

$\chi^2 = 0.648$.

TABLE 7. *Correlation between BCG-FA and R-HI tests.*

R-HI test	BCG-FA test		
	Positive	Negative	Total
Positive	28	2	30
Negative	97	41	138
Total	125	43	168

$\chi^2 = 5.71$, $p < 0.002$.

test to be useful in detecting subclinical infection with *M. leprae* in contacts, however, the test must be highly specific because almost all leprosy contacts in endemic areas are probably exposed to infections with other mycobacteria, which may cause the production of cross-reacting antibodies. In fact, the FLA-ABS test proved to be specific for leprosy because the test was entirely negative in patients with pulmonary tuberculosis and in healthy noncontacts. Although a cross-reaction with *M. smegmatis* was still observed in some sera after absorption with BCG and *M. vaccae*, such a reaction could be easily differentiated from the specific reaction with *M. leprae* by an additional absorption with *M. smegmatis*. The specificity of the reaction with *M. leprae* was also corroborated by its complete absorption with *M. leprae*, as shown by the 3 cases in Table 8. Therefore, the positive reactions in nonleprosy cases shown in Table 2 may be considered as indications of subclinical infections with *M. leprae*. It should be pointed out that various other species and strains of mycobacteria may be needed for the verification of specificity in other countries because different saprophytic and pathogenic mycobacteria are found in nature in different geographic locations. This problem can be addressed by tailoring the mycobacteria used in the absorption procedure to the respective regions and also by finding a cultivable mycobacterium that has an antigenic composition similar to *M. leprae*.

In household contacts, antibody titers showed significant differences according to age and according to the density of infection. Therefore, young contacts with high antibody titers should be carefully observed and considered to have been recently exposed to dense infections. By the antibody titer alone, however, it is difficult to distinguish the humoral immune response occurring at an early stage of the infection from that remaining after spontaneous cure. The lepromin reaction should be tested simultaneously. If it is negative or doubtful, then chemoprophylaxis or vaccination should be tried. When both the FLA-ABS and the lepromin tests are positive, it is reasonable to assume that both humoral and cell-mediated immunity have developed. Such cases may be omitted from leprosy control because they are presumed to be in little danger of leprosy attack or already in spontaneous cure.

The results of the serological tests in schoolchildren have several implications. The high percentage of positive FLA-ABS tests in the children who had a palpable auricular nerve or a suspicious skin eruption seems to indicate that these often overlooked, non-diagnostic clinical signs may be primary lesions caused by the infection with *M. leprae*. In fact, one case with a suspicious skin eruption and high antibody titer was later diagnosed as having leprosy. Except for this case, the other 57 sera that were positive in both the FLA-ABS and BCG-FA tests showed no crossreaction with 6 species of mycobacteria other than *M. leprae*. Since schoolchildren without any clinical signs were not examined serologically in this survey, it was not possible to determine an exact correlation between the FLA-ABS test and clinical findings. However, a significant correlation has been obtained from a subsequent survey in another area of Okinawa although this survey is still in progress. Therefore, it is conceivable that a positive FLA-ABS test in school children is an indication of subclinical infection with *M. leprae*.

FLA-ABS test results showed no corre-

TABLE 8. *Specificity of positive reactions in schoolchildren and suspicious cases.*

Mycobacteria	No. of positive reactions tested[a]	Antibody titer of 3 cases									
		S28[b]	+Msm[c]	+Mav[d]	+Mla[e]	K50	+Msm[c]	+Mla[e]	K70	+Msm[c]	+Mla[e]
M. leprae	60	4	4	4	<0	2	2	<0	1	1	<0
M. tuberculosis	1	3	<0	<0	2	<0	<0	<0	<0	<0	<0
M. kansasii	1	2	<0	<0	2	<0	<0	<0	<0	<0	<0
M. marinum	1	2	<0	<0	1	<0	<0	<0	<0	<0	<0
M. smegmatis	3	4	2	2	2	1	<0	<0	1	<0	<0
M. phlei	1	3	<0	<0	1	<0	<0	<0	<0	<0	<0
M. avium	1	4	4	2	2	<0	<0	<0	<0	<0	<0

[a] Fifty-eight cases were random samples from 83 schoolchildren who were positive in both FLA-ABS and BCG-FA tests. The other 2 cases (K50 and K70) were found from general hospitals described in Table 2.

[b] One case (S28), a middle school girl with suspicious eruptions on her back, was later diagnosed as having leprosy.

[c] The titer after additional absorption with 20% (v/v) suspension of *M. smegmatis*.

[d] The titer after additional absorption with 10% (w/v) suspension of *M. avium*.

[e] The titer after additional absorption with 6×10^8 bacilli purified from an *M. leprae*-infected armadillo liver.

lation with results of tuberculin tests, a history of BCG vaccination, BCG-FA, or R-HI tests. Therefore, it is unlikely that the humoral immune response to the specific antigen(s) of *M. leprae* is influenced by infections with *M. tuberculosis*, BCG, or rubella virus. This view does not deny a possible role of BCG in the prevention of leprosy or a possible immunosuppressive effect of rubella virus on the leprosy attack but rather is an indication of the specificity and consequently the practical utility of the FLA-ABS test.

