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Specificity of Rabbit IgM and IgG Antibody in Intratypic Differentiation of Poliovirus Type I Strains

By

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Summary

The capacity of rabbit IgM and IgG antibody to differentiate various strains of type I poliovirus has been shown not to depend upon the extent of their purification from other serum proteins. IgM and IgG as well as the original sera did not differ from each other in specificity. Comparison of early and hyperimmune sera from the same rabbits did not reveal significant differences in the differentiating capacity of both whole sera and their fractions. The revealed regularities were manifested in different methods of neutralization tests with all poliovirus strains under study. In the course of repeated immunizations the avidity of antibody increases both for the homologous and heterologous strains.

1. Introduction

Many reports have been published on studies of the specificity of different classes of antibody. The results have been found to be variable. Thus, greater specificity of rabbit 19 S neutralizing antibody as compared with that of 7 S antibody in differentiation of strains of herpesvirus (3, 9) and foot-and-mouth disease virus (2) was demonstrated. With influenza virus, however, it was shown that early 19 S neutralizing antibody was less specific than 7 S (12). At the same time, similar specificity of 7 S and 19 S antibody for strains of southern bean mosaic virus was discovered (10).

In this connection it was interesting to find out the possible causes of such variability of the results obtained. In the present work, the effect of some factors on the specificity of rabbit serum antibody was studied on models of poliovirus type I variants. The work with poliovirus is of independent importance since intratypic differentiation of poliomyelitis virus strains is necessary for analysis of vaccine-associated cases of disease and for the study of genetic stability of vaccine strains.

2. Materials and Methods

2.1. Preparation of Sera and Antibody Fractions

Random bred rabbits weighing 2—3 kg were immunized with the LSc 2ab strain of poliomyelitis virus in a dose of $1—2 \times 10^8$ PFU. The virus was inoculated intravenously six times at 10-day intervals. After the first immunization the blood was collected on the 7th day, after the others on the 5th day. The sera were heated at 56° C for 30 minutes followed by precipitation of the globulin fraction by salting-out with $(NH_4)_2SO_4$ at 40 per cent saturation. The globulin fraction was fractionated by gel filtration on Sephadex G-200 column of 5.3 $cm^2 \times 75$ cm with an eluent of 0.02 M Tris plus 0.28 M NaCl, pH 8.0. The protein peaks I and II were used for preparation of IgM and IgG, respectively. For further purification of IgM, ion-exchange chromatography on a DEAE-cellulose column was used with elution in step-wise gradient: Tris plus NaH_2PO_4, 0.01—0.3 M, pH 8.6—5.0. The peak of protein obtained by elution with 0.2 M buffer solution, pH 7.0 consisted of IgM as determined by immunoelectrophoresis. IgG was purified by elution from a DEAE-Sephadex A-50 column in 0.02 M phosphate buffer solution, pH 6.6. The peak eluted under these conditions was the IgG fraction. The purity of IgM and IgG preparations was determined by immunoelectrophoresis with donkey sera against rabbit globulins. Figure 1 presents the results of immuno-

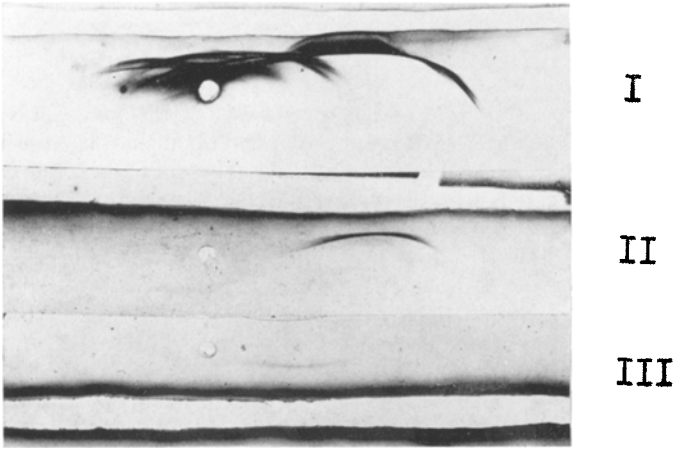

Fig. 1. Immunoelectrophoresis of rabbit serum fractions
I = a fraction of globulins prepared by precipitation with ammonium sulphate at 40 per cent saturation; II = fraction IgG prepared by gel filtration on Sephadex G-200 and ion-exchange chromatography on DEAE-sephadex; III = a fraction of IgM prepared by gel filtration of Sephadex G-200 and ion-exchange chromatography on DEAE-cellulose

electrophoresis of IgM and IgG fractions subjected to all stages of purification. In the course of the study protein solutions were concentrated in an ultrafiltration apparatus Amicon with XM-100 and XM-50 filters.

