Application of monoclonal antibody panels in the virological and epidemiological review of poliomyelitis in Poland, 1981–1990

Z. Jarzabek,[1] J. Zabicka,[2] A. John,[3] J. Howlett,[3] G. Dunn,[3] & D.J. Wood[3]

Monoclonal antibody panels developed to differentiate vaccine-derived and wild-type strains of polioviruses were applied to isolates from cases of paralytic poliomyelitis, non-paralytic poliomyelitis, and healthy excreters of poliovirus from Poland. All isolates from poliomyelitis cases were shown to be vaccine-derived, as were most other strains. However, two strains associated with meningitis had wild-type antigenic phenotypes and, as shown by partial genomic sequencing, wild-type genotypes. Correlation of laboratory and epidemiological data suggested that residual cases of paralytic poliomyelitis in Poland between 1981 and 1990 were vaccine-related. Study of the non-paralytic cases, however, helped identify the circulation of endemic wild-type viruses in a well-vaccinated community.

Introduction

In 1988 the World Health Assembly adopted the goal of global eradication of poliomyelitis by the year 2000 (*1*). Four stages in the progress of a country towards eradication have been described (*2*). Once countries have attained a stage where they are presumed to be polio-free (i.e., they have a reliable reporting system for cases of polio, have reported no indigenous cases of poliomyelitis for the last 3 years, and have achieved polio immunization coverage of 80% or greater), concerted efforts must be made to track every potential case of poliomyelitis related to the wild poliovirus. Laboratory support for countries in this situation should include, for every case, type identification and intratypic characterization of strains, including relation to vaccine strains and evidence of origin (local or imported) of wild strains (*3*).

Poland has had a low incidence of paralytic poliomyelitis (0–4 cases per year) from 1979 through 1990. Epidemiological and virological analysis of cases up to 1982 suggested that most paralytic poliomyelitis cases (PPC) were vaccine-associated (*4*). Virological analysis of isolates was based on McBride intratypic differentiation tests (*5*). Newer methods for intratypic characterization of strains are now available and include cross-adsorbed strain-specific hyperimmune sera (*6*), panels of monoclonal antibodies (*7*), and genetic methods such as primer extension sequencing of selected genomic regions (*8*).

Epidemiological and virological review of the paralytic poliomyelitis cases that occurred in Poland between 1981 and 1990 was initiated to substantiate the view that residual poliomyelitis was vaccine-associated. Intratypic characterization of isolates was carried out with monoclonal antibody panels. Confirmation of selected results from the monoclonal antibody analysis was provided by partial genomic sequencing.

Materials and methods

Disease surveillance

Poliomyelitis is a notifiable disease in Poland. A case is defined as any patient with paralysis typical of poliomyelitis that persists for more than 6 weeks. For each case demographic data, vaccination status, contacts with vaccinees and other epidemiological details were collected by the Department of Epidemiology, National Institute of Hygiene (NIH), Warsaw. Until 1984 cases of meningitis and/or encephalitis in which poliovirus was isolated were also reported.

Diagnostic procedures

Primary virus isolation from faeces or cerebrospinal fluid and neutralizing antibody tests were performed

[1] Department of Virology, National Institute of Hygiene, Warsaw, Poland.

[2] Department of Epidemiology, National Institute of Hygiene, Warsaw, Poland.

[3] Virology Division, National Institute for Biological Standards and Control, Blanche Lane, South Mimms, Potters Bar, Herts EN6 3QG, England. Requests for reprints should be sent to Dr D.J. Wood at this address.

Reprint No. **5283**

in peripheral laboratories. Diagnostic results were confirmed by the Department of Virology, NIH, Warsaw.

Specimens were inoculated into either *Cercopithecus aethiops* primary monkey kidney cells or GMK cells in tube cultures. If no cytopathic effect developed, two or three blind passes were made before a negative result was recorded. Virus isolates were identified by neutralization tests in primary monkey kidney cells and neutralizing antibody titres in paired sera were determined against 100 $TCID_{50}$ virus challenge (*9*). A fourfold or greater rise in titre was considered significant.

Epidemiological classification

Epidemiological and virological data were collated and reviewed by a panel of experts at NIH, Warsaw. Cases were classified according to WHO criteria (*10*) as vaccine recipient, vaccine contact/possible contact, or no known vaccine contact.

Intratypic characterization with monoclonal antibody panels

Selected virus isolates from paralytic cases together with some strains from non-paralytic cases and healthy excreters were further characterized with monoclonal antibody panels at the National Institute for Biological Standards and Control, Potters Bar, England. Three panels, or for each serotype, were used. Each panel included six monoclonal antibodies. Strains were characterized after one additional passage in Hep2C cells.

