INFECTION AND IMMUNITY, Sept. 1983, p. 1217–1225
0019-9567/83/091217-09$02.00/0

Vol. 41, No. 3

Natural Variation of Poliovirus Neutralization Epitopes

RADU CRAINIC,[1]* PHILIPPE COUILLIN,[2] BRUNO BLONDEL,[1] NICOLE CABAU,[2] ANDRÉ BOUÉ,[2] AND FLORIAN HORODNICEANU[1]

Unité de Virologie Médicale, Institut Pasteur, 75724 Paris Cedex 15,[1] and Unité 73 Institut National de la Santé et de la Recherche Médicale, Château de Longchamp, 75016 Paris,[2] France

Received 18 March 1983/Accepted 10 June 1983

Poliovirus-neutralizing monoclonal antibodies were prepared against type 1, type 2, and type 3 wild laboratory (Mahoney, MEF1, and Saukett) and Sabin vaccine strains. Fifty-five poliovirus laboratory strains and field isolates were assayed by neutralization index test with a panel of homotypic monoclonal antibodies. A total of 27 monoclonal antibodies were used. Two categories of neutralization epitopes were found, i.e., cross-reacting (*K*), which is present on almost all strains of the same serotype, and strain-specific (*V*, variable), either wild (*VW*) or Sabin (*VS*). Several distinct neutralization epitopes were defined for each of the three poliovirus serotypes in almost every category. The study of antigenic variation of the Sabin type 1 vaccine virus during replication in human intestine showed that the *VS* neutralization epitope may be lost and even replaced by the *VW* epitopes of the parental Mahoney virus. A late isolate from a vaccine-fed infant recovered the complete neutralization epitope pattern of the Mahoney strain. Upon in vivo virus replication, a different kind of antigenic variation was also detected in which an epitope lost its function in virus neutralization but kept its antigenic conformation unaltered. Neutralization epitope analysis demonstrated that the presence of *VS* epitopes on a field isolate suggests the Sabin origin of the strain when the isolate displays the same epitope pattern as the original Sabin virus, or confirms it when the *VS* epitope(s) is mutually exclusive of *VW* epitopes. The lack of *VS* epitopes on a field isolate does not rule out its being of Sabin origin.

Poliovirus is a picornavirus composed of a naked, icosahedral particle, an RNA^+ genome, and four structural polypeptides: VP1, VP2, VP3, and VP4 (17).

Poliovirus is highly mutable (4), and its broad antigenic variation reflects its phenotypic variation during replication in the human intestine (19). The major inducement for change is exerted upon the virion surface by the selective pressure of virus-induced antibodies (23).

The ecology of poliovirus, a virus having humans as a unique natural reservoir, became more complex 25 years ago, when the mass administration of live oral polio vaccine was introduced.

Since then, durable efforts have been made for the phenotypic characterization of poliovirus isolates and for the analysis of the antigenic relatedness existing between wild strains and virus excreted by subjects that received live poliovaccine. Using serological methods, it has been possible to distinguish numerous serological subtypes (18, 22, 27, 28). However, because of the polyclonal nature of antibodies used in these studies, it has not been possible to provide unambiguous evidence for the intratypic antigenic relatedness of virus circulating in human communities. Moreover, the antigenic structures of poliovirus responsible for the induction of neutralizing antibodies were, until recently, unknown.

The recent development of hybridoma technology has allowed a re-evaluation of poliovirus antigenic variation through the use of monospecific antibodies capable of recognizing neutralization epitopes present on the surface of virus particles (5, 6, 8–12, 24).

We have developed a panel of neutralizing monoclonal antibodies against the three serological types of Sabin vaccine strains (LSc2ab, P712Ch2ab, and Leon12a1b) and wild laboratory strains Mahoney, MEF1, and Saukett. All of these monoclonal antibodies are type specific. Some, but not all, differentiate wild from Sabin-attenuated strains. The above-mentioned properties show that these monoclonal antibodies identified different epitopes on the surface of poliovirus.

This paper reports studies that had as their aim the analysis of the antigenic variation of poliovirus by identification and listing of neutralization epitopes. We found that, by using a

TABLE 1. Poliovirus strains used

Strains	Origin and characteristics[a]	Source
Type 1		
P1-3, P1-6	Batches of commercially available type 1 Sabin vaccine	A. Chippaux, L.A.S. Paris
A4-A28, C2-C22, E2-E14, F2-F21	Strains serially isolated from Sabin vaccine-fed children (A,C,E,F). Figures stand for days after vaccination (for more details, see Table 4)	O. M. Kew, CDC, Atlanta (22)
Brunenders	Wild laboratory strain	B. Larsson, SBL, Stockholm
1342	Wild laboratory strain	Pasteur Inst. (VM), Paris
14002/77	"Wild" virus	G. C. Schild, NIBSC, London
Type 2		
628, 4389, 6139, 8008, 8071	Isolates of Sabin origin	G. C. Schild, NIBSC, London
77726, 77728, 77686, 77687, 2-215, 2-316, 2-318, 2-364, 2-375	Field isolates from different countries before the introduction of Sabin vaccine strains	D. I. Magrath, NIBSC, London
Type 3		
106, 116, 119, 132, 285, 20278, 5229, 11340, 3-12, 158	Isolates of Sabin origin	G. C. Schild and D. I. Magrath, NIBSC, London (10, 11)
Saukett A, D, G, H, E, Leon ATCC	Wild laboratory strains of different origins	D. I. Magrath, NIBSC, London (21)
3-10, 3-694, 3-1, 3-674, 77692, 3-715	Field isolates from different countries before the introduction of Sabin vaccine, characterized as "wild" (except for strain 3-715)	G. C. Schild, NIBSC, London (10, 11)

[a] Strains were classified as "wild" or "Sabin-like" according to their characterization by T1 oligonucleotide mapping (G. C. Schild, personal communication).

sufficiently large panel of monoclonal antibodies, it was possible to characterize in detail a poliovirus strain by analysis of the epitopes simultaneously present on the surface of infectious particles.

