Joint ESACT/IABS Meeting on the Use of Heteroploid and other Cell Substrates for the Production of Biologicals, Heidelberg, F.R.Germany, 1981, Develop. biol. Standard. 50, pp. 221-228 (S. Karger, Basel 1982)

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PRODUCTION AND POTENTIAL USE OF MONOCLONAL ANTIBODIES AGAINST POLIO VIRUSES

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ABSTRACT

Lymphocyte hybridomas secreting monoclonal antibodies against different strains of polio virus types 1, 2 or 3 have been produced. For this purpose Balb/C mice were immunized with purified and inactivated virus suspensions and their splenocytes were fused with P3X63Ag8 mouse myeloma cells. Screening for antibody production was performed in an enzyme-linked immunosorbent assay (ELISA). Antibodies were produced either in cell culture or in Balb/C mice by passaging the hybridomas as solid or ascitic tumors, after they had been cloned at least three times by limiting dilutions in microtiter plates.

Specificities of a number of these monoclonal antibodies were determined in the ELISA and in a neutralization test using different polio virus subtypes. The results indicate that for *epidemiological studies* monoclonal antibodies may prove to be very useful tools. Also the use of monoclonal antibodies for *vaccine production* (affinity chromatography; characterization of viral substructures) and routine *vaccine control* purpose (antigen quantification; neutralization of vaccine virus) seems attractive. Two of the neutralizing monoclonal antibodies against polio virus type 1, showed a selective immunoprecipitation with VP_1, which suggests that VP_1 is an important polypeptide for the induction of neutralizing antibody *in vivo*.

INTRODUCTION

Although polio virus is still a major threat to health in most developing countries, the initiation and maintenance during a quarter of a century of mass vaccination against polio has led to the almost complete disappearance of this disease in the industrialized world. The broad antigenic variation within the three types of polio virus has not been a major obstacle to the development of effective vaccines against this disease. In contrast, the detection of antigenic differences between different subtypes or isolates of polio virus is of great importance for epidemiological reasons. Especially for the differentiation of Sabin-like from non-Sabin-like viruses a system using absorbed antisera in neutralization tests (NT) gel diffusion (GD) tests or ELISA (5, 10) has proven to be successful.

However, the use of monoclonal antibodies produced by lymphocyte hybridomas for the epidemiological study of polio virus may be expected to offer several advantages. Probably the most important among these is the opportunity to characterize the antigenic relationship among different strains at the level of individual antigenic determinants to identify variant viruses that were previously unrecognizable, as proved to be possible with several other viruses. Another important advantage

would be the production of large quantities of high-titred homogenous standard antibody preparations for reference purposes and intratypic differentiation. Apart from the epidemiological importance, anti-polio virus monoclonal antibodies may be expected to become a useful tool in vaccine technology and to facilitate the isolation of viral components for their biochemical characterization.

In the present paper we describe the production of a number of monoclonal antibodies against several strains of the three polio virus types, the evaluation of the specificity of some of these and the selective immunoprecipitation of VP_1 by two neutralizing monoclonal antibodies, suggesting that this polypeptide plays a role in the induction of neutralizing antibodies *in vivo*.

PRODUCTION OF MONOCLONAL ANTIBODIES

Monoclonal antibodies were produced against different strains of polio virus types 1, 2 or 3 essentially as described previously for the production of monoclonal antibodies against polio virus type 1 and rabies virus (8).

Briefly, Balb/C mice were immunized with polio viruses which had been produced, purified and inactivated according to standard procedures used for vaccine production at our Institute (9). The animals were inoculated three times intramuscularly with 0.5 ml suspensions at weekly intervals and four to twelve weeks after the last inoculation they were boosted intravenously with 0.3 ml of the same suspension. Three or four days later their splenocytes were fused with P3X63Ag8 mouse myeloma cells using PEG 4000. Screening of the hybridomas for antibody production was routinely carried out in an indirect ELISA and incidentally in a micro-NT or a GD test (7). Hybridomas producing monoclonal antibodies were cloned at least two times by terminal dilutions in microtiter trays (≈ 0.2 cells/cup) using mouse macrophages or thymocytes as feeder cells. Monoclonal antibodies were produced either in cell culture or in Balb/C mice by passaging the hybridomas as solid or ascitic tumors. The number of stable hybridomas secreting anti-polio virus monoclonal antibodies which we produced so far is shown in Table I.