Among 15,000 schoolchildren, 109 cases were found to be positive in the FLA-ABS test. This rate, 0.7%, does not indicate an exact rate of infection because children without any clinical signs were not examined serologically. On the other hand, if one accepts that a positive result indicates infection with *M. leprae*, then the infection rate is at least 0.7% since any children without clinical signs with positive results would only serve to increase the infection rate. Taking into account the fact that the test was entirely negative in healthy noncontacts, the rate of infection in these children is thus at least 0.7%, and this rate is almost 200 times higher than the recent leprosy incidence rate in Okinawa of 0.004%. Therefore, it is suggested that most of the FLA-ABS positive children have acquired or will acquire protective immunity to *M. leprae* without overt signs or symptoms of leprosy. The FLA-ABS test will be useful for such an immuno-epidemiological study of leprosy and, when used in conjunction with the lepromin test or a suitable *in vitro* test of cell-mediated immunity to *M. leprae*, the FLA-ABS test will be useful in identifying individuals who may benefit from prophylactic measures to prevent overt leprosy.

SUMMARY

The fluorescent leprosy antibody absorption (FLA-ABS) test was improved by absorbing the serum with suspensions of BCG and *M. vaccae*. This test was positive in nearly 100% of patients with bacteriologically positive forms of leprosy and in approximately 80% of tuberculoid cases but negative in 18 patients with pulmonary tuberculosis, in 50 healthy noncontacts, and in 136 of 138 non-leprosy patients in general hospitals. The 2 positive sera of the last group showed a cross-reaction with *M. smegmatis*, but *M. smegmatis* were incapable of absorbing the positive reactions to *M. leprae*. Therefore, the serological sensitivity and the specificity of the FLA-ABS test proved to be satisfactory for detecting subclinical infections with *M. leprae*.

The test was positive in 57 of 62 (91.9%) household contacts of leprosy patients. The mean antibody titers were higher in infants, in grandchildren and children of patients, and in contacts of lepromatous patients than in comparison groups. Among 39 of

these household contacts who were tested with lepromin, 7 showed doubtful Mitsuda reactions but were positive in the FLA-ABS test. Such cases should be carefully observed since they are apparently infected with *M. leprae* but have not developed cell-mediated immunity.

Among 15,000 school children in a leprosy endemic area, 173 children were tested with FLA-ABS because they had a palpable auricular nerve or a suspicious skin eruption. A positive reaction was found in 109 (63%) of them. The percentage of positivity was slightly higher in the villages than in an urban area, corresponding to a higher leprosy incidence rate in the former. The FLA-ABS test results showed no correlation with the results of tuberculin tests or a history of BCG vaccination in these children. Their sera were also examined by indirect immunofluorescence with smears of BCG (BCG-FA test) and by the rubella virus hemagglutination inhibition test, and these tests showed no correlation with the FLA-ABS test. Among 58 sera in which both the FLA-ABS and the BCG-FA tests were positive, 57 sera did not react with 6 species of mycobacteria other than *M. leprae*; one serum did react with the other mycobacterial species, and this person was later found to have leprosy. From these observations it is presumed that the rate of subclinical infection with *M. leprae* in the schoolchildren in this area is at least 0.7% and is almost 200 times higher than the leprosy incidence rate in this area.

RESÚMEN

Se mejoró la prueba de la absorción del anticuerpo fluorescente para la lepra (fluorescent leprosy antibody absorption test, FLA-ABS) por la absorción del suero con suspensiones de BCG y de *M. vaccae*. Esta prueba fue positiva en casi el 100% de los pacientes con lepra bacteriologicamente positivos y en aproximadamente el 80% de los casos tuberculoides pero fue negativa en 18 pacientes con tuberculosis pulmonar, en 50 sujetos sanos (no contactos), y en 136 de 138 pacientes de hospital no leprosos. Los 2 sueros positivos del último grupo dieron una reacción cruzada con *M. smegmatis* pero éste fue incapaz de absorber la reactividad con el *M. leprae*. Por lo tanto, la sensibilidad y especificidad de la prueba FLA-ABS demostraron ser satisfactorias para la detección de infecciones subclínicas con el *M. leprae*.

La prueba fue positiva en 57 de 62 (91.9%) contactos convivientes con los pacientes con lepra. Los títulos promedio de anticuerpos fueron más altos en los infantes, en los nietos y en los hijos de los pacientes, y en los contactos convivientes con los pacientes lepromatosos, que en los otros grupos estudiados. Entre los 39 convivientes que se probaron con lepromina, 7 mostraron reacciones de Mitsuda dudosas pero fueron positivos a la prueba FLA-ABS. Tales casos deben observarse cuidadosamente puesto que aparentemente están infectados con el *M. leprae* y no han desarrollado inmunidad celular.

