TRANSITORY APPEARANCE OF TYPE 2 NEUTRALIZING ANTIBODY IN PATIENTS INFECTED WITH TYPE 1 POLIOMYELITIS VIRUS*

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Previous studies in this laboratory (1) and elsewhere (2) have established that neutralizing antibodies for the infecting strain of poliomyelitis virus are as a rule demonstrable in patients at, or shortly after, onset of first symptoms and continue to increase in titer during convalescence. Further studies on the same patients have indicated that the high titers of homologous antibody which were found at 3 months were still present at 3 years after onset (3). The recent work on immunologic classification of the poliomyelitis viruses, which indicated the existence of 3 distinct types, did not reveal a common antigen among them (4). Similarly, recently reported observations on complement-fixing (CF) antigens derived from the different types of poliomyelitis virus, yielded no evidence of a common antigen when sera from hyperimmunized or from intracerebrally inoculated convalescent monkeys were used for the tests (5–8). Evidence for the existence of group-specific CF antibodies was, however, obtained in studies on patients. Persons, proved to be infected with Type 1 (Brunhilde-like) poliomyelitis virus, who exhibited no evidence of past or concurrent infection with Type 2 (Lansing-like) virus, nevertheless, either developed Type 2, CF antibody in increasing titer, or, very shortly after onset of first symptoms, were found to possess such antibody in titers of a magnitude only rarely encountered in healthy individuals tested as controls (9). This heterotypic CF antibody response completely disappeared in a few patients 3 months after onset, and in 50 per cent of the patients the titers exhibited a fourfold or greater drop by 3 months (9). During the course of this latter study (9) it was observed that a patient, who was proved to have been infected with a Type 1 poliomyelitis virus, had Type 2 neutralizing antibody, 3 days after onset, which completely disappeared between 2 and 4 weeks after onset. This observation led to the study, to be reported here, in which the Type 2 neutralizing antibody response was determined at various times during the first 3 months after onset of poliomeylitis in 18 patients from whom Type 1 virus was recovered.

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Materials and Methods

Patients Studied.—The 18 patients are part of a group described in a previous communication (1). 9 had the paralytic disease and 9 were diagnosed as having non-paralytic poliomyelitis. All had their illness in 1947; 8 were derived from an epidemic in Cincinnati, and

TABLE I

Type 2 Neutralizing Antibody in Poliomyelitis Patients from Whom Type 1 Virus Was Recovered

Type 2 neutralizing antibody in acute phase	Test for Type 2 neutralizing antibody	Patient			Age	Time after onset 1st serum obtained	Type 2 neutralizing antibody				Type 2, CF antibody				Type 1 neutralizing antibody	
		No.	Name	Illness*			1st serum	2 wks.	4 wks.	3 mos.	1st serum	2 wks.	4 wks.	3 mos.	1st serum	3 mos.
					yrs.	*days*										
Positive	Serum dilutions *vs.* 50 LD_{50} of virus	1	Sic.	NP	15	1	1:6	1:32	1:62	1:38	96	64	64	32	—	—
		2	Col.	NP	12	1	1:16	1:64	1:85	1:32	12	32	32	32	—	—
		3	Pet.	NP	13	2	1:3	1:8	1:25	1:17	16	128	64	24	—	—
		4	Hopk.	P	2	2	1:12	1:24	1:36	1:11	8	24	24	4	1:32	1:214+
		5	Kau.	P	11	3	1:32‡ / 1:20	1:2 / <1:2	0 / 0	0 / 0	128	128	96	64	1:32	1:128
		6	Fro.	P	6/12	3	1:40	—	—	1:16 / 1:2	—	2	2	—	1:2?	1:6‖ / 1:128
Negative	Undiluted serum *vs.* various dilutions of virus	7	Fin.	P	8	1	13—	32	50	6—	<2	4	4¶	—	1:12	1:194+
		8	Obe.	P	13	1	5—	1000	250	5—	2	12	8	4	1:2	1:64
		9	Kni.	NP	2	1	10—	50	16—	20—	192	128	96	48	—	—
		10	Yea.	NP	2	0.3	6	10	50	200	4	8	4	2	—	—
		11	Wal.	NP	2	2	5—	50	—	4—	—	—	—	—	16**	1600**
		12	Ris.	P	18/12	3	13—	63	32	4—	—	8	4¶	—	1:6	1:26
		13	Fal.	NP	36	3	6—	20	10	10—	16	32	32	32	—	—
		14	Hopp.	P	18/12	4	5—	25	5—	5—	6	12	8	6	—	—
		15	Hof.	NP	4	3	6—	16—	10—	5—	—	<2	—	—	10—**	63**
		16	Vau.	NP	8	5	6—	13—	6—	100+‡‡	16	8	12	48	—	—
		17	Ric.	P	6/12	5	5—	20—	16—	160+‡‡	—	—	—	—	1:32	1:214+
		18	Ten.	P	7	2	25	20	10	800‡‡	—	—	—	—	1:2	1:52

