

✓
228
HEMAGGLUTINATION AND LEPROMIN REACTIVITY
IN HEALTHY FILIPINO CHILDREN¹

M. S. RHEINS, PH. D., J. M. BIRKELAND, PH. D.,
R. G. BURRELL, M. SC.
*Department of Bacteriology, Ohio State University
Columbus, Ohio*

AND R. S. GUINTO, M.D.
*Epidemiologist, Leonard Wood Memorial
Cebu, Philippines*

For some years one of the authors (R.S.G.) and Dr. J. A. Doull, medical director of the Leonard Wood Memorial, have been interested in the response of healthy children to lepromin and tuberculin. Studies in progress at the epidemiology unit at Cebu, Philippines, have shown a good but not absolute correlation between the two tests (4). Moreover, it is the general rule for healthy adults at Cebu to react positively to lepromin (5, 6).

Early in 1955 blood samples were obtained from 135 school children 6-9 years of age, living on Mactan Island, Cebu. These children had not been subjected to any kind of skin test prior to the taking of the specimens. The sera were recovered and stored in sealed ampules for future serologic testing.

These children were later included among 1,004 who were given preliminary tests with 10 TU of PPD tuberculin (Parke, Davis and Co.), to which dose 288 children (28.7%) gave positive reactions. The 716 negatives were then divided and assigned at random to seven groups which varied in numbers from 89 to 95. Each child was given an injection of lepromin (prepared by Dr. E. Mabalay), and those of the seven test groups received further PPD tests with higher concentrations as follows: 20, 30, 40, 50, 100, 150, or 200 TU. The children from whom blood samples were procured were distributed randomly throughout these groups.

In this report are presented the relationships between reactivity to tuberculin and to lepromin and the serologic findings with respect to the presence of hemagglutinating and hemolytic antituberculo-polysaccharides. The tests were made by modifications (12) of the Middlebrook-Dubos technique (10), the hemolytic test proposed by Middlebrook (11), and the antiglobulin test described by Hinson *et al.* (8).

Although Levine and his co-workers (9) found high titers of hemagglutinins in the sera from lepromatous patients (presumably lepromin negative), the titers of sera from arrested lepromatous cases, and those of tuberculoid and indeterminate nature, were significantly lower, approach-

¹ This study was supported in part by a grant from the Ohio Tuberculosis and Health Association.

ing those observed in normal individuals. These findings were confirmed by Gernez-Rieux *et al.* (3). Since the dynamics of the infectious process conceivably could influence the serologic response, there is but limited justification for assuming the absence of circulating antipolysaccharides in the sera from lepromin-positive but otherwise apparently normal individuals.

MATERIALS AND METHODS

Sera.—All samples were heat-inactivated at 56°C for 30 minutes and twice adsorbed with 0.4 volumes of washed, packed sheep cells to remove naturally-occurring antibodies (each adsorption, 10 minutes at room temperature.)

Hemagglutination and hemolysis tests.—The details of the technique as employed in this study (12) include the following features: (a) sensitization of normal, packed sheep erythrocytes with 1:15 OT.²

(b) admixing of 2 drops of each two-fold saline dilution of adsorbed test serum and 2 drops of a 0.5 per cent suspension of OT-sensitized erythrocytes; and (c) incubation in a 37°C water bath for 30 minutes, followed by light centrifugation and examination for evidence of definite aggregation. The hemolytic modification of this test requires the addition of 1 drop of guinea-pig complement, diluted 1:9 and previously adsorbed in the cold (4°C) with packed sheep cells. After incubation, the titration end-point is determined as the last serum dilution in which hemolysis is complete. Antigen, complement and serum controls are included in the titrations.

Antiglobulin test.—The procedure described by Hinson *et al.* (8) was altered to confirm with the antigen and volumes of reagents commonly used in this laboratory. Antihuman serum, produced in a rabbit, was inactivated at 56°C for 30 minutes and adsorbed twice with 0.4 volumes of sheep cells as outlined above. A 1:4 saline dilution of this serum was found to be adequate for testing.

