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Monoclonal Antibodies of Four Different Specificities for Neutralization of Type 1 Polioviruses

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Four independent hybridoma clones have been established that produce neutralizing antibodies specific to type 1 poliovirus. Each clone produced antibody which neutralized a distinct set of type 1 test strains: (i) all 15 strains tested; (ii) the inducer strain only; (iii) predominantly wild strains; or (iv) all vaccine-related and some wild strains.

Administration of live poliovirus vaccines to individuals is sometimes accompanied by spread of the vaccine strains to other family and community members (2). Accurate surveillance of this process is complicated by the occasional concurrent presence of wild polioviruses in the community, a problem most frequently encountered with type 1 strains. Thus, differentiation of wild poliovirus from vaccine-related strains is a critical surveillance capability. The most widely used methods of intratypic strain differentiation are serological (9) and, for type 1 polioviruses, are based on the recognition that the Sabin 1 vaccine strain (LSc 2ab) possesses an unusual set of antigens shared by few, if any, wild strains (4, 8). Although this unusual antigenicity is occasionally lost upon replication in the intestine (8), its presence appears to be diagnostic for Sabin 1 (4, 8).

Previous differentiation methods, although generally reliable (8), require substantial experience to establish and interpret (8) or use reagents that are laborious to prepare and maintain (4). The advent of hybridoma technology opened the way for the production of highly specific diagnostic reagents which, once selected, can be readily produced in large quantities. We report here the selection of a hybridoma clone which produces high-titered neutralizing antibody to wild type 1 poliovirus strains but is inactive against Sabin 1 and most Sabin 1-related isolates. Three other clones which produced neutralizing antibodies of differing specificities were also selected. A preliminary description of these findings has been presented (D. D. Humphrey and P. M. Feorino, Abstr. Annu. Meet. Am. Soc. Microbiol. 1981, T62, p. 247).

Type 1 poliovirus strain 3983, an isolate from the spinal cord of a person who died from poliomyelitis, was propagated in Chang liver cell culture monolayers in Eagle medium without calf serum. Culture fluids containing virus were concentrated overnight by pervaporation and pelleted at 100,000 × *g* for 2 h. Pellets were suspended in isotonic salt solution and layered onto 30% glycerol–50% potassium tartrate gradients (7). After centrifugation for 18 h at 35,000 rpm in an SW41 rotor, the visible virus band was collected, dialyzed against phosphate-buffered saline, and frozen at −20°C for subsequent mouse inoculation. The final preparation contained approximately 10^{10} particles per ml when viewed by electron microscopy.

Ten- to twelve-week-old BALB/c mice (Jackson Laboratories, Bar Harbor, Maine) were immunized by the method of Zweig et al. (11). The mice were given a booster inoculation 2 months after the original series of three injections. Spleens were harvested 3 days later and fused with P3-X63-Ag8 mouse myeloma cells (5). Hybridoma culture fluids were tested for antibody by a microneutralization assay (1). Positive cultures were cloned and again tested for neutralizing activity with the poliovirus strains listed in Table 1.

Four hybridomas were obtained that grew well and produced large amounts of neutralizing antibody. One representative clone from each hybridoma was examined for neutralizing activity against 19 poliovirus test strains: 15 strains of type 1 and 2 each of types 2 and 3. The isolates from vaccinated individuals were confirmed to be related to Sabin 1 by oligonucleotide mapping. All other strains were shown to be genetically distinct from each other by the same technique (10).

No neutralizing activity against types 2 and 3 polioviruses was detected (Table 1). All clones produced neutralizing antibodies against the inducer strain. However, the pattern in which the remaining type 1 strains were neutralized differed for the antibody from each clone.

Antibody from clone 3 neutralized all wild type 1 strains tested. In contrast, Sabin 1 was not neutralized, and activity was detected against only one (I-331) of the isolates from

TABLE 1. Neutralizing antibody produced by anti-polio 1 hybridomas[a]

Virus description and origin	Classification[b]	Neutralization titers for antibody source			
		Clone 3	Clone 11	Clone 15	Clone 16
Type 1					
Fatal case, Trinidad 3983[c]	NSL	1,280	160	320	2,560
Mahoney	NSL	2,560	320	<10	1,280
Sabin 1 (LSc 2ab)	SL	<10	1,280	<10	320
4-Day Postvaccine I-193	SL	<10	160	<10	320
28-Day Postvaccine I-331	NSL	80	160	<10	80
2-Day Postvaccine I-220	SL	<10	640	<10	320
21-Day Postvaccine I-366	SL	<10	320	<10	640
2-Day Postvaccine I-219	SL	<10	1,280	<10	320
28-Day Postvaccine I-360	NSL	<10	1,280	<10	320
10-Day Postvaccine I-335	NSL	<10	320	<10	80
Fatal case, New York I-2171	NSL	320	320	<10	40
Paralytic case, Nicaragua I-2191	NSL	320	1,280	<10	20
Paralytic case, Bahamas I-2177	NSL	2,560	1,280	<10	80
Paralytic case, Kuwait I-1117	NSL	2,560	640	<10	<10
Paralytic case, Pennsylvania D-581	NSL	2,560	640	<10	<10
Type 2					
MEF-1	NSL	<10	<10	<10	<10
Sabin 2 (P712 Ch 2ab)	SL	<10	<10	<10	<10
Type 3					
Saukett	NSL	<10	<10	<10	<10
Sabin 3 (Leon $12a_1b$)	SL	<10	<10	<10	<10

[a] All wild strains were shown to be genetically different by oligonucleotide mapping. All postvaccine isolates were derived from Sabin 1.