Table I. Number of stable hybridomas secreting anti-polio virus monoclonal antibodies (ELISA : $E_{450} \geqslant 300, = 2$ x blank)

type	subytpe	number of stable hybridomas	number of fusions
1	Mahoney	2	1
	Sabin	7	2
	78.9030	4	1
2	MEF_1	6	2
	Sabin	30	1
3	Saukett	1	3
	Sabin	33	1

Fusion of splenocytes from mice immunized with the vaccine strains Sabin 2 and Sabin 3 yielded many more polio virus antibody secreting hybridomas than fusions with splenocytes from mice immunized in the same way with other polio virus strains. This was not only concluded from the results of the fusions mentioned in Table I but was also confirmed by a number of additional fusions, of which the resulting hybridomas have not yet been cloned (unpublished observations). Monoclonal antibody titers measured in the NT ranged up to 10^4 in cell culture fluids and up to 10^7 in mouse body fluids.

SPECIFICITY AND POTENTIAL USE OF MONOCLONAL ANTIBODIES

As has been described previously (8), specificities of a number of the anti-polio virus type 1 monoclonal antibodies have been determined in the ELISA, GD test and NT, using homologous and heterologous polio virus type 1 antigens or viruses. It was concluded that the monoclonal antibodies tested reacted with various degrees of specificity with one or more of the different virus subtypes. So it could be shown that one of the monoclonal antibodies reacted specifically with subtype Sabin 1, one reacted specifically with almost all subtypes of polio virus type 1 and others reacted in variable degrees with different wild type 1 strains (Fig. 1). With some of the monoclonal antibodies quantitative and qualitative differences were shown between virus strains which are known to be closely related on the basis of epidemiological data. These results indicate that these monoclonal antibodies may become useful tools for epidemiological studies, and their practical application for intratypic differentiation of a large number of different type 1 virus strains is being studied in neutralization tests at present. Apart from the intratypic reactions also intertypic reactions especially between polio virus type 1 and type 2 were demonstrated in the ELISA or GD test with some monoclonal antibodies. The results of the three different assays (ELISA, NT and GD test) obtained with the monoclonal antibodies coincide rather well, although their correlation is not absolute : some monoclonal antibodies which react well in the ELISA, do not show neutralizing activity. A start has also been made with the testing of monoclonal antibodies raised against polio virus types 2 and 3 in the ELISA for their intratypic specificity. For this purpose homologous and heterologous antigens as mentioned in Table I were used. Most of the monoclonal antibodies raised against subtype Sabin 2, also reacted with subtype MEF_1, against Sabin 3 also with Saukett and vice versa. However extinction values (E_{450}) measured in homologous reactions exceeded those of heterologous reactions in most cases. Only for one of the anti-Sabin 2 and for four of the anti-Sabin 3 hybridomas the E_{450} measured in the homologous system was more than two times the E_{450} measured in the heterologous system. Evaluation of the newly developed hybridomas which have not yet been cloned may be needed for practical application in epidemiological studies.

High specificity and high titers obtained with monoclonal antibodies produced by lymphocyte hybridomas are also attractive for polio virus vaccine production technology. For vaccine production the use of these anti-polio virus monoclonal antibodies in affinity chromatographical procedures is being investigated at present. Differences in affinity of the antibodies seem to be important for the determination of elution conditions. Also the use of monoclonal antibodies for the identification of virus substructures and recombinant DNA products to be used for vaccination studies

will be considered. The use of monoclonal antibodies for vaccine control purposes, especially in tests for extraneous agents is attractive, since antibody preparations used in these tests should only contain neutralizing antibodies against the vaccine virus. At present experiments are carried out to determine the usefulness of these monoclonal antibodies for this purpose.