MATERIALS AND METHODS

Cells and media. Details concerning cells and media used in this study have been described elsewhere (5, 6). The mouse myeloma cell line Sp2/O-Ag 14 (26) (kindly provided by G. Buttin) was maintained in Eagle minimum essential medium supplemented with 15% heat-inactivated colt serum. Selective medium was prepared by adding 0.01 mM azaserine and 0.05 mM hypoxanthine to minimal essential medium. For growth of established hybridoma cells, Dulbecco modified medium supplemented with 5 or 10% aseptic calf serum (Medical and Veterinary Supplies, Slough, England) was used.

Viruses. Wild reference type 1 Mahoney (Mah), type 2 MEF1 (MEF), and type 3 Saukett (Sauk) poliovirus strains were kindly provided by A. L. van Wezel (National Institute of Public Health, Bilthoven, Netherlands). Sabin attenuated type 1 LSc2ab (LSc), type 2 P712Ch2ab (P712), and type 3 Leon12a1b (Leon-S) oral poliovirus vaccine strains were sent to us by B. Vacher (Institut Pasteur Production, Marnes la Coquette, France). The origins of other poliovirus laboratory strains and field isolates used in this study are given in Table 1.

Virus suspensions were prepared and titered on HEp-2 cells.

Titration of virus suspensions and the standard virus neutralization test were done by using a micromethod according to the technique already described (20).

Preparation of labeled, purified virions. Virions were labeled with [^{35}S]methionine (7) and purified by isopycnic CsCl gradient centrifugation as previously described (1).

Preparation and characterization of hybridoma cell lines secreting neutralizing monoclonal antibodies. The technique used for the construction of hybridomas has been described in detail in a separate paper (5). Briefly, splenocyte donor mice were immunized with poliovirus suspensions containing about 10^9 50% tissue culture infective doses ($TCID_{50}$) per ml. Each mouse received two injections of 0.2 ml of antigen at a 4-week interval, the first intraperitoneally and the second intravenously. Spleens were harvested 3 to 4 days after the last inoculation. Selection of hybrids was done essentially according to the technique already described (3). Hybridoma cell populations were screened for homologous neutralizing poliovirus antibodies by a microneutralization assay in 96-well microplates. Positive cell colonies were then cloned by limiting dilution. Hybridoma culture fluid was used throughout this study as monoclonal antibody suspension.

Neutralization index (NI) assay. The titer (VT) of the reference virus was determined in parallel in the absence (VT_0) and in the presence (VT_{dA}) of each

TABLE 2. Titers and specificities of poliovirus-neutralizing monoclonal antibodies

Immunizing poliovirus	Monoclonal antibody	Neutralizing titer (log_2)[a]	
		Wild laboratory virus	Sabin virus
Type 1			
Mahoney	Io	9.5	2.5
	Ia	7.5	0
	Ib	8.5	10.5
	Ic	8.0	0
	Id	8.0	3.5
	Ie[b]	8.5	9.0
LSc	1o	2.0	10.5
	1a	0	12.0
	1b	0	10.0
	1c	7.5	6.0
	1d	1.0	6.0
	1e	8.0	10.0
Type 2			
MEF	IIo	9.0	0
	Ia	10.0	4.0
	IIb	10.0	12.5
	IIc	4.5	4.5
P712	2o	0	9.5
	2a	0	8.5
	2b	6.5	10.5
	2c	3.5	8.0
	2d	6.0	2.5
Type 3			
Saukett	IIIo	10.5	0
	IIIa	7.5	9.0
Leon-S	3o	1.0	13.0
	3a	11.5	12.0
	3b	11.0	13.0
	3c	3.0	9.0

[a] Reciprocal of the endpoint dilution (log_2 of hybridoma supernatant that neutralized 50% of one dose of virus (100 $TCID_{50}$).

[b] This monoclonal antibody, named H3 by Emini et al. (8), was kindly sent to us by Eckard Wimmer.

dilution of serially diluted hybridoma supernatant. The NI of each dilution (NI_{dA}) was calculated according to the formula $NI_{dA} = \log VT_0 - \log VT_{dA}$, where dA stands for the dilution of hybridoma supernatant. The NI_{dA}, as calculated above, was taken as the activity of the respective antibody.

When a virus strain was to be characterized according to its antigenic properties, the NI of the virus (determined against a given concentration of a given monoclonal antibody) was taken into consideration. The monoclonal antibody concentration used was the highest dilution of the hybridoma supernatant giving at least 3 NI units ($NI_{dA} > 3$) when tested against the inducing virus.

Immunoprecipitation. Immunoprecipitation was performed as previously described (2, 13). Mouse ascitic fluids (25 μl) were added to [^{35}S]methionine-labeled, purified poliovirions. Immune complexes were precipitated by using *Staphylococcus aureus* (Cowan I strain) and were assayed for radioactivity.

RESULTS

Grouping of monoclonal antibodies. Monoclonal antibodies were raised against wild laboratory or Sabin vaccine polioviruses of types 1, 2, and 3. Hybridoma cell lines were selected by neutralization of the immunizing virus. Their neutralizing activity was strictly type specific (data not shown).

