International Symposium on Monoclonal Antibodies :
Standardization of their characterization and use, Paris, France, 1983
Develop. biol. Standard. Vol. 57, p. 135-139 (S. Karger, Basel, 1984)

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STRAIN DIFFERENTIATION OF POLIOVIRUSES WITH MONOCLONAL ANTIBODIES

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ABSTRACT

Panels of monoclonal antibodies raised against different poliovirus type 1, 2 and 3 strains, were tested in a micro-neutralization test and in a micro-enzyme linked immunosorbent assay against a large number of poliovirus strains. The results were compared with those obtained with the classical system of serodifferentiation using strain specific cross-absorbed antisera. For this purpose a theoretical pattern fitting computer program was developed, in which each strain could be compared with all the other strains of which the serological data had been stored in the memory of the computer. The results obtained with the panels of monoclonal antibodies coincided well with those obtained with the cross-absorbed antisera. Especially for the identification of virus isolates related to the Sabin vaccine strains, these panels of monoclonal antibodies proved to be valuable tools.

INTRODUCTION

Since the introduction of poliomyelitis vaccination in developed countries, epidemics of poliomyelitis are mainly found in the developing world. In these unvaccinated populations poliomyelitis epidemics are usually caused by poliovirus type 1. However, cases associated with the use of attenuated vaccines are often caused by types 2 or 3 (3). The intratypic strain characterization of virus isolates is of major importance to epidemiological surveillance of poliomyelitis and to studies of vaccine efficacy and safety. For this purpose biochemical analysis of the viral genomes and/or serodifferentiation with cross-absorbed antisera are the methods of choice at the moment (3,5,8). Since the former method is restricted to more specialized laboratories the latter is most widely used. Rabbit antisera cross-absorbed with heterologous virus strains have proved to be valuable tools for serodifferentiation. The use of monoclonal antibodies instead of these strain specific antisera has recently been suggested by different groups (1,3,4,6,7) and it was demonstrated that certain cross-absorbed antisera may be replaced by monoclonal antibodies of the same specificity (7). Other monoclonal antibodies, like, for example those developed against the Sabin type 1 and 2 vaccine strains, proved to be more specific than the cross-absorbed antisera which were routinely used for intratypic serodifferentiation (7). Therefore we decided that broader panels of monoclonal antibodies with different specificities should be used in order to perform a more proper characterization on the basis of an antigenic mapping.

In the present paper we present the results obtained with a panel of monoclonal antibodies raised against different type 2 strains of poliomyelitis virus. They were tested in a micro-ELISA and in a micro-NT and the serological data were compared with these obtained with strain specific cross-absorbed antisera in a theoretical pattern fitting (TPF) computer program.

MATERIAL AND METHODS

Viruses

The 29 different poliomyelitis virus type 2 strains used were classified as Sabin-like (SL), non-Sabin-like (NSL) and intermediate (IM) according to results obtained with cross-absorbed antisera (8). They were selected on the basis of geographical distribution and in fact obtained from laboratories all over the world. They were propagated in Vero cell monolayer cultures. After a complete cytopathic effect had developed the cultures were frozen and thawed twice and the supernatants were used as antigens in ELISA after low speed centrifugation. These preparations were also used in neutralization tests after dilution to 100 $TCID_{50}$ per inoculum.

Serological tests

Micro-neutralization tests were carried out in microtiter plates using 100 $TCID_{50}$ per well, essentially as previously described (8). They were read 5 days after inoculation. Also the micro-ELISA was carried out as previously described (7) with minor modifications : a conventional bovine anti-poliovirus type 2 antiserum was used for coating. The following steps were applied respectively : the virus, the monoclonal antibody, a sheep antimouse Ig coupled to horse radish peroxidase and tetramethylbenzidine in DMSO and H_2O_2. The reaction was stopped by adding H_2SO_4 and the results were read at 450 mn in a micro-ELISA reader. Between all steps the plates were extensively washed with distilled water containing 0.05% Tween 80. For serological testing of the different type 2 strains hybridoma culture supernatants were used.

Production of monoclonal antibodies

A panel of murine hybridomas producing monoclonal antibodies against different poliomyelitis virus type 2 strains was generated essentially in the same way as described for the production of hybridomas specific for the Sabin-vaccine strains (6). Immunizations of Balb/c mice were carried out with 4 different type 2 strains (see Table I) and the initial selection of the hybridomas was performed on the basis of micro-ELISA results. Micro-NT were carried out later.