2.2. Neutralization Test

The method of neutralization kinetics was used (11). In the majority of tests the N 209 strain isolated in 1959 from a patient with a severe form of poliomyelitis was used as the heterologous strain. In some tests other strains were also utilized as test-viruses (Table 2). 0.2 ml of homologous (LSc 2ab) and heterologous strains containing $2—5 + 10^6$ PFU were mixed with equal amounts of immune serum or its IgM and IgG fractions in the working dilution. After contact for 1, 2, 4, 6 and 8 minutes the mixture was immediately diluted 1000-fold with Earle's salt solution cooled to 4° C. The result-

ing diluted mixture was inoculated into bottle cultures of primary rhesus monkey kidney tissue subsequently overlayed with agar. After reading of the plaque numbers in the bottles, the constant of neutralization was calculated by the formula

$$K = \frac{D}{T} \times 2.3 \times 1^{\circ} g \frac{V_0}{Vt}$$

where V_0 and Vt are virus titers before and after contact with the serum, respectively, D is the reciprocal of the serum dilution and T is the time of virus contact with serum. Then, the normalized constant (K_n) was determined by comparison of the rate of neutralization of the strain under study with that of the prototype strain assigored the value of 100. K_n was calculated from points lying on the exponential part of neutralization curves.

In some experiments, the differentiating capacity of sera was determined by comparison of maximum serum dilutions reducing plague counts by 50 per cent or more of the homologous and heterologous strains respectively. In such cases the virus was in contact with the serum at 37° C for 1 hour.

The avidity of the sera was determined by comparative titration in the neutralization test by the cytopathic effect in monolayer cultures and by the pH color test (8).

The considerably higher titers observed in the pH color test were regarded as an indication of the presence of low avidity antibody, since both kinds of antibody were detected in the metabolic test but only high avidity antibody in monolayer cultures. The tests were run with an equal dose of testvirus corresponding to 100 and 1000 TCPD as determined in metabolic and cytopathic tests, respectively.

3. Results

The data presented in Figure 2 show that the differentiating capacity of IgM and IgG antibody preparations at different stages of their preparation and purifica-

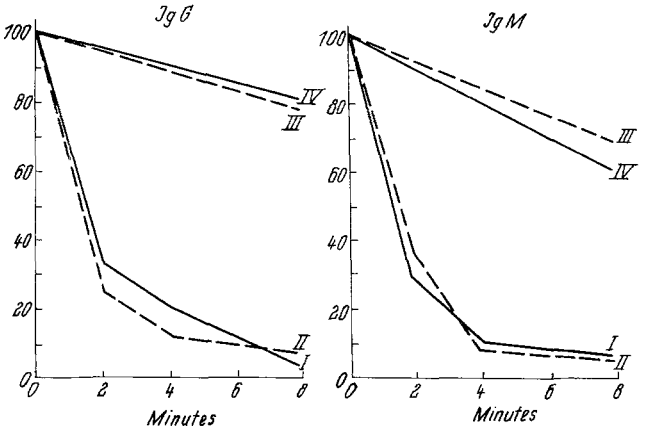

Fig. 2. Differentiating capacity of IgM and IgG fractions at different stages of their purification
———— preparations of IgM and IgG after gel filtration and ion-exchange chromatography; — — — preparations of IgM and IgG after gel filtration; I and II = neutralization of a homologous strain; III and IV = neutralization of a heterologous strain

tion does not differ. IgM and IgG obtained after gel filtration on Sephadex G-200 and after final purification by ion-exchange chromatography gave similar neutralization curves for the LSc2ab and 209 strains.

It will be seen in Table 1 that the specificity of IgM and IgG antibody prepared from any one serum is similar and shows no significant difference from the specificity of the whole serum. Only in one instance (hyperimmune serum No. 1) was the

Table 1. *Specificity of Whole Sera and Their IgM and IgG Fractions*

Rabbit No.	Early Sera			Hyperimmune Sera[a]		
	K_n			K_n		
	whole	IgM	IgG	whole	IgM	IgG
1	21	28	—	20	25	7
2	17	12	14	14	14	14
3	21	19	17	20	29	30
4	5	8	12	7	10	7
5	30	33	25	37	22	31
				28[b]	34	30
6	20	—	—	43	—	—
7				33	25	23

[a] Obtained after 6 immunizing doses.
[b] In this case the serum was collected after 8 injections of antigen.