Each hybridoma antibody was tested for the specificity of its neutralizing activity against cloned wild reference and vaccine homotypic polioviruses (Table 2). Accordingly, monoclonal antibodies which neutralized both wild laboratory and Sabin strain were classified as cross-reacting (*K*), and those recognizing only the inducing virus were classified as strain specific (*V*), be it wild (*VW*) or Sabin (*VS*) (Table 3). A finer characterization of monoclonal antibody neutralizing specificities was obtained by testing them against a battery of different poliovirus variants, as will be described below. This analysis allowed a more detailed classification of the antibodies corresponding to the epitope they recognize. This classification was also given in Table 3.

Neutralization index as a simple assay for the differentiation of poliovirus antigenic variants. To study naturally occurring antigenic variation of numerous laboratory strains and field isolates of poliovirus, we investigated whether the NI test

TABLE 3. Grouping of monoclonal antibodies by neutralizing specificities[a]

Antibodies neutralizing poliovirus of:	Antibodies that were:		
	Type specific (*K*)	Strain specific (*V*)	
		Wild (*VW*)	Sabin (*VS*)
Type 1	Ib (*K*)	Io (*VWa*)	1o (*VS*)
	Ie (*K*)	Ia (*VWd*)	1a (*VS*)
	1c (*K*)	Ic (*VWb*)	1b (*VS*)
	1e (*K*)	Id (*VWc*)	1d (*VS*)
Type 2	IIb (*Ka*)	IIo (*VWa*)	2o (*VSb*)
	IIc (*Kb*)	IIa (*VWb*)	2a (*VSc*)
	2b (*Ka*)		2c (*VSb*)
			2d (*VSa*)
Type 3	IIIa (*Kc*)	IIIo (*VWa*)	3o (*Sa*)
	3a (*Ka*)		3c (*Sb*)
	3b (*Kb*)		

[a] Antibodies were grouped on the basis of their capacity to neutralize homotypic (wild-reference and Sabin) viruses, according to results recorded in Table 2. The symbols in parentheses represent the name of the epitope defined by each monoclonal antibody given according to results shown in Fig. 4. In previous publications (5, 6, 9), some monoclonal antibodies directed against poliovirus type 1 were used under a different name: *Io* = CJ27, *Ia* = CJ12, *Ib* = CJ31, *Ic* = H8-4, *Id* = H8-25, *1o* = BA12, *1a* = BL25, *1c* = BM55, and *1d* = BL7.

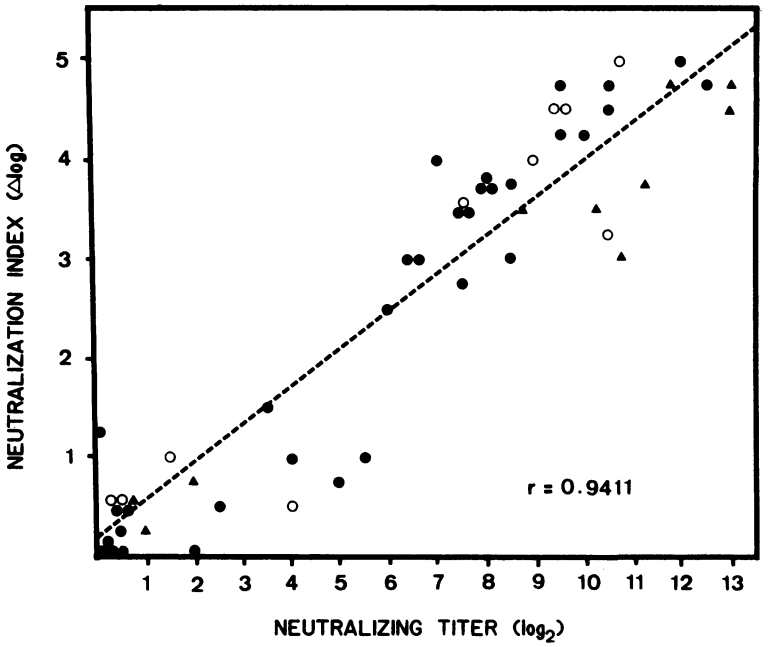

FIG. 1. Correlation between the NI and standard neutralization tests for antigenic characterization of laboratory poliovirus strains. Each virus strain was tested against a set of homotypic monoclonal antibodies by both NI and standard neutralization tests. The NI of a given type 1 (●), type 2 (○), or type 3 (▲) strain was calculated as the difference of the log virus titer (Δlog = NI units) in the absence and in the presence of a constant concentration of hybridoma supernatant. The antibody concentration was chosen to give at least 3 NI units when tested against the inducing viral strain. The neutralizing titer of a hybridoma supernatant was estimated as the reciprocal of the log 2 endpoint dilution that neutralized 50% of a virus dose (100 $TCID_{50}$). The diagonal straight line represents the computed linear regression of the recorded values (r = correlation coefficient).

could give reliable results for neutralization epitope analysis.

Because several authors used mainly the constant virus/variable antibody (standard virus neutralization) test, we performed an experiment in which this method was compared with the NI test, i.e., the difference of the log virus titer in the absence and presence of a constant concentration of antibodies. The results (Fig. 1) indicated that the correlation between the NI test and the endpoint titration of neutralizing capacity of monoclonal antibodies is statistically highly significant for all three poliovirus serotypes.