Theoretical pattern fitting computer program

For the detection of similarities in serological (micro-NT and micro-ELISA) reaction patterns between two virus strains of poliovirus type 1 a theoretical pattern fitting (TPF) computer program was developed (7). The difference obtained with each antibody preparation between reference virus and test virus was quadrated and summarated with the differences obtained with other antibody preparations. This resulted in (non) fitting values (nfv) between virus strains. Based on test results with eight monoclonal antibodies produced during the course of these studies, nfv limits were chosen to classify each of the virus strains tested, into one of the three following categories of fitting with the reference strain used :

− = « non related » ; + = « related » ; + + = « perfect fit ».

RESULTS AND DISCUSSION

Fusions with spleen cells from Balb/c mice immunized with each of the Sabin vaccine strains have resulted in the generation of hybridomas producing neutralizing monoclonal antibodies with different degrees of specificity for the respective Sabin vaccine strains. Three of the anti-Sabin 1 (3D8 ; 4E6 ; 1D4) and one of the anti-Sabin 2 (4-20D10) monoclonal antibodies were finally selected on the basis of an even higher specificity for the respective vaccine strains than the routinely used cross-absorbed antisera. An anti-Sabin 3 (6-10D7) monoclonal antibody recognised the same type 3 Sabin-like strains as the Sabin 3-specific cross-absorbed antiserum. Likewise a monoclonal antibody (7E8) has been produced against a Dutch type 1 isolate from an epidemic in 1978 (78-9030) which on the basis of reactivities with cross-absorbed antisera was classified as non-Sabin-like (NSL), Kuwait-like (KL).

Table Ia. Testing of 29 poliomyelitis virus type 2 strains with 8 anti-type 2 monoclonal antibodies in a micro NT

monoclonal antibody	1	2	3	4	5	6	7	8	TPF (reference Sabin 2)
raised against	77-728	77-728	LS 2188	77-728	81-4789	77-728	Sabin 2	LS 2188	
virus strains									
NSL									
MEF	30	30	30	-	-	-	-	30	-
77-728	10	100	-	10	-	100	-	-	-
77-686	10	30	10	-	-	-	-	30	-
II-316	-	30	30	-	-	-	-	30	-
II-364	-	-	30	-	-	-	-	100	-
6400	30	30	-	-	-	-	-	30	-
EHARA I	-	100	10	-	-	-	-	30	-
LS 1462	30	300	30	-	-	-	-	100	-
LS 2188	100	30	100	-	-	-	-	100	-
81-4789	-	-	-	-	-	10	10	-	+
64-80	10	100	10	-	-	-	-	30	-
LS 1885	30	100	10	-	-	-	-	100	-
IM									
PMH 25714	-	-	10	10	-	100	-	-	+
81-15072	-	-	-	10	-	100	-	-	+
SL									
Sabin 2	-	-	10	10	-	100	300	-	++
120/0/8	-	-	-	10	-	30	300	-	++
188/4/3	-	-	-	10	-	100	-	-	+
154/1/10	-	-	-	10	-	30	300	-	++
72 H 1	-	-	-	-	-	30	-	30	+
73 Hi 3	-	-	-	10	-	30	-	-	+
73 H 6	-	-	-	10	-	30	-	-	+
70-I-3	-	-	-	10	-	100	300	-	++
LJ	-	-	-	10	-	100	-	-	+
P(H)	-	-	-	10	-	100	300	-	++
VS	-	-	-	-	-	100	-	-	+
81 S 18	-	-	-	10	-	100	-	-	+
81 S 521	-	-	-	10	-	100	-	-	+
1498	-	-	-	-	-	30	-	-	+
1863 a	-	-	-	10	-	100	-	-	+

- = <10

The epidemic occurred in a group of people who refused vaccination on religious grounds. Monoclonal antibody 7E8 could replace a specific KL cross-absorbed antiserum since it showed the same pattern of serological reactivities with more than 80 type 1 strains tested. However, since a system of poliovirus strain differentiation based upon the presence of one single epitope - which would be the case when only strain specific monoclonal antibodies were used - may be too selective to study relationships between strains, we introduced a new system. This does not only use the results obtained with strain specific antibody preparations, but also uses the information obtained with monoclonal antibodies reacting with less unique antigenic determinants. In a theoretical pattern fitting computer program each virus strain can be compared with all the other strains of which the serological data have been stored in the memory of the computer. Initially this system was worked out for intratypic characterization of poliovirus type 1 strains. The results were seen to coincide well with those obtained with the classical system (7). At present the system is also adapted for type 2 and type 3 strain characterization, by selecting proper panels of monoclonal antibodies. Table I shows an example of preliminary data obtained in micro-ELISA and micro-NT with a panel of monoclonal antibodies raised against different poliovirus type 2 strains. The last column shows the TPF non fitting values obtained with this panel of eight monoclonal antibodies and a selection of NSL, IM and SL type 2 strains using the Sabin 2 vaccine strain as a ref-