Each isolate was tested against the panel appropriate to its serotype in a microneutralization assay.[a] Approximately 100 $TCID_{50}$ of virus was incubated with serial twofold dilutions of monoclonal antibody at 35°C for 3 hours after which Hep2C cells were added. Microtitration plates were sealed and incubated at 35°C for 7 days. The highest antibody dilution giving complete inhibition of viral cytopathic effect was considered as the end-point titre. A titre of 1:20 or greater was regarded as significant reaction between virus and antibody.

Nucleotide sequence analysis

Viruses propagated in Hep2C cells were purified in sucrose gradients and the partial sequence of the phenol-extract RNA was determined by the dideoxy primer extension method, as described previously (*11*, *12*).

[a] *Manual for the virological investigation of poliomyelitis.* Unpublished document, WHO/EPI/CDS/Polio/90.1, 1990.

Results

Epidemiological features

In the ten-year period 1981–90, 21 cases of paralytic poliomyelitis occurred, ranging from 0 to 4 cases per year. On epidemiological grounds 18 cases were classified as vaccine-related; 5 occurred in recent recipients of trivalent oral poliomyelitis vaccine (TOPV) (range, 11–34 days between vaccine administration and onset of illness) and 13 occurred in contacts or possible contacts of vaccinees (range, 12–36 days between contact and onset). A further three non-epidemiologically linked cases occurred in patients with no known contact with vaccinees (Table 1). A 34-day incubation period to onset of paralysis after the 4th dose of TOPV was seen in one recipient case subsequently shown to be immunodeficient. Paralysis is well documented to occur in immunodeficient patients after multiple doses of TOPV and outside the usual 30-day maximum incubation period (*10*). TOPV is also known to fail to protect a disproportionately large number of immunodeficient cases (*10*), which may explain case 10 who was classified as a contact case despite four previous immunizations.

The age distribution of recipient cases was 3–20 months and 12/13 contact cases occurred in children under three years of age (Table 1). The presumed source of contact in 11/12 of these cases was with other children in nurseries or during hospital admissions for unrelated, mainly acute respiratory tract illness. Only 2/12 children had been previously vaccinated. The remaining contact case occurred in a 25-year-old man, the parent of a recently vaccinated infant. In 2/3 cases with no known vaccine contact, the patient was unvaccinated and less than 3 years of age (Table 1). The remaining patient was a two-year-old boy who had received four doses of TOPV. Laboratory confirmation in this case was a fourfold rise in antibody titre.

Risk of vaccine-associated disease

The birth cohort during the study period was in the range of 600 000 to 700 000 per year with a total number of 6.4 million births. The acceptance rate for 3 doses of TOPV in the first year of life was 95% and 4 recipient cases occurred in children under 1 year. The risk of vaccine-associated disease in children who received primary vaccination was therefore 0.66 per million, which is an improvement on the 1.57 per million risk seen between 1970 and 1979 (*13*). A higher rate of vaccine-associated disease was seen in contacts or possible contacts of vaccinees since there were 1.25 cases per million children under 1 year.

Table 1: **Epidemiological and virological features of patients with paralytic poliomyelitis**