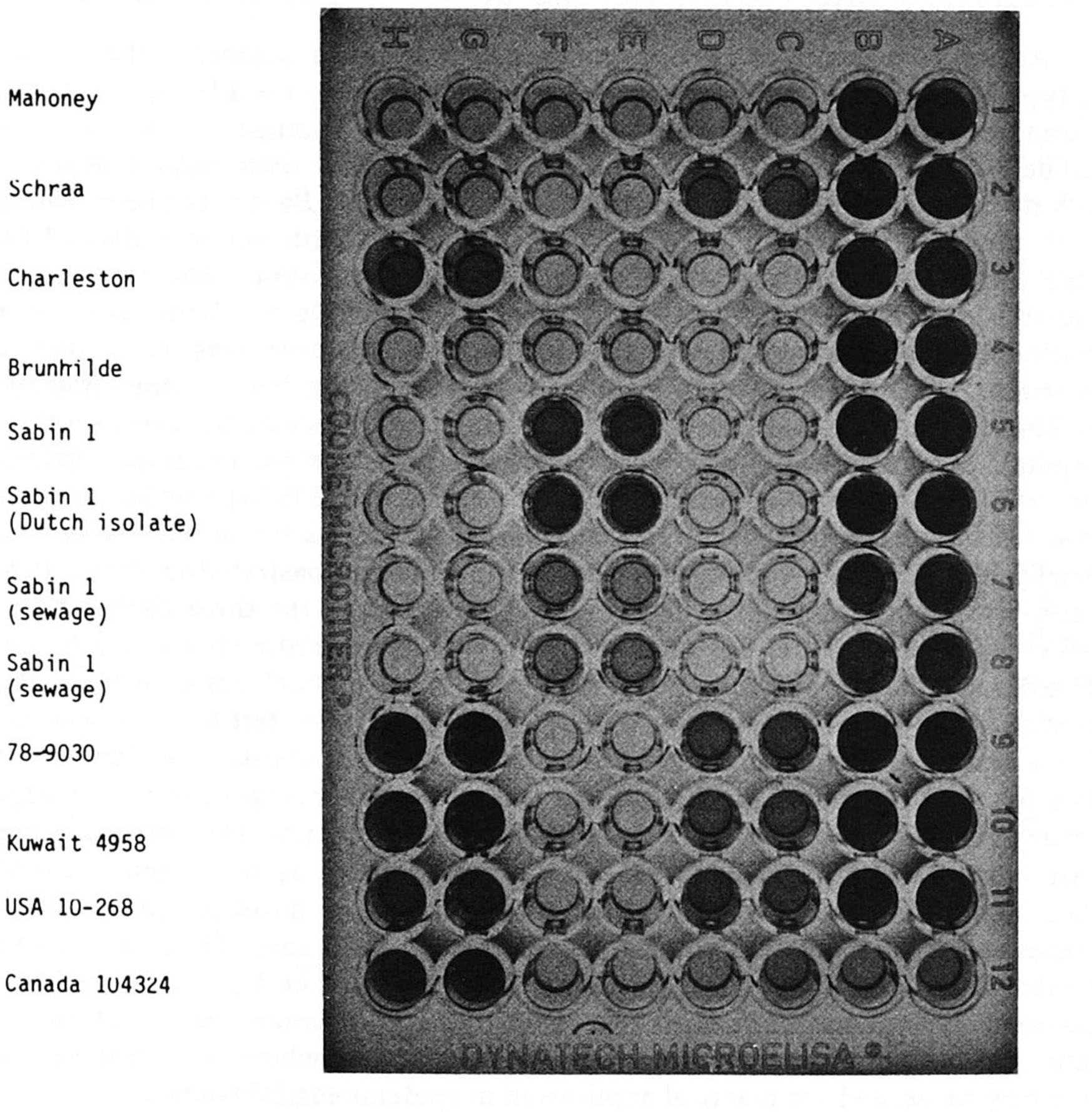

Fig. 1. Indirect ELISA carried out in duplicate with twelve different polio virus type 1 antigens and four different anti-type 1 monoclonal antibodies (D8106 7E8, D8106 7D5 and D8106 5D9 raised against Dutch isolate 78.9030 and S8106 3D8 raised against Sabin type 1 virus). Note varying degrees of specificity of different monoclonal antibodies with different antigens.

SELECTIVE IMMUNOPRECIPITATION OF VP_1 OF POLIO VIRUS TYPE 1 WITH TWO NEUTRALIZING MONOCLONAL ANTIBODIES

The relative absence of neutralizing antibody following immunization with disrupted virus is thought to be a general property of picornaviruses (3, 4), although FMDV and Coxsackie B_3 viruses have recently been considered exceptions (1, 2, 6). Consequently, it has not been possible to determine which of the viral polypeptides of polio virus is responsible for induction of neutralizing antibody.

The use of neutralizing monoclonal antibodies could be another approach to discover viral polypeptides which induce neutralizing antibodies since they may be expected to react specifically with one antigenic determinant. This determinant should be present on the polypeptide or polypeptides which induce neutralizing antibodies.

