PROPERTIES OF SECONDARY CYTOPHILIC MOUSE Vi-ANTIBODIES

V. I. Levenson, N. I. Braude, and
E. V. Chernokhvostova

UDC 612.017.1:576.8.097.5

Antibodies appearing in the blood serum of mice in the early period after immunization with Vi-antigen of Salmonella typhi, and described as secondary cytophilic antibodies because of their property of producing fixation of erythrocytes loaded with Vi-antigen on macrophages, belong to the class of γM-globuline.

* * *

Investigation of immune sera obtained from mice on the 3rd-4th day after injection of Vi-antigen of Salmonella typhi [1, 2] showed that the "early" antibodies contained in them are incapable of opsonizing homologous erythrocytes loaded with Vi-antigen, but in vitro they cause adhesion of erythrocytes to the surface of macrophages with the formation of the characteristic rosettes. Such erythrocytes fixed on macrophages are not ingested for a long time. Vi-antibodies causing fixation of antigen on macrophages differ from the classical cytophilic antibodies [6, 9] in that their affinity for the surface of the macrophages is exhibited only after interaction with antigen. They may therefore be described as "secondary cytophilic antibodies."

The object of the present investigation was to determine the class of immune gobulins to which these antibodies belong.

EXPERIMENTAL METHOD

Peritoneal macrophages of normal mice, mouse erythrocytes loaded with Vi-antigen, and mouse Vi-antisera were obtained in accordance with the published data [1, 2]. The following methods were used to identify the Vi-antibodies producing immune fixation or phagocytosis of the antigen in vitro: 1) treatment of the sera with reducing substances: cysteine [3] and 2-mercaptoethanol (2-ME, Schuchardt, 0.1 M solution, incubation for 2 h at 37°); 2) chromatographic separation of serum proteins on columns with DEAE-cellulose [4]. The results of immunoelectrophoresis showed that the method of fractionation adopted yielded the following fractions of immune globulins: fraction I – slow γG-globulins, fraction II – fast γG- and γA-globulins, fraction III – γM-globulins with small traces of the fastest moving γG-globulins. The essential feature is that immune globulins of the γM type were eluted only from fraction III.

Experimental procedure. A suspension of mouse Vi-erythrocytes (10 million) in 1 ml of the test dilution of serum (fraction) was incubated for 1 h at 37° and added to peritoneal cells transplanted 1 h previously into flasks with cover slips (1 million cells per flask). The flasks were then incubated at 37° for a further hour and specimens stained by Romanovsky's method were examined under the microscope [1, 2]. The intensity of opsonization was determined from the number of macrophages containing at least one erythrocyte in their cytoplasm. The activity of the secondary cytophilic Vi-antibodies causing the formation of erythrocyte rosettes around the macrophages was estimated from the number of cells to which at least 3 erythrocytes were adherent. The results were expressed semiquantitatively in accordance with the following scale: ++++ over 20% of active macrophages; +++ 10-20% of active macrophages; ++ 5-10%; + 1-5%, and 0 less than 1% of active cells.

The results of determination of Vi-antibodies in the passive hemagglutination reaction were expressed on a scale of conventional units: 1) 1/10, 2) 1/20, 3) 1/40, 4) 1/80, and so on.

Moscow Research Institute of Epidemiology and Microbiology, Ministry of Health of the RSFSR (Presented by Academician Z. V. Ermol'eva, Academy of Medical Sciences of the USSR.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 68, No. 7, pp. 65-68, July, 1969. Original article submitted May 18, 1968.

TABLE 1. Effect of Treatment with Cysteine and 2-ME on Activity of Secondary Cytophilic Vi-Antibodies

Serum No.	Without treatment		After treatment with cysteine		After treatment with 2-ME	
	Titer in PHR	Secondary cytophilic activity	Titer in PHR	Secondary cytophilic activity	Titer in PHR	Secondary cytophilic activity
M-15	7	++++	–	–	0	0
M-19	9	+++	–	–	0	0
M-22	12	+++	–	–	0	0
M-104	9	++++	–	–	0	0
M-76	8	++++	0	0	–	–
M-81	11	++++	0	0	–	–
M-82	12	++++	0	0	–	–

EXPERIMENTAL RESULTS

Treatment of the sera with reducing substances (cysteine, 2-ME) caused complete activation of the secondary cytophilic Vi-antibodies (Table 1), indicating that these antibodies belong to the class of γM-globulins. However, this conclusion cannot be final, because treatment in this manner is known to cause injury also to type γG-antibodies, notably a decrease in their complement-fixing activity [14].