Epidemiological characterization and case number[a]	Sex/age at onset	Year of onset	No. of OPV doses	Interval (days)[b]	Virus culture				Serology[e]				Laboratory confirmation[f]
					Specimens[c] collected (days from onset)	Virus type	Intratypic characterization[d]		I	II	III	Days from onset	
							Mab	Seq					
Vaccine-associated: recipient													
1	F/20 m	1982	4	34	F (7,9)	Neg	—[g]	—	16/16	8/8	32/32	7/28	No
2	M/6 m	1984	1	11	F (20)	3	3S	—	8/16	128/128	64/128	20/34	V3
3	M/7 m	1985	2	11	F, CSF (8)	Neg	—	—	<4/32	<4/4	<4/8	8/34	S1,2,3
4	M/3 m	1987	1	20	F (2)	2	2S	—	<16/<16	32/256	256/512	2/20	V,2,3
					TS (2)	3	3S	—					S2
5	M/4 m	1988	2	14	F (10)	Neg	—	—	128/128	32/128	4/128	10/25	S2,3
Vaccine-associated: possible contact													
6	M/25 y	1982	?	29	F (8)	2	2S	—	—	12/48	—	8/22	V2,S2
7	F/18 m	1982	0	18	F (4)	2	—	—	<4/<4	32/128	<4/<4	4/30	V2,S2
8	M/3 y	1982	4	?	F (19)	3	3S	3S	32/32	32/64	8/8	16/33	V3
9	M/10 m	1983	0	12	F (21)	2	—	—	512/384	128/64	<4/<4	21/39	V2
10	M/23 m	1983	4	18	F (6)	2	2S	—	<4/4	<4/4	<4/8	4/26	V2,S1,2,3
11	F/4 m	1984	0	21	F (7)	Neg	—	—	4/4	4/4	64/256	9/34	S3
12	M/4 m	1985	0	12	F (4)	Neg	—	—	8/8	16/16	64/256	4/20	S3
13	M/11 m	1985	0	16	F (12,14,18)	Neg	—	—	64/64	128/128	128/64	12/31	No
14	M/11 m	1986	0	20	F (30)	2	2S	—	—	64/256	—	30/46	V2,S2
15	M/6 m	1988	0	30	F (36)	2	2S	—	<4/<4	256/1024	<4/<4	36/56	V2,S2
16	M/20 m	1988	0	?	F (1)	2	2S	2S	<4/<4	32/64	<4/<4	30/50	V2
17	M/10 m	1988	0	36	F (10)	Neg	—	—	<4/<4	256/1024	<4/<4	12/39	S2
18	M/2 m	1990	0	19	F (1)	Neg	—	—	<4/<4	<4/<4	128/512	15/29	S3
Source unknown													
19	M/14 m	1981	0	?	F (6)	2	2S	—	<4/<4	1024/1024	<4/<4	6/20	V2
20	M/28 m	1986	4	?	F (11)	Neg	—	—	128/128	64/64	128/512	7/17	S3
21	M/5 m	1987	0	?	F (4)	Neg	—	—	<16/<16	256/1024	<16/<16	4/42	S2

[a] Cases 1, 2, 3 and 10 were shown to have immunodeficiencies; patient 3 died.
[b] From last dose of OPV and onset of paralysis (recipient cases); from first date of possible contact and onset of paralysis in the case (contact cases).
[c] F, faeces; CSF, cerebrospinal fluid; TS, throat swab.
[d] Mab = monoclonal antibody panel. Seq = partial genomic sequencing.
[e] Neutralizing antibody; ≥4-fold rise in titre was significant.
[f] V, by virus isolation; S, by serology.
[g] — = not done.

Diagnostic investigations

Faecal samples were obtained from all patients with paralysis and virus was isolated in 11/21 cases. Unsually, positive cultures were only made from 6/16 (37%) stools collected less than 14 days after the onset of illness, whereas 5/5 samples collected later than 14 days were positive. One isolate was also made from a throat swab.

Poliovirus type 2 predominated in contact cases (8/13), whereas recipient cases were often (3/5) associated with infection with more than one serotype. Types 2 and 3 were both detected in cases with no known contact. Poliovirus type 1 was not implicated in PPC during this period. The strains that were characterized (Table 1) were all shown to be vaccine-like (see below) including the only strain analysed from the 'no known contact' category.

A fourfold or greater rise in neutralizing antibody to one or more serotypes occurred in 14/21 patients. Serology was especially useful in possible contact cases where there was no prior history of immunization. Thus 6/10 contact cases with no previous TOPV had a significant seroresponse to a single type (Table 1). In four of these cases no virus was isolated.

Intratypic characterization with monoclonal antibody panels

Ten poliovirus strains isolated from 9 PPC patients (seven strains of type 2 and three strains of type 3) together with 11 strains isolated from 10 patients with non-paralytic poliovirus CNS infections or healthy excretors (one type 1 strain, two type 2 and eight type 3) were analysed (Tables 1 and 2). A type-1 isolate (Pol/449/84; case 3, Table 2) taken in 1984 from a patient with meningitis had a wild-type antigenic phenotype as did a 1982 type-2 isolate (Pol/432/sf82; case 1, Table 2), also from a case of meningitis. All other strains had vaccine-like antigenic phenotype although 2/11 type-3 isolates showed extensive antigenic drift. Thus strain Pol/466/87 (case 5, Table 2) was not neutralized by monoclonals which recognize antigenic sites 2, 3 and 4, and strain Pol/432/f82 (case 1, Table 2) escaped neutralization by monoclonals which recognize antigenic sites 1, 3 and 4. One patient (case 1, Table 2) was therefore excreting a vaccine-like type-3 strain in stool but had a wild-type-2 CNS infection and seroconversion. In the absence of virological data this case was classified as possibly vaccine-related, but, considering the intratypic characterization, was most likely related to the wild type-2 strain.