* NP = non-paralytic; P = paralytic.

‡ The results of the first test are shown in the numerator, and those of the second test in the denominator.

§ The first test in 1948 yielded a titer of 1:16 and on repetition in 1951 the titer was 1:2; a test performed simultaneously on the 3-year specimen of this patient yielded a negative result.

‖ The higher titer in the denominator represents the result of a repeat test on this serum after storage for 3 years in frozen state.

¶ The CF tests were negative on the serum obtained 3 years after onset.

** The values represent the neutralization index of undiluted serum.

‡‡ The 50 per cent serum dilution titer *vs.* 50 LD_{50} of Type 2 virus was: 1:16 for Vau. 1:200 for Ric.; also 1:200, 3 years after onset. 1:4 for Ten.; 1:2, 3 years after onset.

10 from an epidemic in Akron. The 18 strains of poliomyelitis virus recovered from the alimentary tract of these patients were all subsequently (4) classified as Type 1. 11 of these patients were also included in a subsequent study with Type 2 poliomyelitis CF antigen (9), and 7 of them were also investigated for persistence of neutralizing antibody against their own strain of virus 3 years after onset (3).

Serum Specimens.—The specimens were all collected in 1947 (1) and stored in the frozen state in a chest containing solid CO_2. Acute phase specimens were obtained between 8 hours and 5 days after onset (Table I), and the others at 2 weeks, 4 weeks, and 3 months after onset.

Tests for Type 2 Neutralizing Antibody.—Qualitative and quantitative tests were performed in mice using aliquots of a frozen preparation of the Lansing strain of virus. In the qualitative tests the mixtures consisted of undiluted serum and 3 fivefold or tenfold serial dilutions of virus, incubated at room temperature for 1 hour prior to inoculation into groups of 8 mice. A control titration of the virus in 0.9 per cent solution of NaCl accompanied each test, but the neutralization index was calculated on the basis of a cumulative LD_{50} derived from multiple control titrations using 8 to 20 mice per dilution. The neutralization index is the control LD_{50} titer divided by the LD_{50} titer of the virus in the presence of serum, and a value of 50 or over was regarded as indicating the presence of antibody. Neutralization indexes of less than 30 were regarded as negative and those between 30 and 50, as questionable. In the quantitative tests the mixtures consisted of serial fourfold dilutions of serum and 50 LD_{50} of virus per 0.03 ml. of mixture, the result being expressed as the final dilution of serum capable of protecting 50 per cent of the mice; the 50 per cent end-point was calculated by the method of Reed and Muench.

In both the qualitative and quantitative tests the mice were observed for 35 days. This was done because repeated observations on control titrations with 20 to 140 mice per dilution of virus on at least 4 different pools of the Lansing virus (about 200 mouse passages) revealed that the LD_{50} titer at 35 days was consistently from 0.3 to 0.6 of a log higher than at 21 days. The longer period of observation may result in somewhat lower values for neutralizing antibody in both the qualitative and quantitative tests because the proportion of mice exhibiting incubation periods of 21 to 35 days is much higher among those inoculated with antibody-virus mixtures than among controls. An examination of the protocols revealed that sera regarded as negative on the 35 day basis did not become positive on the 21 day basis. However, sera with neutralization indexes between 30 and 50 on the 35 day basis occasionally yielded indexes of 50 on the 21 day basis. Thus, the 2 week serum of patient Fin., recorded as having a neutralization index of 32 on the 35 day basis (Table I), revealed an index of 63 on the 21 day basis.

In the quantitative test all 4 sera of one patient were examined in a single test, because when that is done, fourfold or greater differences in titer can be regarded as representing real differences in concentration of antibody. This reproducibility of the 50 per cent neutralization end-point of a serum has been found not only in duplicate tests in this laboratory employing fourfold dilutions of serum, but also by Bell (10) with tenfold dilutions of serum.