De 15,000 escolares estudiados en un área endémica, 173 niños se probaron con la prueba FLA-ABS debido a que presentaron un nervio auricular palpable o erupción dérmica sospechosa. En 109 casos (63%) se observó una reacción positiva. El porcentaje de positividad fue ligeramente mayor en los pequeños poblados que en las áreas urbanas, coincidiendo con una mayor incidencia de lepra en los primeros. Los resultados de la prueba FLA-ABS no mostraron correlación con la reactividad a la tuberculina o con la historia de vacunación con BCG en estos niños. Sus sueros también fueron examinados por inmunofluorescencia indirecta en extendidos de BCG (prueba BCG-FA) y por la prueba de inhibición de la hemaglutinación por el virus de la rubeola. Ninguna de estas pruebas mostró correlación con la prueba FLA-ABS. De 58 sueros en los cuales ambas pruebas (FLA-ABS y BCG-FA) fueron positivas, 57 no reaccionaron con 6 especies de Mycobacterium diferentes al *M. leprae*; un suero sí reaccionó con las otras micobacterias pero posteriormente se encontró que esta persona tenía lepra. De estas observaciones se infiere que la incidencia de infección subclínica por el *M. leprae* en los escolares del área endémica estudiada es cuando menos del 0.7%, lo cual resulta casi 200 veces mayor que la incidencia de lepra en la misma área.

RÉSUMÉ

L'épreuve d'absorption aux anticorps fluorescents dans la lèpre (FLA-ABS) a été améliorée lorsque l'on recourait à une méthode d'absorption du sérum par des suspensions de BCG et de *M. vaccae*. Cette épreuve était positive chez près de 100% des malades présentant des formes bactériologiquement positives de la lèpre, et chez approximativement 80% des cas tuberculoïdes. Par contre, elle s'est révélée négative chez 18 malades atteints de tuberculose pulmonaire, chez 50 personnes normales non contact de malades de la lèpre, et chez 136 parmi 138 malades non-lépreux étudiés dans les hôpitaux généraux. Les 2 échantillons de sérum positifs de ce dernier groupe montraient une réaction croisée avec *M. smegmatis*, mais *M. smegmatis* était toutefois incapable d'absorber les réactions positives à *M. leprae*. Dès lors, la sensibilité et la spécificité sérologiques de l'épreuve FLA-ABS se sont révélées être satisfaisantes pour détecter des infections sub-cliniques par *M. leprae*.

L'épreuve a été positive chez 57 contacts domiciliaires de malades de la lèpre (91,9%) sur 62. Les titres moyens d'anticorps étaient plus élevés dans les classes

d'âge les plus jeunes, de même que chez les enfants et petits-enfants de malades, et chez les contacts de malades lépromateux, lorsque l'on comparait ces groupes à des groupes témoins. Parmi les 39 contacts domiciliaires qui ont été étudiés par la lépromine, 7 ont montré des réactions de Mitsuda douteuses mais étaient cependant positifs pour l'épreuve FLA-ABS. De tels cas devraient être soigneusement suivis, car ils sont vraisemblablement infectés par *M. leprae*, mais n'ont pas encore développé d'immunité à médiation cellulaire.

Parmi les 15.000 écoliers d'une région endémique pour la lèpre, 173 enfants ont été testés par cette épreuve FLA-ABS, car ils présentaient soit un nerf auriculaire palpable, ou bien une éruption cutanée suspecte. Une réaction positive a été observée chez 109 (63%) d'entre eux. Le pourcentage de positivité était légèrement plus élevée dans les villages que dans la région urbaine, ce qui correspond à un taux d'incidence plus élevé pour la lèpre dans les villes. Les résultats d l'épreuve FLA-ABS chez ces enfants n'ont montré aucune corrélation avec les résultats des épreuves à la tuberculine, non plus qu'avec des antécédents de vaccination par le BCG. Le sérum de ces enfants a été examiné par des méthodes d'immunofluorescence indirecte, avec des frottis de BCG (épreuve BCG-FA) et par l'épreuve d'inhibition de l'hémagglutination du virus de la rubéole; ces épreuves n'ont montré aucune corrélation avec l'épreuve FLA-ABS. Parmi 58 échantillons de sérum, trouvés positifs à la fois pour l'épreuve FLA-ABS et pour l'épreuve BCG-FA, 57 ne réagissaient pas avec 6 espèces de mycobactéries autres que *M. leprae*; un sérum réagissait avec les autres espèces mycobactériennes, mais il a été trouvé ultérieurement que ce sujet souffrait de la lèpre. On peut, à partir de ces observations, présumer que le taux d'infection subclinique par *M. leprae* chez les écoliers, dans la région étudiée, est au moins de 0,7%, et donc presque 200 fois plus élevé que le taux d'incidence de la lèpre dans la zone.

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