IgG fraction more specific than IgM and the native serum. In the majority of cases no changes were found in specificity of antibody in the course of repeated immunizations of rabbits. The sera and fractions there of after the first and the sixth immunization had no statistically significant differences in K_n. Only in rabbit No. 6 the hyperimmune serum was more specific than the earlier one. Further experiments were performed to find out whether the discovered facts of IgM and IgG specificity were the general regularity. For this purpose, K_n were calculated

Table 2. *Intratypic Antigenic Differentiation of Different Strains of Type I Poliovirus by Hyperimmune Sera and Their IgM and IgG Fractions*

Strain No.	Origin	Serum No. 2			Serum No. 8		
		K_n			K_n		
		Whole	IgM	IgG	Whole	IgM	IgG
	From poliomyelitis patient:						
Lugovskoy	1954	20	22	27	22	30	26
209	1959	30	33	22	33	33	27
1959	1970	40	55	44	66	56	50
1976	From a vaccinated child, 1970	40	44	44	44	50	37
1783	From sewage, 1970	80	88	90			

for two sera and a whole series of heterologous poliovirus type I strains (Table 2). In all cases the specificity of the native serum and its IgM and IgG fractions was found to be similar.

The differentiating capacity of the sera was further compared by the kinetic neutralization test and by calculation of maximum dilutions neutralizing 50 per cent or more plaques (Table 3). No differences in the specificity of whole sera and their IgM and IgG fractions were found by these two methods.

Table 3. *Comparison of the Specificity of Sera and Their Fractions in Two Types of Neutralization Test*

Serum No.	Strain	Kinetica of neutralization K_n			Neutralization for 1 hour Negative $\log_2$ [a]		
		Whole serum	IgM	IgG	Whole serum	IgM	IgG
1	Homologous				13	5	10
	Heterologous	25	19	27	10	2	7
2	Homologous				12	5	9
	Heterologous	15	12	10	10	2	7

[a] Log of the maximum serum dilution giving neutralization of 50 per cent or more plaques.

In studies of avidity of early and hyperimmune antibody it was found that the avidity of antibody increased in the course of repeated immunizations both for homologous and heterologous strains. Thus, in early sera antibody was detectable only in the more sensitive color test in titers of 1:16—1:64, whereas in hyperimmune sera the titers in the color test did not exceed more than 4-fold the titers of the cytopathic test in the monolayer cultures. Thus, the difference in antibody titers observed in the two test systems is not a constant value, but depended on the different quality, i.e. avidity of antibody in early and hyperimmune sera.

4. Discussion

Investigation of the comparative specificity of IgM and IgG antibody by different researchers gave variable results. It may be assumed that one of the reasons of this variability consisted in the fact that those studies were carried out with antibody preparations obtained by different methods giving different degrees of purification. Thus, HAMPAR *et al.* (3, 4, 5), TREMAINE and WRIGHT (10) obtained IgM and IgG after gel filtration of sera on Sephadex G-200, whereas WAGNER and COWAN (11) separated serum fractions by density gradient centrifugation.

In the present study we examined the specificity of IgM and IgG at different stages of their purification using homologous and heterologous virus strains in kinetic neutralization tests. The first stage was precipitation of globulins in $(NH_4)_2SO_4$ solution at 40 per cent saturation followed by gel filtration on Sephadex G-200 column. The second stage was ion-exchange chromatography on DEAE-cellulose (IgM) and DEAE-Sephadex (IgG). After the second stage of purification IgM and IgG gave one precipitation band in immunoelectrophoresis. These fractions were found to have similar capacity for differentiation of intratypic antigenic differences in poliovirus type I strains after both stages of purification.

In reports so far published, the specificity of neutralizing antibody was studied either by the kinetic neutralization (3, 4, 5) or by determination of the maximum serum dilutions giving neutralization of 50 per cent or more plaques (9). It might be suggested that the method of neutralization test affected the results. In the present study these methods were compared using two (early and hyperimmune) serum samples. Yet no differences were found in the specificity of the whole sera and their IgM and IgG fractions by these two methods.

In the majority of early and hyperimmune sera the differentiating capacity of IgM and IgG was shown to be similar[1]. Analogous results were obtained by TREMAINE and WRIGHT (10) in the precipitation test with southern bean mosaic virus. However, there are many reports recording higher specificity of either IgM (3, 4, 9, 11) or IgG (12). This variability of the results appears to be dependent to a considerable extent upon the nature and properties of the antigens. Also the importance of the species of animals tested cannot be excluded, since there are reports in the literature on differences in specificity of IgM and IgG antibody obtained from different animal species immunized with the same antigens (2, 11).