MEASURING TUBERCULIN AND LEPROMIN REACTIVITY

Tuberculin reactions.—0-4 mm., negative (—); 5-10 mm., weak positive (1+); 11-20 mm., moderately positive (2+); 21 mm. and larger, strong positive (3+).

Lepromin reactions.—(a) Early or Fernandez reaction. Measured after 48 hours; diameter of erythema with definite edema. 0-4 mm., negative (—); 5-9 mm., doubtful (\pm); 10-14 mm., weak positive (1+); 15-19 mm., moderate positive (2+); 20 mm. or larger, strong positive (3+). (b) Late or Mitsuda reaction. Measured after 21 days (some after 22-23 days). Less than 3 mm., negative (—); 3-4 mm., doubtful (\pm); 5-7 mm., weak positive (1+); 8-9 mm., moderate positive (2+); 10 mm. or larger, or any reaction with ulceration, strong positive (3+).

RESULTS

Tuberculin and lepromin reactivity.—The data regarding the tuberculin and lepromin reactivity of the children from whom blood was withdrawn for serologic testing are compiled in Table 1.

It is interesting to note that while 90 of the individuals tested (68.1%) failed to give an early reaction to lepromin, only 5 persons (3.7%) had not responded at the time of the later observation (i.e., late, or Mitsuda reac-

² Old tuberculin (4 \times international standard), generously provided by Dr. H. D. Piersma of Lederle Laboratories Division, American Cyanamid Co., Pearl River, New York.

tion). If doubtful reactions are excluded from the latter calculations, 84.4 per cent of the children reacted positively to lepromin, thus attesting to the early age at which sensitivity is developed. It should be considered,

TABLE 1. *Skin reactivity of 135 children to tuberculin (PPD) and lepromin (Mabalay).*

Children tested. Grouped by original screening test (10 TU)		Tuberculin test ^a				Lepromin reaction									
		Test dose (TU)	Reactivity				Early				Late				
			—	1+	2+	3+	—	+	1+	2+	—	±	1+	2+	3+
Positive	Negative														
42	—	10	—	17	6	19	15	17	8	2	1	—	35	4	2
	13	20	9	4	—	—	9	3	1	—	1	2	8	2	—
	9	30	6	1	2	—	9	—	—	—	—	1	8	—	—
	12	40	7	4	1	—	11	1	—	—	—	2	9	—	1
	12	50	7	5	—	—	11	1	—	—	1	4	5	2	—
	8	100	4	4	—	—	6	2	—	—	—	—	7	—	1
	14	150	8	4	2	—	11	3	—	—	1	1	11	1	—
	16	200	6	7	2	1	11	4	—	1	1	5	7	2	1
	9 ^b	—	—	—	—	—	7	1	1	—	—	1	8	—	—

^a The results in the 42 children positive to 10 TU are those of the original screening test; those in the 84 children of the other tested groups are of the retests with larger doses.

^b Children negative to 10 TU but not retested with tuberculin.

however, that late reactions may not reflect a pre-existing sensitization to mycobacteria, but rather an allergic response following the intracutaneous injection of the test dose of heat-killed leprosy bacilli (lepromin).

Since the 135 children represent but a small proportion of the 1,004 who participated in the lepromin—and tuberculin-testing program, a further analysis of this aspect of the experiment will not be attempted at this time. It is expected that the results with the entire group will be published at a later date.

Serologic testing.—The 135 sera were examined by the Middlebrook-Dubos test, its hemolytic modification, and the antiglobulin test as outlined above. The results tabulated in Table 2 concern only the distribution of the test sera with regard to the serologic tests. By far the greatest reactivity was observed with the hemagglutination test alone. However, the highest titer recorded was only 1:16, and the majority ranged from 1:2 to 1:4. While these titers are not considered to be of diagnostic significance for tuberculous infections, they may be of importance in the present study in which it was assumed that the subjects were free from tuberculosis.