A well-defined concentration of each monoclonal antibody which optimally differentiated between a pair of reference wild and Sabin homotypic strains had to be established before the test was carried out. Once this concentration was determined, it was used throughout this study. Results of a representative experiment performed for the establishment of optimal dilution to be used in NI tests for six monoclonal antibodies directed against reference wild and Sabin poliovirus strains of all three serotypes are shown in Fig. 2. According to these results, we decided to use in the NI test the dilution of hybridoma supernatant fluid which differentiated homologous from heterologous viruses of at least 3 NI units. Therefore, a strain being neutralized by a certain antibody at more than 2 NI units was considered antigenically homologous. Conversely, strains displaying < 2 NI units (in most cases, no detectable neutralization) were classified as heterologous.

This criterion of the differentiation is in agreement with the statistical findings shown in Fig. 1, where strains were grouped in two distinct clusters, clearly separated by NI = 2.00.

Analysis of neutralization epitopes on natural variants of poliovirus. The possible coexistence of many different epitopes on a single virion was further investigated for various poliovirus strains. For this purpose, poliovirus strains of different origin (listed in Table 1) were tested with a large panel of neutralizing monoclonal antibodies (listed in Table 2). A synthesis of the results of NI tests for each virus strain with every homotypic monoclonal antibody is recorded in Fig. 3. Results can be summarized as follows. (i) Our battery of monoclonal antibodies was able to detect a variable number of distinct

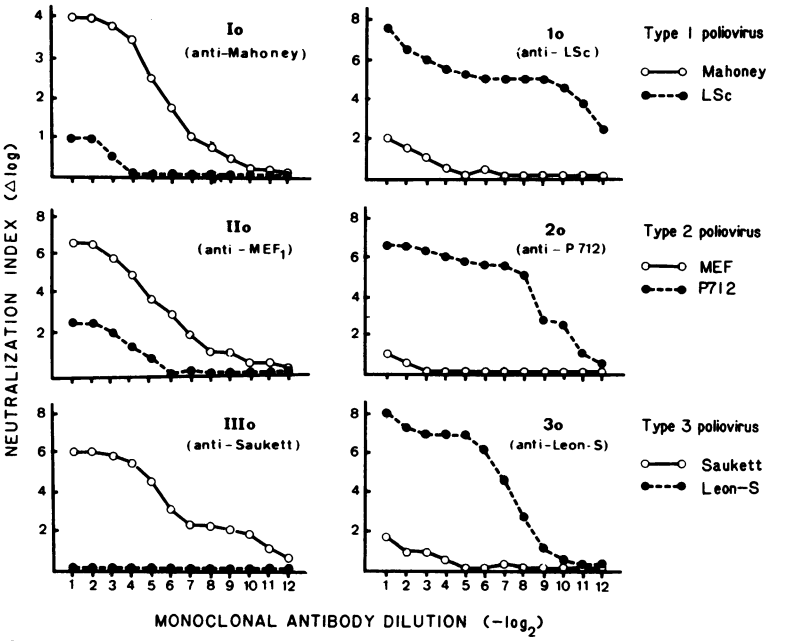

FIG. 2. Specificity of the neutralizing activity of six strain-specific antipolio monoclonal antibodies. Supernatant fluids of hybridoma cultures were tested by NI assay against a pair of homotypic (homologous or heterologous) viruses. The NI were calculated as the difference (Δ) between the log virus titer in the absence and in the presence of each dilution of hybridoma supernatant.

neutralization epitopes simultaneously present on the virion. This number varied from two to five. (ii) All strains tested, irrespective of their serotype, confirmed the existence of two categories of epitopes, i.e., cross-reacting (*K*) and strain specific (*V*). In the case of type 1, an exception was found for 1 strain of 14 tested (strain 14002/77) which lacked the *K* epitope. In the case of type 2 and type 3 viruses, several distinct *K* epitopes were detected: two for type 3, and three for type 2 polioviruses. (iii) There were several distinct *V* epitopes, differently distributed among the various strains tested. (iv) Differences were found in the epitope number and distribution among the three poliovirus types. For type 1 Sabin virus, a unique *VS* epitope was found. In contrast, several distinct *VS* epitopes were found for type 2 and type 3 Sabin viruses (three for type 2 and two for type 3). The Sabin-specific epitope(s) was mutually exclusive of the *VW* epitope(s) in type 1 and type 3 viruses, but not in type 2 strains, where coexistence of *VS* and *VW* epitopes was found in some cases.

Comparison of antigenic characterization of poliovirus isolates with monoclonal and strain-specific polyclonal antibodies. The frequent antigenic drift of Sabin vaccine poliovirus strains upon replication in vaccinees has so far been demonstrated by using strain-specific polyclonal antibodies (22, 27). Poliovirus strains serially isolated from infants at several intervals after oral type 1 vaccine administration were studied with strain-specific neutralizing monoclonal antibodies. The isolates have been previously characterized antigenically by modified Wecker and McBride intratypic serodifferentiation tests with strain-specific polyclonal sera (22). Two homotypic, strain-specific monoclonal antibodies were used for the antigenic analysis of these viruses: one recognizing an epitope (*VS*) specific of the LSc2ab Sabin vaccine strain (*Io* antibody), and another directly against an epitope (*VWa*) specific for wild Mahoney virus (*Io* antibody). The previous antigenic characterization of viruses by intratypic serodifferentiation with polyclonal antibodies was confirmed, with little exception, by an NI test using these monoclonal antibodies (Table 4). All 11 strains previously defined as vaccine-like were found to carry the LSc2ab-specific antigenic determinant. Similarly, the six strains previously identified as non-vaccine-like or intermediate no longer possessed this epitope. Moreover, on the two final isolates (A21 and A28) of the infant 1, which proved to be non-vaccine-like in modified Wecker and McBride tests, the *VWa* Mahoney-specific neutralization epitope was present. When tested with a larger panel of monoclonal antibodies (see Fig. 3), the strain A28 was found to possess all four Mahoney neutralization epitopes (*VWa*, *VWb*, *VWc*, and *VWd*), and two of them (*VWb*