There are also quite contradictory reports with regard to the comparative specificity of early and hyperimmune sera. KRUEGER (5), explaining the greater specificity of rabbit hyperimmune antisera for MS2 bacteriophage as compared with early sera, suggested that in the course of reimmunization there occurred stimulation of one particular clone of antibody-producing cells to the dominant antigenic determinant. WEBSTER (12) explained the greater specificity of early sera in comparison with hyperimmune sera by the following hypothesis. Early sera having low avidity can interact only with the homologous virus strain. In the hyperimmune serum, the affinity of the active center of antibody to antigenic determinants increases, thus not only homologous but also heterologous determinants having certain similarity with them can react with this antibody. The present study showed the specificity of the majority of early and late sera as well as their IgM and IgG fractions to be similar[2]. On the other hand, it was clearly shown that the avidity of antibody increased in the course of hyperimmunization both for homologous and heterologous strains of poliovirus. In this case, apparently many virus determinants, including those common or closely related to heterologous strain, take part in the antigenic stimulation. Since the increase in avidity of antibody may occur for all these determinants, no differences in the kinetics of neutralization concerning homologous and heterologous virus strains were observed.

References

1. COWAN, K. M.: Immunochemical studies of foot-and-mouth disease. V. Antigenic variants of virus demonstrated by immunodiffusion analyses with 19S and 7S antibodies. J. exp. Med. **129**, 333 (1969).

[1] However, one exception was observed, for the IgG fraction of the hyperimmune serum from rabbit No. 1 (cf. Table 1) which proved to be more specific than the IgM and the native serum. Unfortunately, the specificity of the corresponding antibody fraction of the early serum was not tested.

[2] Only in rabbit No. 6 (cf. Table 1) the hyperimmune serum was more specific than the early serum which was apparently due to individual characteristics of this animal.

2. COWAN, K. M.: Immunochemical studies of foot-and-mouth disease. VI. Differences in antigenic determinant site recognition by guinea pig 19S and 7S antibodies. J. Immunol. **104**, 423—431 (1970).
3. HAMPAR, B., M. MAGE, and M. KEEHN: Heterogeneity in the ability of rabbit 7S and 19S neutralizing antibodies to differentiate between intratypic antigenic variants of herpes simplex virus. J. Immunol. **101**, 256—260 (1968).
4. HAMPAR, B., L. M. MARTOS, D. ABLASHI, R. SIGUENZA, and G. WELLS: Differentiation of cross-reacting simian cytomegalovirus strains by late 19S rabbit neutralizing antibodies. J. Immunol. **103**, 1155—1156 (1968).
5. HAMPAR, B., L. M. MARTOS, M. CHAKRABORTY, and M. BURROUGHS: Late 19S rabbit antibody neutralization test for differentiating herpes simplex virus types 1 and 2. J. Immunol. **104**, 593—598 (1970).
6. KRUEGER, R.: Effect of antigenic stimulation on the specificity of antibody produced by rabbits immunized with bacteriophage MA-2. J. Immunol. **104**, 1117—1124 (1970).
7. MCBRIDE, W.: Antigenic analysis of poliovirus by kinetic studies of serum neutralization. Virology **7**, 45 (1959).
8. SABIN, A.: Present status of attenuated live virus poliomyelitis vaccine. J. Amer. med. Ass. **162**, 1589—1596 (1956).
9. STEVENS, T., T. PINCUS, R. BURROUGHS, and B. HAMPAR: Serologic relationship of a simian herpesvirus (SA 8) and herpes simplex virus: heterogeneity in the degree of reciprocal cross-reactivity. J. Immunol. **101**, 979—983 (1968).
10. TREMAINE, J., and N. WRIGHT: Cross-reactive antibodies in antisera to two strains of southern bean mosaic virus. Virology **31**, 481—488 (1967).
11. WAGNER, G. G., and K. M. COWAN: Immunochemical studies of foot-and-mouth disease. IX. Differences in neutralizing activities of guinea pig and bovine 19S and 7S antibodies. J. Immunol. **106**, 656—660 (1971).
12. WEBSTER, R. G.: The immune response to influenza virus. III. Changes in the activity and specificity of early IgM and IgG antibody. Immunology **14**, 39—52 (1968).
13. WESTAWAY, E. G.: Greater specificity of 19S than 7S antibodies in hemagglutination-inhibition test with closely related group B arboviruses. Nature (Lond.) **219**, 78 (1968).

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