After pelleting ^{35}S methionine labelled polio virus type 1 (Mahoney) from an infected Vero cell culture medium the virus was purified in an isopyknic CsCl density gradient. A clear peak of infectivity and radioactivity at a density of 1.35 g/ml was collected and used for the preparation of viral polypeptides. For this purpose the virus was disrupted in SDS-urea and polypeptides were separated in a tube polyacrylamide gel. As reference non-labelled polio virus was treated in the same way, run in a parallel gel and the polypeptides were stained with Coomassie blue. The gel containing the labelled polypeptides was divided into five regions : A – E. Region B was cut into 17 equal slices and region D into seven. After elution of the slices in 250 μl TESV-buffer the labelled polypeptides were localized by liquid scintillation counting (Fig. 2). Peak fractions containing VP_1, VP_2 and VP_3 respectively were used in immuno-precipitation experiments. Too little label was present in VP_4 to be used for this purpose.

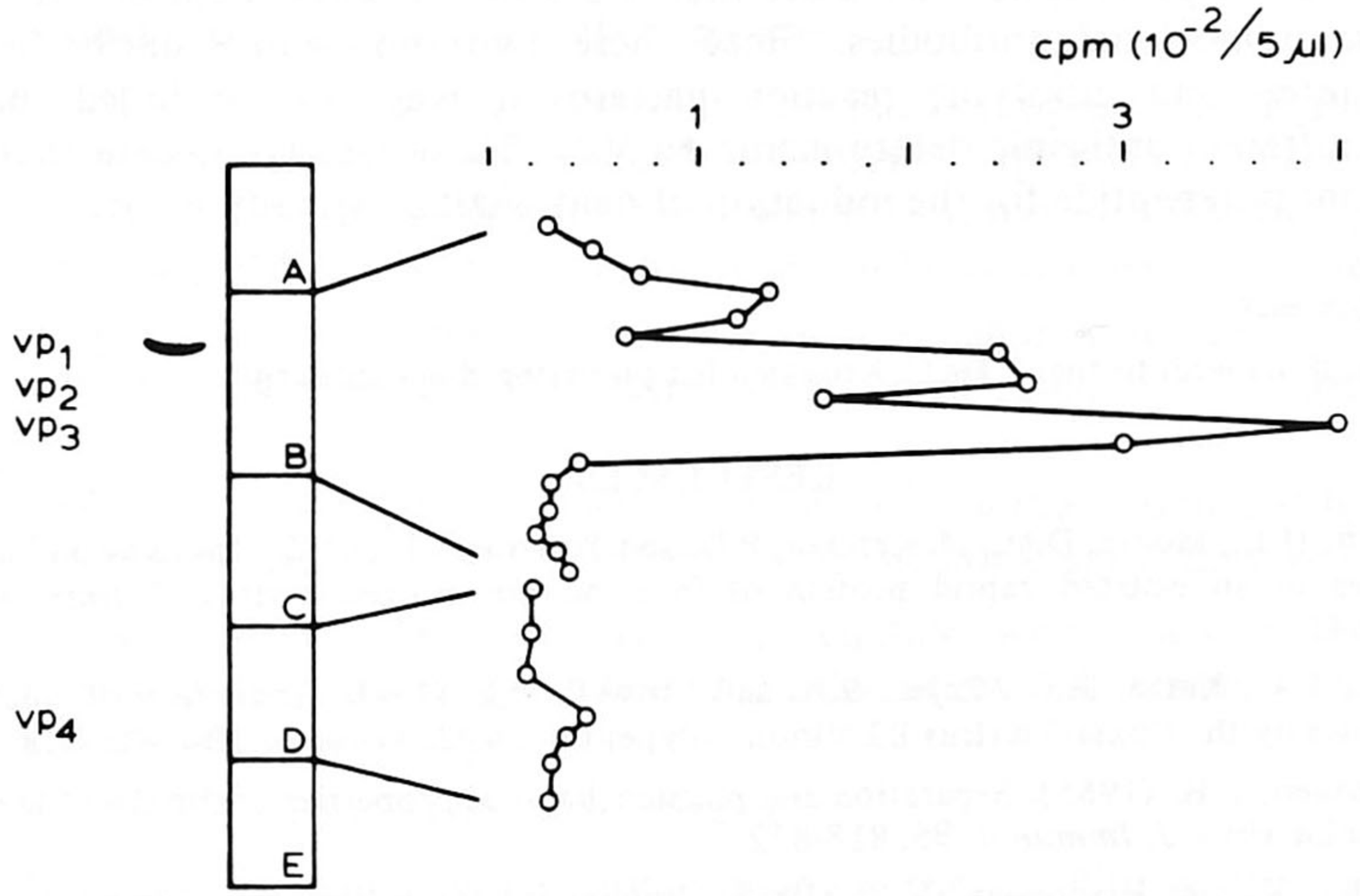

Fig. 2. Two cylindrical polyacrylamide gels, one containing non-labelled polio virus type 1 (Mahoney) polypeptides stained with Coomassie blue (left) and one containing ^{35}S methionine labelled polypeptides from the same virus (A-E) which were eluted from the fractionated gel and localized by liquid scintillation counting.

