A Hemolytic Reaction of Erythrocytes Sensitized With Tuberculin and Components Extracted from Leprosy Nodules

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In recent years, Middlebrook and Dubos¹⁾ has published a hemagglutination reaction of erythrocytes sensitized with extracts of tubercle bacilli and it was retested by many investigators. Subsequently the test was made in leprosy by Levine²⁾ with sheep red cells sensitized with tuberculin. It was revealed that the test was applicable for measuring a serological response in leprosy patients and that antibody titers in active cases were significantly higher than those in negative cases and frequently much higher than those encountered in active cases of tuberculosis and the reason of which was thought to be due to the fact that *M. leprae* and *M. tuberculosis* possess a common antigenic structure. As compared with this work, Koji³⁾ made a same experiment and reported on the lower titer in leprosy than in tuberculosis.

Recently, Mayama and Fukuda⁴⁾ made a test with hen's erythrocytes and concluded that the titers in lepromatous type were higher than those in neuro-macular type, and that the more advanced the clinical conditions of patients, the higher the titer of sera.

We⁵⁾ have made a fundamental experiment on a hemolytic reaction between the cattle erythrocytes sensitized by body components of typhoid bacilli and anti-typhoid sera with addition of complement, and found that the hemolytic reaction was more sensitive and specific than the other hitherto known serological reactions, and the author attempted to apply this reaction for leprosy. Because the artificial cultivation of *M. leprae* is not yet successful, instead of extracts of this bacilli that from leprosy nodules must be used. In connexion with this, were reported formerly some detailed investigations by Nakamura⁶⁾ in our laboratory and Toda.⁷⁾ But it is very difficult to gather a large quantity of nodules on account of the recent development of chemotherapy in leprosy. Thereby, on the other hand is described here a hemolytic reaction of erythrocytes

sensitized with tuberculin as reported by Levine, in order to know whether tuberculin can substitute for extracts of *M. leprae* or not.

EXPERIMENTAL

Materials and Methods

1) Experiment using erythrocytes sensitized with tuberculin

The technic employed for determination of titers of hemolytic reaction is that described by our co-workers.⁸⁾

2) Experiment using erythrocytes sensitized with extracts from leprosy nodules

Leprosy nodules 5.2 g. (wet weight) are immediately cut into thin slices and then placed in five volumes of a mixture of 96 per cent. alcohol and ether (1:1 by volume). After a lapse of one week, the mixture was centrifuged and the residue was then treated with about ten volumes of boiling alcohol for 17 hours, and then the mixture was centrifuged again and the residue dried in a desiccator. It was then treated with boiling water five times (1.5 hours) and the supernatant was dialyzed. The water extract was concentrated under reduced pressure, the appearing precipitates centrifuged off.

To the yellowish supernatant liquid were added equal volume of 1/2 *N* trichloroacetic acid and the flocculent precipitates centrifuged. The supernatant was dialyzed and added with three volumes of acetone and the appearing precipitates was dried in vacuo (CF).

The precipitates produced by trichloroacetic acid were redissolved again in water with addition of small amount of alkali and precipitated in its IEP and dried using lyophilisation (PF₁).

The precipitates appearing during concentration of the original water extract under reduced pressure gave positive biuret and ninhydrin tests and were dissolved in water in alkaline condition and precipitated at IEP (pH 6.0). The precipitates were dried by lyophilisation (PF₂). The supernatant was again precipitated with acetone and dried (PF₃). The last supernatant was dialyzed and dried (PF₄).

The cold and hot alcohol extracts were evaporated together under reduced pressure to dryness, and the remainder again treated with boiling alcohol. To the hot alcohol soluble part were added five volumes of cold acetone and the appearing white precipitates were dried (LF). The outline of these procedures and yields of each fraction were showed in Table I.

Approximately 1 cc of washed 10 per cent red cells was added to 2 cc. of the neutral isotonic solutions containing 1 mg. of each of these fractions. The cells were well suspended and placed in a water bath

TABLE I
Separation of Several Components from Leprosy Nodules

at 37°C, for 2 hours being frequently agitated, then the suspension was centrifuged and the clear supernatant fluid was discarded. The packed treated red cells were washed three times with 30 cc. of saline and finally suspended in 20 cc. of saline (0.5 per cent "sensitized red cell suspension").

Results

Experiment using erythrocytes sensitized with tuberculin. As shown in Table II, 12 sera of the 40 lepromatous type and 5 sera of the 25 neuro-

macular type showed positive hemolysis in dilution of 10 to 100 times of sera. Namely, in contrast with tuberculous cases by Takasawa *et al.* it was recognized that leprosy was lower in positive percentage and the same fact was found in hemolysis as shown in hemagglutination. Although somewhat differences were seen in titer, no difference in positive percentage was found between lepromatous and neuromacular type. Furthermore, distribution of titers in leprosy cases is more variable and labile than in tuberculous cases.

Table III gives no significant relationship between clinical conditions of patients and positive hemolytic reaction.

TABLE II
Comparison of Positive Percentage in Leprosy

	Sera tested	Positive sera	Positive percentage	Confidence limit of positive perc.	Averaged titer
Lepromatous type	40	12	33.3	50.2—12.3	2.68
Neuromacular type	25	5	20.0	42.6—4.2	1.99
Total	65	17	26.1	40.8—13.5	2.38

TABLE III
Relationship between Clinical Condition of Leprosy
Patients and Hemolytic Reaction

Lepromatous type	Sera tested	Positive sera	Positive percent.	Number of positive cases at the serum dilution of				
				1:10	1:20	1:40	1:80	1:100
Far advanced	17	7	41.1	4				3
Mediately advanced	18	4	27.7	2		1	1	
Slightly advanced	5	1	20.0			1		
Total	40	12	33.3	6		2	1	3
Neuro-macular type	Sera tested	Positive sera	Positive percent.	Number of positive cases at the serum dilution of				
				1:10	1:20	1:40	1:80	1:100
Far advanced	4	1	25.0			1		
Mediately advanced	13	2	15.3			1		1
Slightly advanced	8	2	25.0	1	1			
Total	25	5	20.0	1	1	2		1

In addition to this experiment, a hemolytic reaction was conducted between the erythrocytes sensitized with a polysaccharide fraction of

typhoid bacilli (Ashida⁹⁾, alkali treated Boivin antigen) and the sera of leprosy patients. Consequently 2 cases of 8 lepromatous type and 1 case of 6 neuro-macular type showed titer of 1:10.

Experiment using erythrocytes sensitized with extracts from leprosy nodules. Using erythrocytes sensitized with above described fractions from leprosy nodules, each 5 sera of lepromatous and neuro-macular type were tested in 1:10 to 1:100 of serum dilution and the results were all negative.

SUMMARY

From the hemolytic reaction of cattle erythrocytes sensitized with tuberculin by sera of leprosy patients, it was recognized that leprosy was lower than tuberculosis in positive percentage and serum titer against Levine's report. Namely, in contrast with Takasawa and his coworker's investigations in tuberculosis, it was confirmed that tuberculin was more specific to tuberculosis in hemolytic reaction than leprosy, and there was found no difference of positive percentage between lepromatous and neuro-macular type. Further, the significant relationship between clinical conditions of patients and positive hemolytic reactions was not proved.

The author could not also obtain an expected result in hemolytic reaction using erythrocytes sensitized with each component extracted from leprosy nodules because of a small amount of yields. Considering from the fundamental experiment of hemolytic reaction, however, if more large quantity of the extract of nodules would be obtained, the specific hemolytic reaction would appear in leprosy as in cases of typhoid. Now, this problem will be revealed in future.

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