A more definite conclusion regarding the nature of the secondary cytophilic Vi-antibodies can be drawn on the basis of chromatographic investigation (Table 2). During fractionation of the "early" Vi-antisera all the antibodies are found in the macroglobulin fraction III. They are sensitive to the action of cysteine and exhibit marked secondary cytophilic activity in a culture of macrophages. Even more demonstrative results were obtained by the investigation of "late" Vi-antisera (M-4, M-30, M-23, M-21). After chromatographic fractionation, Vi-antibodies were found in all three fractions, although only fraction III, containing the macroglobulins, possessed secondary cytophilic activity in a culture of macrophages. Fraction I (slow γG-globulins) exhibited marked opsonizing properties in vitro, while fraction II, when tested in a culture of macrophages, as a rule was completely inactive, despite its high content of hemagglutinating Vi-antibodies. These experiments showed that antibodies belonging to different classes of immune globulins differ considerably also in their effect on the reaction between antigen and macrophages. The opsonizing activity demonstrates a link with antibodies of the γG type, while secondary cytophilic Vi-antibodies are linked with the macroglobulin fraction.

TABLE 2. Investigation of Activity of Chromatographic Fractions

Test material	Index	Serum No.				
		M-22	M-4	M-30	M-23	M-21
Original serum	Titer of PHR	9/0*	12/6	11/11	11/10	12/8
	Secondary cytophilic activity	+++	++++	++++	+	++++
	Opsonizing activity	0	++	++	+	++
Fraction I	Titer of PHR	0	0	6/5	– –	2/2
	Secondary cytophilic activity	0	0	+	0	0
	Opsonizing activity	0	+	+++	++	++
Fraction II	Titer of PHR	0	3/3	10/7	7/7	9/5
	Secondary cytophillic activity	0	0	+	0	+
	Opsonizing activity	0	++	0	0	0
Fraction III	Titer of PHR	10/0	10/0	11/2	7/0	11/0
	Secondary cytophilic activity	++++	+++	++++	++++	++++
	Opsonizing activity	0	0	0	+	0

* Titer of antibodies without cysteine treatment/titer after treatment with cysteine.

Discussion of the results will be limited to their comparison with the known data concerning the nature of opsonizing antibodies. These data are contradictory. Investigations based on determination of the blood clearance (in vitro) lead to the conclusion that antibodies of type γM possess the strongest opsonizing activity [8, 12]. However, in vitro investigations lead to the opposite conclusion—higher opsonizing activity of type γG antibodies [11, 13], in agreement with the results of the present investigation. This contradiction can be removed if it is accepted that immune fixation of antigen on macrophages can take place not only in vitro, but also in vivo [10]. This phenomenon, linked with γM-antibodies, is probably capable of yielding very high blood clearance indices, although it is based not on ingestion, but merely on fixation of the antigen by the reticulo-endothelial cells. High activity of the macroglobulin antibodies in the experiments with determination of the blood clearance thus received an alternative explanation, removing the contradiction with the result of direct tests of opsonizing activity in vitro.

The concept has recently been developed that induction of antibody formation is a two-stage process: in the first stage macrophages interact with antigen, liberating a highly immunogenic material the nature of which is at present the subject of discussion [5,7]. This material is the inducer of antibody formation in the lymphoid cells in the second stage of the process. Data indicating that the reaction between macrophages and antigen does not always take place in the same manner, but that it may occur not only as phagocytosis but also as fixation of the antigen on the surface of the cells, also raise another question: what is the importance of each of these types of interaction for the fate of the antigen and induction of antibody formation.

LITERATURE CITED

1. V. I. Levenson and N. I. Braude, Dokl. Akad. Nauk SSSR, 174, No. 2, 468 (1967).
2. V. I. Levenson and N. I. Braude, Folia Biol. (Prague), 13, 113 (1967).
3. E. V. Chernokhvostova, Lab. Delo, No. 6, 323 (1965).
4. M. Adinolfi et al., J. Exp. Med., 123, 951 (1966).
5. B. Askonas and J. Rhodes. Nature, 205, 470 (1965).
6. S. Boyden, in: Cell-Bound Antibodies, B. Amos and H. Kprowsky (editors), Philadephia (1963), p. 7.
7. M. Fishman et al., in: Molecular and Cellular Basis of Antibody Formation, Prague (1965), p. 491.
8. P. L. Mollison, in: 11th Congress of the International Society of Haematology, Papers presented, Sydney (1966), p. 234.
9. D. Nelson and P. Mildenhall, Aust. J. Exp. Biol. Med. Sci., 45, 113 (1967).
10. G. Nossal and A. Abbot, in: 11th Congress of the International Society of Haematology, Papers presented, Sydney (1966), p. 5.
11. W. Parish, Nature, 208, 594 (1965).
12. J. Robbins et al., J. Exp. Med., 122, 385 (1965).
13. J. Smith, et al., J. Immunol., 98, 336 (1967).
14. G. Wiedermann et al., Proc. Soc. Exp. Biol. (N.Y.) 116, 448 (1964).