Lepromin and serologic reactivity.—The serologically reactive sera, as measured with OT-sensitized erythrocytes, are grouped with respect to the lepromin reactivity of the donors in Table 3. Little can be concluded from

this table because the majority of children gave late positive or doubtful reactions to lepromin, thus influencing the significance of the serologic tests in such a comparison.

TABLE 2. *Serologic reactivity of the 135 test sera.*

Test	Positive results	
	Number	Per cent
Hemagglutination	55	40.7
Hemolysis	4	2.9
Antiglobulin	16	11.8
Hemagglutination and hemolysis	4	2.9
Hemagglutination and antiglobulin	14	10.4
Hemolysis and antiglobulin	0	0.0
Hemagglutination, hemolysis, and antiglobulin	2	1.5

Tuberculin and lepromin reactivity and serologic tests.—It seemed advisable to determine the relationship between the degree of tuberculin hypersensitivity and lepromin reactivity, and to correlate the results with the

TABLE 3.—*Correlation of hemagglutination and antiglobulin reactions of 135 sera, with the results for the lepromin test in the donor individuals.*

	Early reaction			Late reaction		
	Positive	Doubtful	Negative	Positive	Doubtful	Negative
Lepromin reaction of donors	13 (9.6 %)	32 (23.7 %)	90 (66.7 %)	114 (84.4 %)	16 (11.8 %)	5 (3.7 %)
Hemagglutination reaction positive	2 (15.4 %)	12 (37.5 %)	41 (45.5 %)	45 (39.5 %)	7 (43.7 %)	1 (20.0 %)
Antiglobulin reaction, positive	0 (0.0 %)	0 (0.0 %)	0 (0.0 %)	1 (0.08 %)	1 (0.06 %)	0 (0.0 %)

serologic findings. The 135 donors were grouped according to their response to the minimal test dose of tuberculin (10 TU), and the number of late lepromin reactors was calculated for each group. The assigning of the positive serologic reactions completed this phase of the study.

As can be seen in Table 4, the children who were markedly hypersensitive to PPD gave a higher percentage of positive lepromin reactions than did those who were negative to the 10 TU dose. The difference between the percentage of positive serologic tests in each of the groups is probably of no significance, in keeping with the reported independence of the antibodies responsible for the tuberculin reaction and those that are measured with OT-sensitized cells (7).

TABLE 4.—Correlation of lepromin reactivity of donors and hemagglutination reactions of 135 sera with the results of the tuberculin test (10 TU of PPD).

	Positive	Negative
Tuberculin reaction of donors	42 (31.1%)	93 (68.9%)
Late lepromin reaction (positives)	40 (95.2%)	74 (79.6%)
Hemagglutination test, positive	15 (35.7%)	38 (40.9%)

DISCUSSION

Since approximately 40 per cent of the 135 sera tested in the study contained hemagglutinating antibodies while 85 per cent of the donors gave positive lepromin reactions, it is obvious that the development or persistence of reactivity to lepromin is not necessarily reflected in detectable serum hemagglutinins. The explanation of the presence of hemagglutinins (9), and of reactivity to lepromin, in these children is not known. The most rational one is prior infection with one or another species of mycobacterium—*M. tuberculosis*, *M. leprae*, or some unknown variety. The explanation of the difference in proportions probably lies in the nature of the antigens responsible for the two tests, one being protein while the other is of polysaccharide nature.

The independence of tuberculin hypersensitivity and the Middlebrook-Dubos antibodies has already been established by Haley and associates. Cole and Favour (2) further confirmed this observation by examining serum fractions, obtained from guinea-pigs which had been injected with *M. tuberculosis* for evidence of serologic activity and capacity to incite hypersensitivity upon passive transfer. The antibodies detected by the Middlebrook-Dubos test and those responsible for tuberculopolysaccharide hypersensitivity resided in the gamma globulin fraction. The antibodies directed toward the so-called Boyden antigens (1), protein hemosensitans, and those which induced tuberculin hypersensitivity were associated with a new serum fraction, designated IV-10.