Partial nucleotide sequencing

Nine strains were also sequenced in VP1 around antigenic site 1 (Tables 1 and 2). Pol/449/84 (case 3, Table 2) was confirmed as a wild type-1 virus and also shown to be unrelated to Mahoney, which is used as a laboratory strain in diagnostic laboratories in Poland. Pol/472/88 (case 16, Table 1) was shown to be identical to Sabin II vaccine strain whereas Pol/432/sf82 (case 2, Table 2) was confirmed as wild type. Six type-3 isolates were sequenced and were identical to Sabin III (two isolates) or showed one (three isolates) or two (one isolate) base changes over a range of 78–300 bases sequenced. All were therefore confirmed as vaccine-like. Pol/432/f82 (case 2, Table 2) had a G to A change at base 2771 which results in Ala— Thr at amino acid 99 in VP1. Monoclonal antibody 520 (number 35 in the type-3 panel) is known to be sensitive to changes in this residue (*14*).

Discussion

A high level of vaccine coverage (92–98% of children between 1975 and 1989 received three doses in the first year of life plus a booster in the second year) (*15*) resulted in the virtual eradication of paralytic poliomyelitis from Poland. Only 21 PPC were reported between 1981 and 1990, and 18 could be epidemiologically related to vaccine usage. The remaining three may also have had contact with vaccinees, since vaccination is carried out throughout the year, but these could not be documented. Although a causal relationship between paralysis and vaccination can be questioned it is generally accepted that the association is more than coincidental (*10*). However, one patient with meningitis (case 2, Table 2) was excreting a vaccine-like type-3 strain in stool while a wild-type-2 strain was isolated from CSF. The isolation of poliovirus from CSF is rare but not unprecedented (*16*) and this case illustrates the need for improved diagnostic tests to unequivocally establish a causal relationship. The simultaneous isolation of vaccine and wild-type viruses in the same individual also implies heavy environmental contamination with polioviruses.

One striking difference between PPC in Poland and other countries is the age distribution of contact cases. Whereas elsewhere contact cases often occur in adults, especially parents of vaccinated children (*13*), in Poland contact cases predominantly occurred in young children (< 2 years old). The majority of these infections were apparently acquired from other children in nurseries or hospitals (Table 1). An effective system for screening immunization status among children attending nurseries and kindergartens is needed. Each child who is going to attend a nursery, kindergarten, or other child-gathering place should be screened for polio (and other) immunizations and be immunized, if needed. A similar screening should

Table 2: **Epidemiological and virological features of patients without paralytic poliomyelitis**[a]

Epidemiological characterization and case number	Sex/age at onset	Year of onset	No. of OPV doses[b]	Interval (days)[c]	Virus culture				Serology				Laboratory confirmation
					Specimens collected (days from onset)	Virus type	Intratypic characterization		I	II	III	Days from onset	
							Mab	Seq					
Meningitis/encephalitis possible contact													
1	M/5 y	1982	3 TOPV	11	CSF (4)	2	2W	2W	16/32	<4/64	2/2	4/16	V2,3
					F (4)	3	3S	3S					S2
Meningitis/encephalitis source unknown													
2	M/3 y	1982	3 MOPV 1 TOPV	—	CSF (1)	2	2S	—	—/16	—/16	—/8	—/68	V2
3	M/14 y	1984	3 MOPV 1 TOPV	—	CSF (1)	1	1W	1W	32/16	16/16	8/8	1/9	V1
Conditions not related to poliomyelitis[d]													
4		1987	—	—	F	3	3S	3S	—	—	—	—	V3
5		1987	—	—	F	3	3S	3S	—	—	—	—	V3
6		1988	—	—	F	3	3S	—	—	—	—	—	V3
7		1989	—	—	F	3	3S	—	—	—	—	—	V3
8		1989	—	—	F	3	3S	—	—	—	—	—	V3
9		1989	—	—	F	3	3S	3S	—	—	—	—	V3
10		1989	—	—	F	3	3S	3S	—	—	—	—	V3

[a] Footnotes in Table 1 apply here also.
[b] MOPV, monovalent oral poliovaccine; TOPV, trivalent oral poliovaccine.
[c] From dose of OPV in contact to onset of meningitis in case.
[d] All children <3 years.

be performed in health settings (hospitals, health centres, and outpatient departments) where children are reporting for curative purposes.