[b] NSL, Non-Sabin-like by modified Wecker and McBride tests; SL, Sabin-like by modified Wecker and McBride tests.

[c] Inducer strain.

vaccinated individuals. Isolate I-331, taken later after immunization, was known to be antigenically altered (8). Although I-331 is neutralized by clone 3 antibody, the required antibody levels are 4- to 32-fold higher than for the wild strains. Isolates I-360 and I-335 from vaccinated individuals, also believed to have altered antigens (8), were not neutralized by clone 3 antibody. Apparently, these strains are altered at sites different from those modified in I-331.

Clone 11 produced high titers (160 to 1,280) of neutralizing antibody to all type 1 polioviruses tested. Wild-type and vaccine-related isolates were neutralized with approximately equal efficiency. Interestingly, several strains, including Sabin 1, gave reproducibly higher titers with clone 11 than did the inducer strain. It appears that the site neutralized by clone 11 antibody is quite conserved among type 1 strains.

Only the inducer strain was neutralized by antibody from clone 15. Monoclonal antibodies of narrow, inducer strain specificity have been described for other viral systems (3). Other isolates from the Trinidad outbreak were also neutralized by clone 15 antibody (data not shown). Monoclonal antibodies that have narrow strain specificity may be valuable in following the spread of poliovirus strains in human populations.

Most type 1 strains were neutralized by clone 16 antibody, although the titers varied widely. For example, neutralization of Sabin 1 and the Sabin-like isolates from vaccinated individuals required similar antibody titers. Drifted vaccine-related strains I-331 and I-335 gave lower titers with clone 16 antibody. Significantly, Kuwait strain 2171 and Pennsylvania isolate D518 (related genetically, serologically, and epidemiologically to the Netherlands epidemic isolate P78-56) were not neutralized by antibody from clone 16. The antigenic similarity of I-2171 and P78-56 was previously demonstrated by Kapsenberg et al. (4), although the two strains appear to be genetically distinct (10). The site normally neutralized by clone 16-type antibody is apparently absent in these two strains.

The varied specificities of the four monoclonal antibody classes suggest that at least four distinct sites detectable by neutralization are present on the 3983 strain of type 1 poliovirus. The topological relationship and chemical nature of these sites are, at present, unknown.

The total number of different surface antigenic sites through which neutralization of type 1 polioviruses may occur is also unknown, although this may be determined after a larger collection of monoclonal antibodies has been examined.

Of greatest epidemiological interest is the antibody from clone 3, which clearly appears to be of value in differentating type 1 vaccine-related polioviruses from wild strains. Sabin 1 virions are believed to differ from those of the parental Mahoney strain at only 10 to 13 amino acid residues (5). Our laboratory is very interested in identifying the chemical nature of the site of action of clone 3 antibodies. Furthermore, detailed molecular characterization of the potential type-specific antigen neutralized by clone 11 antibody may be useful in developing chemically defined vaccines to type 1 poliovirus.

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LITERATURE CITED

1. **Ballew, H., F. T. Forrester, H. C. Lyerla, W. M. Velleca, and B. R. Bird.** 1977. Microneutralization test for the detection of antibody to enteroviruses, p. 114–120. *In* Laboratory diagnosis of viral diseases. Centers for Disease Control, Atlanta, Ga.
2. **Centers for Disease Control.** 1980. Poliomyelitis surveillance summary 1977–78. Centers for Disease Control, Atlanta, Ga.
3. **Flamand, A., T. J. Wiktor, and H. Koprowski.** 1980. Use of hybridoma monoclonal antibodies in detection of antigenic difference between rabies and rabies-related virus proteins. II. The glycoprotein. J. Gen. Virol. **48**:105–109.
4. **Kapsenberg, J. G., R. A. Coutinho, A. G. Hazendonk, A. B. R. Ran, and A. L. van Wezel.** 1981. Epidemiological implications of the isolations and intratypic serodifferentiation of poliovirus strains in the Netherlands. Dev. Biol. Stand. **47**:293–301.
5. **Kearney, J. F., A. Radbruck, B. Liesgang, and K. Rajewsky.** 1979. A new mouse myeloma cell line that has lost immunoglobulin expression but permits the construction of antibody-secreting hybrid cell lines. J. Immunol. **123**:1548–1550.
6. **Kew, O. M., M. A. Pallansch, D. R. Omilianowski, and R. R. Rueckert.** 1980. Changes in three of the four coat proteins of oral poliovaccine strain derived from type 1 poliovirus. J. Virol. **33**:256–263.
7. **Martin, M. L., and E. L. Palmer.** 1973. Complement-fixing antigens produced by varicella-zoster virus in tissue culture. Appl. Microbiol. **26**:410–413.
8. **Nakano, J. H., and H. M. Gelfand.** 1962. The use of a modified Wecker technique for the serodifferentiation of type 1 poliovirus related and unrelated to Sabin's vaccine stain. I. Standardization and evaluation of the test. Am. J. Hyg. **75**:363–376.
9. **Nakano, J. H., M. H. Hatch, M. L. Thieme, and B. Nottay.** 1978. Parameters for differentiating vaccine-derived and wild poliovirus strains. Prog. Med. Virol. **24**:363–376.
10. **Nottay, B. K., O. M. Kew, M. H. Hatch, J. T. Heyward, and J. F. Obijeski.** 1981. Molecular variation of type 1 vaccine related and wild poliovirus during replication in humans. Virology **107**:405–423.
11. **Zweig, M., C. J. Hellman, Jr., H. Rabin, R. F. Hopkins III, R. H. Neubauer, and B. Hampar.** 1979. Production of monoclonal antibodies against nucleocapsid proteins of herpes simplex virus types 1 and 2. J. Virol. **32**:676–678.