Table II. Immunoprecipitation of ^{35}S methionine labelled polio virus type 1 (Mahoney) polypeptides with monoclonal antibodies raised against polio virus type 1 (Mahoney) : $M8014D_2$ and $M8017C_5$

	total counts	counts precipitated with serum $M8014D_2$	(%)	counts precipitated with serum $M8017C_5$	(%)	counts precipitated with control serum	(%)
VP_1	2591	331	(12.8)	194	(7.5)	36	(1.4)
VP_2	2722	41	(1.5)	42	(1.5)	36	(1.3)
VP_3	4646	50	(1.1)	47	(1.0)	50	(1.1)

Immunoprecipitation of the three major polypeptides was carried out with the sera of two mice in which anti-Mahoney hybridomas had been grown. Neutralizing antibody titres of these sera were $1 \cdot 10^5$ and $2 \cdot 10^4$ respectively, using 100 $TCID_{50}$ of polio virus type 1 (Mahoney). A serum of a normal mouse served as a control and sepharose-protein A was used to precipitate antibody-polypeptide complexes.

As shown in Table II, a selective immunoprecipitation of VP_1 occurs with both monoclonal antibodies : 12.8 and 7.5% of the total label present in VP_1 could be immunoprecipitated, whereas with the control serum only 1.4% was precipitated. From both other polypeptides no more than 1.5% of the label could be precipitated with these monoclonal antibodies. Since these two monoclonal antibodies show different inter- and intratypic reaction patterns it may be concluded that they recognize different antigenic determinants on VP_1. These results indicate that VP_1 is an important polypeptide for the induction of neutralizing antibody in vivo.

Acknowledgments

The authors wish to thank Ms C. Kruyssen for preparing the manuscript.

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DISCUSSION

SCHÖNHERR : Only 12% of your antigen was precipitated by monoclonal antibodies. Did you also do this with normal polyclonal serum and get a better precipitation ?

OSTERHAUS : No, we did not do that.

DOEL : You put antibody onto the plate first in your ELISA test. Have you tried just putting the virus antigen onto the plate and looking at the reactivity of the monoclonal antibody to the virus ?

OSTERHAUS : We have tried but it did not work sufficiently well.

DOEL : When FMDV is put on a plastic plate it opens up and reveals different antigens. Would polio virus behave in the same way ?

VAN DER MAREL : We have looked at this but D antigen does not absorb onto the plate very well. The structural change that occurs in FMDV is an indication that it is not the intact virus but a degraded virus which is absorbed, and with polio this would be the C antigen. It is safer for us to use an antibody coat to absorb the virus.

HORODNICEANU : When you tested with anti-Mahoney and anti-Sabin monoclonal antibodies did you find that some wild strains could not be identified as either ?

OSTERHAUS : We did not check them all in a neutralisation test but certain strains do react very weakly or not at all with the monoclonal antibodies we raise.

HORODNICEANU : Did you try to precipitate with monoclonal antibody precursors, such as cytoplasmic extracts of the cells, at say 3 hours post-infection, to see what sort of precursors you get ?

OSTERHAUS : This has not been done yet.

PETRICCIANI : Can you say anything about the stability of the clones with respect to the antibody and also whether you have observed any drift in antigenic specificity of the antibodies.

OSTERHAUS : We have to sub-clone at least twice to get a stable clone so most clones are therefore not stable. We have not found any drift in specificity so far.

NICHOLLS : Did you confine your ELISA tests to polystyrene plates or did you try PVC plates ?

VAN DER MAREL : We have not found any differences between different types of plates and so we use polystyrene routinely.

NICHOLLS : Have you noticed any difference in virus binding to polystyrene and PVC plates as opposed to binding the serum ?

VAN DER MAREL : No, we have the impression that because the virus preparations always contain C particles as well as infectious particles, the C particles would preferentially be absorbed so we use an indirect assay to prevent this happening.