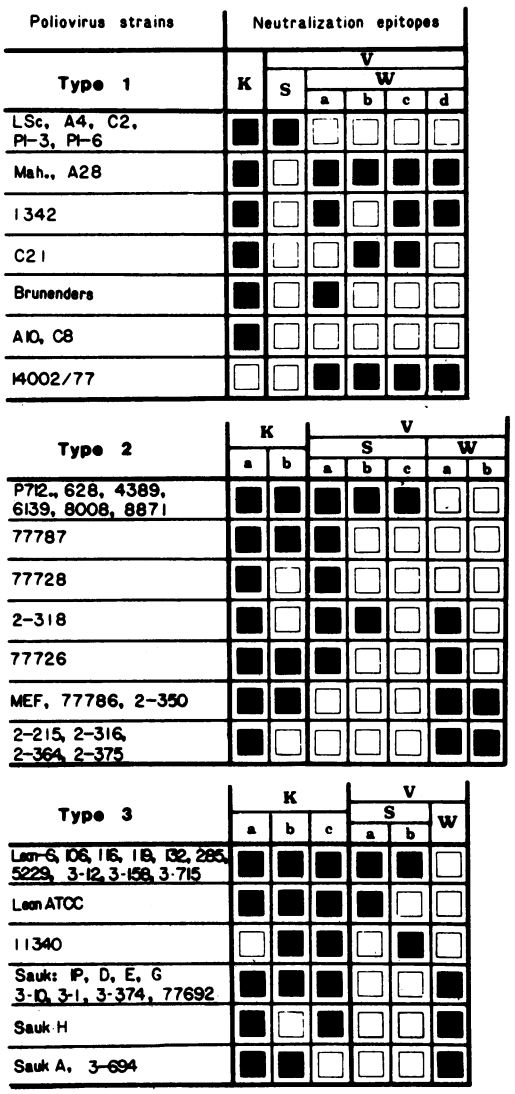

FIG. 3. Analysis of neutralization epitopes on natural variants of poliovirus by NI with monoclonal antibodies. NI was determined for each virus-antibody combination as described in the text. Each viral strain was tested with every homotypic monoclonal antibodies listed in Table 3. Reactions were considered positive (■) for NI > 2.00 and negative (□) for NI < 2.00 (in most cases there was no detectable neutralization). Results were grouped together for monoclonal antibodies that behaved similarly for all viruses tested, thus defining the same neutralization epitope (see Table 3). Poliovirus strains showing similar neutralization patterns with every category of monoclonal antibodies were grouped together.

and *VWc*) were found on isolate C21 of infant 3.

Neutralization versus non-neutralization epitope. The facility with which a poliovirus strain can change its neutralization epitopes during in vivo passages (see Table 4) prompted us to examine whether an epitope could lose its function in virus neutralization while still physically persisting on the viral surface.

To test for this assumption, some type 1 strains serially isolated from the vaccinees were examined for their reactivity with a panel of monoclonal antibodies by NI test. Strains negative in neutralization were tested in immunoprecipitation of radiolabeled particles with the respective monoclonal antibodies. The results (Fig. 4) showed that some epitopes conserved their configuration even if they lost their role in virus neutralization. For instance, monoclonal antibodies that specifically neutralize Sabin virus (*VS*) bound to Mahoney but did not neutralize it. Inversely, on the LSc2ab virus, a Mahoney antigenic determinant (*VWd*) was found that played no role in vaccine virus neutralization. Meaningful results were those obtained with isolates at various intervals after LSc2ab vaccine administration. The virus excreted early after vaccine administration (strains A4 and C2) displayed the same epitope pattern as the original Sabin vaccine strain. At 8 and 10 days after vaccine administration, the Sabin epitope was still present but was no longer critical for neutralization. Similarly, Mahoney-specific antigenic determinants were also present, but, here again, not as neutralization epitopes. After 3 to 4 weeks of virus excretion, some (strain C21) or all (strain A28) of the Mahoney epitopes became critical for virus neutralization. Epitope distribution and function on the A28 strain were identical to those on Mahoney virus, the strain which originally served for production of the attenuated LSc2ab Sabin virus (25).

DISCUSSION

Our previous (5, 6, 9) and present results, as well as the observations of other authors (8, 10–12, 24) showed that poliovirus-neutralizing monoclonal antibodies can differentiate subtypes among strains belonging to the same serotype. This finding opens a new approach to the study of the natural variation of poliovirus upon replication in human intestine.

Here we have focused our attention on the identification and itemization of different neutralization epitopes simultaneously present on the poliovirion. This is of particular interest for the study of the modifications of neutralization patterns of Sabin-derived strains after their passage in humans.