The grouping of isolates from PPC as type-2 or type-3 strains is characteristic of vaccine-associated, rather than wild-type disease (*10*). The association of type-2 infections with contact cases seen in the present study is consistent with the possible explanation that excretion of type-2 revertants is prolonged (*17*). The unusually high frequency of type-2 infections in contact cases (8/13) in this series may, however, imply a higher than usual rate of excretion of type-2 revertants from Polish vaccinees. This hypothesis could be subjected to field-testing.

Tests for neutralizing antibody to poliovirus are no longer recommended in the routine laboratory diagnosis of patients with poliomyelitis.[b] Our results showed, however, that these tests were especially useful in vaccine-associated contact cases and provided a diagnosis where virus isolation failed.

The characteristics of virus strains associated with PPC and other clinical conditions were studied with monoclonal antibody panels.[b] A fixed-virus varying-antibody method was used rather than the fixed-antibody varying-virus method described in the new WHO manual for virological investigation of poliomyelitis.[b] Both methods were found to be equally suitable during developmental work on the monoclonal antibody panels at the National Institute for Biological Standards and Control (NIBSC), England. Results obtained with the monoclonal antibody panels in the present study agreed completely with partial genomic sequencing of selected strains (Tables 1 and 2). An exhaustive evaluation of these monoclonal antibody panels for intratypic characterization of polioviruses is currently being carried out. If the results confirm the promising preliminary studies carried out at NIBSC, then this approach should be very useful for the poliomyelitis eradication initiative.

All viruses associated with PPC that were characterized were shown to be vaccine-like. This included a virus from a case with no known vaccine contact, which confirms the value of incorporating laboratory data into classification schemes for PPC (*18*). Therefore paralytic poliomyelitis in Poland is currently related to TOPV usage. Strategies for reducing vaccine-related cases that are being explored elsewhere (*19*) may also be applicable in Poland.

The identification of wild type 1 and 2 viruses from cases of meningitis in 1984 and 1982, respectively, demonstrates the value of characterizing viruses from cases of non-paralytic neurological infection as a means of detecting wild virus circulation in immune populations. Current WHO/EPI surveillance strategies do not include monitoring of meningitis cases for poliovirus infection and the circulation of wild poliovirus may therefore be missed in some situations. Clearly much work remains to be done before criteria for demonstration of poliomyelitis eradication can be devised.

[b] *Manual for the virological investigation of poliomyelitis.* Unpublished document, WHO/EPI/CDS/Polio/90.1, 1990.

Acknowledgement

Z. Jarzabek gratefully acknowledges financial support from the British Council for a study visit to NIBSC to undertake strain characterization of isolates.

Résumé

Etude virologique et épidémiologique, à l'aide de séries d'anticorps monoclonaux, des cas de poliomyélite survenus en Pologne de 1981 à 1990

Une étude épidémiologique et virologique des cas de poliomyélite paralytique survenus en Pologne de 1981 à 1990 a été entreprise pour vérifier l'opinion selon laquelle ces cas résiduels de poliomyélite étaient liés à la vaccination. Des séries d'anticorps monoclonaux ont été utilisés pour la caractérisation intratypique des isolements de virus.

Vingt et un cas ont été identifiés. Des arguments épidémiologiques ont permis de considérer 18 de ces cas comme liés à la vaccination; 5 sont survenus chez des sujets récemment vaccinés avec un vaccin buccal trivalent et 13 chez des personnes qui avaient été ou avaient pu être en contact avec des sujets vaccinés. Les trois autres cas se sont produits chez les patients qui n'avaient eu aucun contact avec des sujets vaccinés. La majorité (12/13) des infections acquises par contact ont touché des enfants de moins de trois ans.

Dix souches isolées chez des patients atteints de la forme paralytique de la maladie et onze souches provenant de personnes atteintes d'une forme non paralytique ou de porteurs sains ont été caractérisées à l'aide de séries d'anticorps monoclonaux. Toutes les souches isolées chez des malades, de même que la plupart des autres souches, se sont révélées d'origine vaccinale. Toutefois, deux souches associées à une méningite, l'une de type 1 en 1984 et l'autre de type 2 en 1982, avaient des phénotypes antigé-

niques de type sauvage et, comme l'a montré un séquençage partiel du génome, des génotypes également de type sauvage. Enfin, le séquençage partiel de sept autres souches a donné des résultats concordant parfaitement avec la caractérisation par les anticorps monoclonaux.

La concordance des résultats de laboratoire et des données épidémiologiques laisse à penser que les cas résiduels de poliomyélite paralytique survenus en Pologne de 1981 à 1990 étaient liés à la vaccination. Cependant, l'étude des cas de poliomyélite non paralytique a contribué à mettre en évidence la circulation de virus endémiques sauvages dans une communauté bien vaccinée.

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