Table Ib. Testing of 29 poliomyelitis virus type 2 strains with 8 anti-type 2 monoclonal antibodies in a micro ELISA

monoclonal antibody	1	2	3	4	5	6	7	8	TPF (reference Sabin 2)
raised against	77-728	77-728	LS 2188	77-728	81-4789	77-728	Sabin 2	LS 2188	
virus strains									
NSL									
MEF	1955	1867	1382	508	405	370	400	1453	-
77-728	1906	1790	1323	1604	575	1438	699	792	-
77-686	1877	1714	1485	492	406	387	415	1503	-
II-316	1894	1757	1424	499	507	354	371	1465	-
II-364	408	1444	1255	462	402	347	370	1334	-
6400	1749	1695	560	477	551	356	375	1382	-
EHARA I	1614	1623	1205	343	452	320	363	1490	-
LS 1462	1787	1756	1390	499	680	325	364	1428	-
LS 2188	1651	1615	1452	459	485	339	342	1402	-
81-4789	426	366	1004	1670	1794	1456	495	890	+
64-80	1836	1733	1338	505	392	370	386	1422	-
LS 1885	1885	1782	1306	505	645	339	379	1412	-
IM									
PMH 25714	1286	644	1196	1680	1086	1592	520	1093	+
81-15072	1312	739	1189	1792	1155	1505	515	891	+
SL									
Sabin 2	395	365	920	1674	1372	1490	1162	462	++
120/0/8	406	365	867	1649	1390	1416	1144	515	++
188/4/3	372	367	890	1790	1330	1442	458	887	+
154/1/10	434	389	836	1806	1060	1395	1150	727	++
72 H 1	435	369	1028	1040	1590	1292	430	1425	+
73 Hi 3	406	383	698	1560	1034	1360	582	788	+
73 H 6	440	357	278	1668	1764	1430	524	853	+
70-I-3	391	340	890	1674	506	1367	1089	732	+
LJ	420	364	857	1669	1344	1432	510	852	+
P(H)	364	361	908	1645	1443	1431	1150	553	++
VS	410	316	871	1694	1341	1435	544	959	+
81 S 18	449	394	874	1694	1352	1160	472	945	+
81 S 521	397	336	728	1441	1152	1318	544	724	+
1498	399	368	837	1633	1435	1420	401	872	+
1863 a	382	337	1121	906	1667	1249	404	1413	++

erence (− = unrelated; + = related; ++ = perfect fit). The value of the new system is clearly demonstrated with this example: no monoclonal antibodies of absolute strain specificity for either SL or NSL strains were used. By combining them in the TPF system, a serodifferentiation could be made which coincided well with the results obtained when the cross-absorbed antisera were used. The only exception was found in the characterization of strain 81-4789, which had been found, « Sabin-related » in this TPF system but had previously been characterized with cross-absorbed antisera as NSL with some difficulty. Although the panel of monoclonal antibodies shown here is certainly not optimal for this purpose, a good correlation was already observed with the classical system which uses a small number of strain specific cross-absorbed antisera. In this way the monoclonal antibodies offer the opportunity to characterize strains not only on a qualitative basis - the presence of specific antigenic determinants - but also on a quantitative basis, by providing information about the presence of less unique antigenic determinants.

For rapid typing of new poliovirus isolates we have developed another ELISA system in which microtiter plates are precoated with anti-type 1, -type 2 and -type 3 monoclonal antibodies in a standardized way, using an anti – mouse Ig preparation as catching antibody. The distribution of the monoclonal antibodies is standardized in such a way that a rapid typing (in type 1, 2 or 3) but also a rough intratypic differentiation (in SL or NSL) may be performed by just reading the

plate. Since the serological data of all the strains tested will be filed in the memory of the computer it will be possible to compare a new strain directly with all the previously tested strains using the TPF computer program. By connecting the ELISA scanner directly to the computer, it will be possible to combine the scanning of the results and the comparison of these results with stored data, to a one command operation, which will run a scan and immediate respond with the best fitting references.

ACKNOWLEDGEMENTS

The authors wish to thank Mr. J. v.d.Lee and Mr. C.H.J. Siebelink for the skilful technical assistance and Ms. I. Huijgen-Regtien for preparing the manuscript.

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