By testing poliovirus strains of various origin with our panel of neutralizing monoclonal antibodies (Fig. 3), two categories of neutralization epitopes were identified, i.e., cross-reactive (*K*) and strain specific (*V*). The cross-reacting epitopes were type specific and were found to be

TABLE 4. Intratypic differentiation of type 1 poliovirus strains serially isolated from vaccine-fed infants by neutralization index with strain-specific monoclonal antibodies

Source of type 1 poliovirus strains	Days after vaccine administration	Label	Antigenic character[a]	NI with monoclonal antibodies[b]	
				anti-LSc (*Io*) (*VS*)	anti-Mah (*Io*) (*VWa*)
LSc2ab			Reference vaccine	4.50	0.50
Mahoney			Reference wild	0	4.75
Infant 1	4	A4	Vaccine-like	3.00	0.75
	6	A6	Vaccine-like	4.25	0.25
	8	A8	Vaccine-like	3.75	0.25
	10	A10	Non-vaccine-like	0.50	0.75
	14	A14	Non-vaccine-like	0.50	0
	21	A21	Non-vaccine-like	0.50	3.50
	28	A28	Non-vaccine-like	0	3.00
Infant 3	2	C2	Vaccine-like	3.50	0
	4	C4	Vaccine-like	4.50	1.75
	6	C6	Vaccine-like	3.50	0
	8	C8	Intermediate	0	0
	14	C14	Non-vaccine-like	0	0
	21	C21	Non-vaccine-like	0	1.00
Infant 6	2	E2	Vaccine-like	3.00	0
	14	E14	Vaccine-like	3.00	0
Infant 7	2	F2	Vaccine-like	4.25	0
	21	F21	Vaccine-like	3.00	0

[a] Viral isolates were previously classified according to modified Wecker and McBride serodifferentiation test results with strain-specific antisera (22). The infant numbers correspond to those used in the above-mentioned publication.

[b] NI was calculated as described in the text. Abbreviations *Io*, anti-LSc2ab monoclonal antibodies recognizing the *VS* epitope; *Io*, anti-Mahoney monoclonal antibodies recognizing the *VWa* epitope (mean values of two or three individual experiments).

conserved on almost all viruses of the same serotype. For that reason, we call this category *K* (constant) epitopes. A different number of distinct *K* epitopes was found for each of the three poliovirus serotypes: one for type 1 (*K*), two for type 2 (type 2 *Ka* and *Kb*), and three for type 3 (type 3 *Ka*, *Kb*, and *Kc*).

The strain-specific, variable (*V*) epitopes allowed us to distinguish between Sabin vaccine strains and wild viruses. Differences were found in the *V* neutralization pattern in the three poliovirus serotypes. For instance, using four different monoclonal antibodies, we detected only one type 1 Sabin-specific (*VS*) neutralization epitope on the LSc2ab virus. The type 1 *VS* epitope was mutually exclusive of the four *VW* epitopes detected on the wild Mahoney strain. In contrast, Sabin type 2 virus has at least three distinct strain-specific neutralization epitopes, and type 3 has two. In some type 2 and type 3 vaccine-derived strains, the Sabin epitope(s) coexisted with one or two wild epitopes.

In the case of type 1 poliovirus, four (reference 12) up to seven (reference 9) distinct neutralization epitopes have been described previously. Our present observations confirmed the multitude of distinct neutralization epitopes on each of the three poliovirus serotypes.

With our battery of neutralizing monoclonal antibodies, a maximum of five neutralization epitopes simultaneously present on a virion were detected. The topological relationship of these epitopes remains unknown until competition studies, under way in our laboratory, are carried out.

It thus seemed worthwhile to try to reveal the fate of different neutralization epitopes during human passages of poliovirus and, particularly, of those epitopes specific for Sabin vaccine strains. Our results (Fig. 4; Table 4), in agreement with those previously obtained by intratypic sero-differentiation with polyclonal antibodies (22), demonstrated that the type 1 *VS* neutralization epitope may be lost during multiplication of the Sabin virus in human intestine (isolates from children 1 and 3 in Table 4). This fact has a direct implication in the determination of the origin of a type 1 field isolate. For type 1 virus, the situation might be complicated by the reappearance of the parental Mahoney neutralization epitope(s) during virus multiplication in humans.

In the understanding of poliovirus antigenic variation during multiplication in human intestine, the most pertinent finding was that the same epitope can have alternate functions in the neutralization of virus infectivity. By performing neutralization and immunoprecipitation in paral-

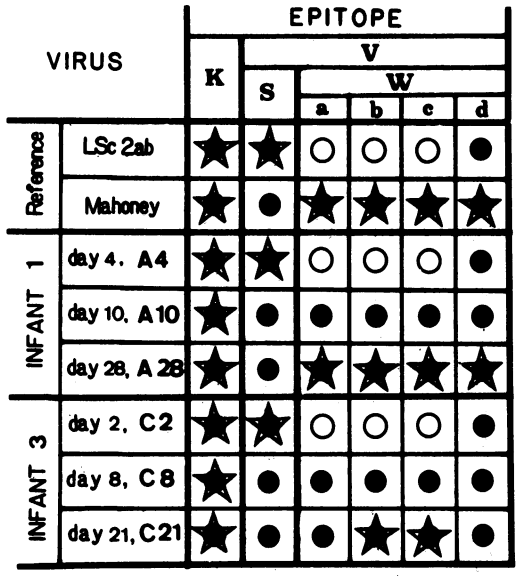

FIG. 4. Modification of epitope distribution and function upon virus passage in humans. Type 1 polioviruses serially isolated from two vaccine-fed infants (strains A and C in Table 4) were tested in neutralization (NI test) and immunoprecipitation (see the text) with a panel of six different monoclonal antibodies. Antibodies were chosen to include every epitope, as defined in Fig. 3, i.e., *Ic* as *K*; *Io* as *VS*; and *I*, *Ic*, *Id*, and *Ia* as *VWa*, *VWb*, *VWc*, and *VWd*, respectively. The two inducing strains used for raising monoclonal antibodies (wild Mahoney and attenuated LSc2ab) were also included in the test as reference viruses. The presence of an epitope (●) was characterized by an immunoprecipitated fraction of >20% (usually >60%). When an epitope was absent (○), 0 to 2% of the radioactivity was precipitated. No values intermediate between 2 and 20% were found. Neutralization epitopes (stars) were defined as described above (see the text), i.e., by NI > 2.00 with the respective monoclonal antibody.

