11450

Specific Nature of Complement Fixing Antibody in Malaria as Demonstrated by Absorption Tests.

ANNA DEAN DULANEY AND WARREN K. STRATMAN-THOMAS.
(Introduced by O. S. Gibbs.)

From the Department of Bacteriology and the Department of Preventive Medicine, University of Tennessee College of Medicine.

We[1] have confirmed the demonstration by Coggeshall and Eaton[2,3] of a specific complement fixation reaction in malaria employing *P. knowlesi* antigens. The parasites were washed as free as possible of hemoglobin and other blood constituents and dried *in vacuo*. When ready for use a standardized amount was rehydrated with physiological saline, frozen and thawed, and the supernatant fluid used as antigen.

We have tested sera from 83 patients in whose blood malaria parasites were demonstrated. Seventy-two percent gave a positive complement fixation for malaria at some time during the course of the disease. The positive reaction was correlated with the presence or recent presence of demonstrable parasites but not with the number of parasites. Our results show that a positive complement fixation reaction with our parasite antigen is probably diagnostic of malaria. However, a negative reaction does not rule out malaria.

Sera from 134 individuals presumably free from malaria yielded 127 negative and 7 weakly positive reactions read as 1+ or ±. Forty-three of these sera were known to give a positive Wassermann reaction, 40, a negative Wassermann. One in each group gave a weakly positive reaction with the malaria antigen. It would appear that syphilis does not provoke non-specific reactions with the malaria parasite antigen.

We have further shown that Wassermann negative patients who received induced malaria remained Wassermann negative throughout the course of their treatment, even after they had developed a strongly positive reactivity for the malaria antigen.

Absorption experiments also indicate distinct and unrelated antibodies since treatment of serum with either the malaria or Wassermann antigen removes the specifically reacting substance from that

1 Stratman-Thomas, Warren K., and Dulaney, Anna Dean, *Am. J. Trop. Med.*, in press.

2 Eagle, Harry, and Hogan, Ralph B., *J. Exp. Med.*, 1940, **71**, 215.

3 Coggeshall, Lowell T., and Eaton, Monroe D., *J. Exp. Med.*, 1938, **67**, 871.

serum without modification of its ability to fix complement in the presence of the other antigen.

For these absorption experiments sera giving 4+ reaction with both the malaria and Wassermann antigens were used. We followed the procedure employed by Eagle[2] in absorption experiments on syphilitic sera using Wassermann and Reiter spirochetal antigens.

The 5 sera tested were obtained from patients who had received induced malaria therapy for the treatment of paresis. Sera obtained from these patients prior to the malaria inoculation gave 4+ Wassermann reactions and no complement fixation with the malaria antigen.

Each serum was divided into 5 portions. Part 1 (2 cc) was absorbed with the sediment from 2 cc of Kahn antigen which had been diluted with 3.5 cc of saline and centrifuged. After incubation for 2 hours at 37.5°C and overnight in the ice box 2 cc of physiological saline were added. Removal of the lipoidal particles was accomplished by centrifugation at high speed and subsequent filtration through a micro-Seitz filter. Part 2 (2 cc) was diluted with an equal volume of physiological saline and served as a control for the filtering process to which Part 1 was subjected. Part 3, (2 cc) was combined with an equal volume of the undiluted *P. knowlesi* antigen, prepared in our routine manner. After incubation for 2 hours at 37.5°, the mixture was centrifuged and the supernatant fluid removed. One cc (KAb_1) was set aside for testing and the remainder used for a second absorption with the undiluted antigen. In some cases this process was repeated again and the final mixture of serum and antigen left in the ice box overnight when it was centrifuged and the supernatant fluid (KAb_3) removed for testing. Part 4 (2 cc) was absorbed 2 or 3 times with an antigen prepared from red blood cells of normal monkeys to rule out species factors which might influence the reaction with the malaria antigen. Part 5 was used as the untreated serum control.

Complement fixation tests on non-absorbed and absorbed portions of the sera were done at the same time with Wassermann antigen and our *P. knowlesi* malaria antigen. The procedure employed for our routine complement fixation tests was followed. Serum and antigen controls were included. Table I gives the detailed results obtained with the serum of patient DIS. Other sera yielded similar data.

These results show: (1) Absorption with the Wassermann antigen removes the so-called syphilitic reagin, responsible for the positive Wassermann test without affecting the reactivity of the serum with

TABLE I.
Effect of Absorption with Wassermann, Malaria, and Normal Monkey Antigens on Reactivity of Serum for Wassermann and Malaria Antigen.

Serum of patient dis.	Antigen	Dilutions of serum: Undiluted	1:2	1:4	1:8	1:16	1:32	1:64
Untreated	*	4+	4+	4+	4+	2+	—	—
	†	4+	4+	4+	1+	—	—	—
Diluted 1:2 with saline and filtered	*	....	4+	4+	3+	2+	—	—
	†	....	4+	3+	—	—	—	—
Absorbed with Kahn antigen, diluted 1:2 with saline, filtered	*	....	4+	4+	3+	1+	—	—
	†	....	—	—	—	—	—	—
Absorbed with malaria antigen 1 x (KAb_1)	*	....	3+	2+	1+	—	—	—
	†	....	4+	4+	—	—	—	—
Absorbed with malaria antigen 2 x (KAb_2)	*	....	....	1+	—	—	—	—
	†	....	....	4+	—	—	—	—
Absorbed with malaria antigen 3 x (KAb_3)	*	....	....	....	—	—	—	—
	*	....	....	....	—	—	—	—
Absorbed with normal monkey antigen 1 x (NAb_1)	*	....	4+	4+	4+	2+	—	—
	†	....	4+	4+	±	—	—	—
Absorbed with normal monkey antigen 2 x (NAb_2)	*	....	....	4+	3+	2+	—	—
	†	....	....	4+	1+	—	—	—
Absorbed with normal monkey antigen 3 x (NAb_3)	*	....	....	....	3+	1+	—	—
	†	....	....	....	—	—	—	—

*Malaria.
†Wassermann.

the malaria antigen to a significant degree. (2) Absorption with the malaria antigen removes the malaria antibody without removal of the syphilitic reagin to a significant degree. (3) Absorption with normal monkey red cell "antigen" does not affect the reactivity of the serum with either the Wassermann or the malaria antigen. 4. On the basis of this investigation the complement fixation test for malaria would appear to be indicative of the presence of a specific malaria antibody in patients' sera. 5. The syphilitic reagin and the malaria antibody would appear to be distinct entities.