In view of these findings it might be of value to examine sera from healthy, tuberculin-negative children for tuberculoprotein antibodies and to correlate these results with lepromin reactivity.

SUMMARY

1. Approximately 40 per cent of the sera from healthy children residing in an area endemic for leprosy gave positive results when tested for anti-tuberculopolysaccharides. The titers approached those seen in normal individuals generally.

2. There was no apparent correlation between lepromin reactivity of the donors and the reactivity of their sera.

3. Differences in specificities of the test antigens is presented by way of explanation.

RESUMEN

1. Aproximadamente 40 por ciento de los sueros de niños sanos que residían en una zona endémica de lepra acusaron resultados positivos al ser ensayados en busca de antituberculopolisacáridos. Los títulos se aproximaron a los observados en individuos normales generalmente.

2. No hubo correlación aparente entre la reactividad de los donantes a la lepromina y la reactividad de sus sueros.

3. A modo de explicación, se presentan las diferencias en las especificidades de los antígenos de ensayo.

REFERENCES

1. BAYDEN, S. V. The adsorption of proteins on erythrocytes treated with tannic acid and subsequent hemagglutination by anti-protein sera. *J. Exper. Med.* **93** (1951) 107-120.
2. COLE, L. R. and FAVOUR, C. B. Correlations between plasma protein fractions, antibody titers, and the passive transfer of delayed and immediate cutaneous reactivity to tuberculin PPD and tuberculopolysaccharides. *J. Exper. Med.* **101** (1955) 391-420.
3. GERNEZ-RIEUX, CH., MONTESTRUC, E. and TACQUET, A. Application des réactions d'hémagglutination et d'hémolyse conditionnée au diagnostic sérologique de la lèpre. *Internat. J. Leprosy* **21** (1953) 21-25.
4. GUINTO, R. S., DOULL, J. A., and MALABY, E. B. Tuberculinization and reactivity to lepromin; association between lepromin and tuberculin reactions in school children in Cordova and Opon, Cebu, Philippines. *Internat. J. Leprosy* **23** (1955) 32-47.
5. GUINTO, R. S., DOULL, J. A., and MABALAY, E. B. A note on the lepromin reaction in males and females of the general population of Cordova, Mactan Island, Cebu, Philippines. *Internat. J. Leprosy* **23** (1955) 131-134.
6. GUINTO, R. S., DOULL, J. A., and MABALAY, E. B. The Mitsuda reaction in persons with and without household exposure to leprosy. *Internat. J. Leprosy* **23** (1955) 135-138.
7. HALEY, R. R., WINTHROP, N. D., ADCOCK, J. and OWEN, C. R. Hemagglutination reaction following BCG vaccination in human subjects. *American Rev. Tuberc.* **66** (1952) 58-62.
8. HINSON, K. F. W., RICHARDSON-JONES, A. and CHAMBERLIN, J. A. The Middlebrook-Dubos reaction in relation to the clinical aspects of pulmonary tuberculosis. *British J. Tuberc.* **46** (1952) 50-59.
9. LEVINE, M., CHUNG-HOON, E. K., ICHIRIU, E., ARAKAKI, J. and BEATTY, M. Sero logical response in various types and stages of Hansen's disease (leprosy) to tuberculin-sensitized sheep red blood cells. *Internat. J. Leprosy* **20** (1952) 201-212.
10. MIDDLEBROOK, G. and DUBOS, R. J. The specific serum agglutination of erythrocytes sensitized with extracts of tubercle bacilli. *J. Exper. Med.* **88** (1948) 521-528.
11. MIDDLEBROOK, G. A hemolytic modification of the hemagglutination test for antibodies against tubercle bacillus antigens. *J. Clin. Invest.* **29** (1950) 1480-1485.
12. RHEINS, M. S., THURSTON, J. R., NEWTON, N. and DODD, M. C. Evaluation of drop techniques for the detection of hemagglutinins and hemolysins in tuberculous sera. *American J. Med. Tech.* **20** (1954) 254-258.