lel (see Fig. 4), we demonstrated that, due to mutation, an epitope can lose its function in viral neutralization without modifying its antigenic specificity. For instance, a surprising result was the detection on Mahoney virus of a Sabin epitope in a non-neutralization form, and, conversely, on the LSc2ab strain the presence of a Mahoney-specific epitope which was no longer critical for neutralization of the Sabin virus. A more comprehensive picture of epitope variation was obtained by analyzing some of the strains serially isolated from vaccine-fed children (see Fig. 4), using immunoprecipitation to test for the presence of an epitope and the NI test to examine its function in virus neutralization. Based on these results, we concluded that a mutation can affect a neutralization site in two ways: either by modifying its antigenic configuration or by negating only its function in virus neutralization. It seems, then, that during selection of the type 1 LSc2ab virus from its wild parent Mahoney (25), some neutralization epitopes (*Wa*, *Wb*, and *Wc* in Fig. 4) were lost by conformational alteration. Another epitope, the *Wd*, retained its conformation but lost its function in virus neutralization. Conversely, upon replication in humans, the LSc2ab virus recovered in some instances the lost Mahoney epitopes, first in conformational terms and, finally, in functional terms.

Despite the significant progress made in the study of the mechanism of virus neutralization (9, 16), no explanation is available for the mechanism by which a viral epitope can conserve its antigenic configuration while losing its function in neutralization. Concerning the difference between a neutralization and a non-neutralization epitope, the only available observation is that the reaction of a neutralization epitope with the corresponding antibody alters the viral isoelectric point from 7.0 to 4.5, whereas a non-neutralizing antibody does not induce such a modification (9, 15).

One of the main problems of epidemiological survey of poliomyelitis is to determine the vaccine or nonvaccine origin of poliovirus isolates (29). Several recently published papers have shown that some of the best methods for identification of the genetic origin of poliovirus isolates are oligonucleotide mapping of poliovirus RNA (14, 21, 23, 30) and antigenic characterization by monoclonal antibodies (5, 7, 10–12, 24).

The results presented here show that for the characterization of poliovirus isolates by neutralizing monoclonal antibodies, some limitations must be taken into consideration, mainly due to the instability of neutralization epitopes during intestinal replication of the virus. This high degree of variability is well illustrated by the complete conversion of the epitope pattern of the LSc2ab strain to that of its parental wild Mahoney virus (see Fig. 4). Consequently, the Sabin origin of a viral isolate cannot be ruled out only by the absence of Sabin epitope(s). On the contrary, the presence of the Sabin-specific antigenic determinant(s) would suggest—but not confirm—the Sabin origin of a poliovirus strain. The unique *VS* epitope that we found for type 1 poliovirus was mutually exclusive of wild Mahoney epitopes. In this particular case, when the Sabin-specific epitope was present on an isolate, the virus could be considered Sabin-derived. The same could be said for the *VSc* epitope of type 2 virus. The situation is complicated in the case of other type 2 and the type 3 polioviruses by the possible coexistence, in various combinations, of Sabin and wild-type epitopes.

An important step in the understanding of the biological significance of poliovirus antigenic variation during its natural replication would be

to ascertain whether a correlation exists between neurovirulence and the presence or absence on the virus surface of certain epitopes with or without function in viral neutralization.

ACKNOWLEDGMENTS

This work was supported by Institut National de la Santé et de la Recherche Médicale (INSERM) grants PRC 124010 and CRL 818004, by a grant from La Fondation pour la Recherche Médicale to F.H., and by INSERM CRL contract no. 818007 to A.B. and N.C.

We wish to express our gratitude to Tadeusz J. Wiktor for helpful discussion and to Susan Michelson for critical reading of the manuscript. The skillful technical assistance of Gabrielle Dufraisse, Marie-Claude Grisard, Chantal Duros, and Monique Coulon is gratefully acknowledged. We wish to thank Nicole Perrin for her expert secretarial assistance.

LITERATURE CITED

1. **Blondel, B., O. Akacem, R. Crainic, P. Coullin, and F. Horodniceanu.** 1983. Detection by monoclonal antibodies of an antigenic determinant critical for poliovirus neutralization present on VP1 and on heat-inactivated virions. Virology **126**:707–710.
2. **Blondel, B., R. Crainic, O. Akacem, P. Bruneau, M. Girard, and F. Horodniceanu.** 1982. Evidence for common, intertypic antigenic determinants on poliovirus capsid polypeptides. Virology **123**:461–463.
3. **Buttin, G., G. Leguern, L. Phalente, E. C. C. Lin, L. Medrano, and P. A. Cazenave.** 1978. Production of hybrid lines secreting monoclonal anti-idiotypic antibodies by cell fusion on membrane filters. Curr. Top. Microbiol. Immunol. **81**:27–36.
4. **Cooper, P. D.** 1969. The genetic analysis of poliovirus, p. 177–217. *In* H. B. Levy (ed.), The biochemistry of viruses. Marcel Dekker, Inc., New York.
5. **Couillin, P., R. Crainic, N. Cabau, F. Horodniceanu, and A. Boué.** 1982. Strain-specific type 1 poliovirus neutralizing monoclonal antibodies. Ann. Virol. **133E**:315–323.
6. **Crainic, R., P. Couillin, N. Cabau, A. Boué, and F. Horodniceanu.** 1982. Determination of type 1 poliovirus subtype classes with neutralizing monoclonal antibodies. Dev. Biol. Stand. **50**:229–234.
7. **Crainic, R., F. Horodniceanu, and M. Barme.** 1981. Quantitation of cell DNA in the evaluation of heteroploid cells as substrate for the preparation of killed viral vaccines. Intervirology **14**:300–309.
8. **Emini, E. A., B. A. Jameson, A. J. Lewis, G. R. Larsen, and E. Wimmer.** 1982. Poliovirus neutralization epitopes: analysis and localization with neutralizing monoclonal antibodies. J. Virol. **43**:997–1005.
9. **Emini, E. A., S.-Y. Kao, A. J. Lewis, R. Crainic, and E. Wimmer.** 1983. Functional basis of poliovirus neutralization determined with monospecific neutralizing antibodies. J. Virol. **46**:466–474.
10. **Ferguson, M., Y.-H. Qi, P. D. Minor, D. I. Magrath, M. Spitz, and G. C. Schild.** 1982. Monoclonal antibodies specific for the Sabin vaccine strain of poliovirus 3. Lancet **ii**:122–124.
11. **Ferguson, M., G. C. Schild, P. D. Minor, P. J. Yates, and M. Spitz.** 1981. A hybridoma cell line secreting antibody to poliovirus type 3 D antigen: detection in virus harvest of two D antigen populations. J. Gen. Virol. **54**:437–442.
12. **Humphrey, D. D., O. M. Kew, and P. M. Feorino.** 1982. Monoclonal antibodies of four different specificities for neutralization of type 1 polioviruses. Infect. Immun. **36**:841–843.
13. **Kessler, S. W.** 1975. Rapid isolation of antigens from cell with a staphylococcal protein A-antibody adsorbent: parameters of the interaction of antibody-antigen complexes with protein A. J. Immunol. **115**:1617–1624.
14. **Kew, O. M., B. K. Nottay, M. H. Hatch, J. H. Nakano, and J. F. Obijeski.** 1981. Multiple genetic changes can occur in the oral polio vaccines upon replication in humans. J. Gen. Virol. **56**:337–347.
15. **Mandel, B.** 1971. Characterization of type 1 poliovirus by electrophoretic analysis. Virology **44**:554–568.
16. **Mandel, B.** 1979. Interaction of viruses with neutralizing antibodies, p. 37–121. *In* H. Fraenkel-Conrat and R. R. Wagner (ed.), Comprehensive virology, vol. 15. Plenum Publishing Corp., New York.
17. **Matthews, R. E. F.** 1982. Fourth report of the International Committee on Taxonomy of Viruses. Classification and Nomenclature of Viruses. Intervirology **17**:129–132.
18. **McBride, W. D.** 1959. Antigenic analysis of poliovirus by kinetic studies of serum neutralization. Virology **7**:45–58.
19. **Melnick, J. L.** 1961. Attenuated poliovirus vaccine: virus stability, p. 384–402. *In* Poliomyelitis, Fifth International Poliomyelitis Conference, Copenhagen. Lippincott, Philadelphia, Pa.
20. **Melnick, J. L., H. A. Wenner, and C. A. Phillips.** 1979. Enteroviruses, p. 471–534. *In* E. H. Lennette and N. J. Schmidt (ed.), Diagnostic procedures for viral, rickettsial and chlamydial infections, 5th ed. American Public Health Association, Inc., Washington, D.C.
21. **Minor, P. D., G. C. Schild, M. Ferguson, A. Mackay, D. I. Magrath, A. John, P. J. Yates, and M. Spitz.** 1982. Genetic and antigenic variation in type 3 polioviruses: characterization of strains by monoclonal antibodies and T1 oligonucleotide mapping. J. Gen. Virol. **61**:167–176.
22. **Nakano, J. H., H. M. Gelfand, and J. T. Cole.** 1963. The use of a modified Wecker technique for serodifferentiation of type 1 polioviruses related and unrelated to Sabin's vaccine strain. II. Antigenic segregation of isolates from specimens collected in field studies. Am. J. Hyg. **78**:214–226.
23. **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 polioviruses during replication in humans. Virology **108**:405–423.
24. **Osterhaus, A. D. M. E., A. L. van Wezel, G. van Steenis, A. G. Hazendonk, and G. Drost.** 1981. Production and potential use of monoclonal antibodies against polioviruses. Dev. Biol. Stand. **50**:221–228.
25. **Sabin, A. B., and L. R. Boulger.** 1973. History of Sabin attenuated poliovirus oral live vaccine strains. J. Biol. Stand. **1**:115–118.
26. **Shulman, M., C. D. Wilde, and G. Köhler.** 1980. A better cell line for making hybridomas cell lines. Nature (London) **276**:269–270.
27. **Van Wezel, A. L., and A. G. Hazendonk.** 1979. Intratypic serodifferentiation of poliomyelitis virus strains by specific antisera. Intervirology **11**:2–8.
28. **Wenner, H. A., I. Archetti, and G. R. Dubes.** 1959. Antigenic variation among type I polioviruses. A study of 16 wild-type strains and 5 variants. Am. J. Hyg. **70**:66–90.
29. **World Health Organization.** 1981. Markers of poliovirus strain isolated from cases temporally associated with the use of live poliovirus vaccine: report on a W.H.O. collaborative study. J. Biol. Stand. **9**:163–184.
30. **Yoneyama, T., A. Hagiwara, M. Hara, and H. Shimojo.** 1982. Alteration of oligonucleotide fingerprint patterns of the viral genome in poliovirus type 2 isolated from paralytic patients. Infect. Immun. **37**:46–53.