Tests for Type 1 Neutralizing Antibody.—The results of simultaneous quantitative tests in monkeys on the acute phase and 3 month convalescent sera using the patient's own virus have already been reported for 9 of the 10 patients (1), and are reproduced in Table I. The titers in Table I are double those in the original report (1), because the final serum dilutions are given here in conformity with those recorded for the Type 2 neutralizing antibody. The tests on the 3 month sera of 7 of the patients were repeated 3 years later as part of another study (3), and were found to be as high or higher than those recorded in Table I. The acute phase and 3 month sera of patient Kau. were examined in a single test during the present study, using a Type 1 virus derived from another patient, Obe., in the same epidemic, because the virus recovered from Kau. was of insufficient potency for quantitative neutralization tests in monkeys.[1] Mixtures consisting of serial, fourfold dilutions of serum and 100 PD_{50}[2] of virus were inoculated into groups of 4 *rhesus* monkeys, and the 50 per cent serum dilution end-point determined by the method of Reed and Muench.

Tests for Type 2, CF Antibody.—The CF tests were performed by Dr. Jordi Casals at the Laboratories of the Rockefeller Institute, and are recorded in Table I to provide a complete

[1] I am indebted to Dr. Johan Winsser for assistance in the performance of this test.

[2] PD_{50} means 50 per cent poliomyelitis dose because the calculation takes into account the animals which are not paralyzed but exhibit poliomyelitic lesions in the nervous system

antibody spectrum on this series of patients. The data on 11 of these patients appear in the report by Casals, Olitsky, and Sabin (9). The data on patients Fro., Fin., Ris., and Hof. were not previously recorded because they were carried out with CF antigens of lower titer, but not less than 4 units.[3]

RESULTS

The data on the 18 patients investigated in this study are presented in Table I. Although Type 1 poliomyelitis virus was recovered from the alimentary tract of all these patients and it is highly probable that it was responsible for the current illness, diagnosed as paralytic or non-paralytic poliomyelitis, one can be certain of this only in the 10 patients whose sera were also tested for homotypic neutralizing antibody.

Patients with Type 2 Neutralizing Antibody during Acute Phase.—6 of the 18 patients had Type 2 neutralizing antibody in the acute phase serum specimens, obtained 1 to 3 days after onset; the titers in these sera ranged from 1:3 to 1:40, and during subsequent weeks the level of this antibody exhibited a somewhat different pattern in each of these patients. In 3 of them (Sic., Col., and Pet.) the titer increased fivefold to tenfold between 1 to 2 days and 4 weeks after onset; while there was a downward trend thereafter, the titers in the 3 month sera were still 2 to 6 times higher than in the acute phase specimens. Bell (10) performed quantitative tests for Lansing antibody on serial serum specimens obtained from 4 normal individuals over a period of 3 to 10 months, and the maximum variation in titer was 0.3 of a log, *i.e.* no more than a twofold difference, which probably represents the limits of accuracy of the test. The changes in titer which exceed this twofold range may, therefore, be regarded as representing a variation in antibody concentration that does not ordinarily occur without antigenic stimulation. In these 3 patients one cannot rule out a concurrent infection with a Type 2 poliomyelitis virus, and the results of the Type 2, CF tests do not help in reaching a decision because similar patterns are found in patients without Type 2 neutralizing antibody. However, in view of the results obtained in the other patients, a concurrent infection with Type 2 virus is not necessarily the real cause of the observed events. In patient Hopk. the Type 2 neutralizing antibody is present in the same titer in the 2 day and 3 month specimens, while the 2 week and 4 week specimens show twofold and threefold greater titers respectively. Although one may question the significance of this twofold and threefold difference, the same fluctuation is apparent in the Type 2, CF antibody titers. In contrast with these data for the Type 2 virus, patient Hopk. exhibits a sevenfold greater titer of homotypic neutralizing antibody in the 3 month than in the 2 day serum specimen. The data on patient Kau. are of special significance, because they show that a person proved to have been infected with Type 1 poliomyelitis

[3] I am indebted to Dr. Jordi Casals for permission to insert these results in the present report.

virus, by both virus recovery and development of increasing and persistent titer of homotypic antibody, can have Type 2 neutralizing antibody in a titer of 1:32 at 3 days after onset which completely disappears within 2 to 4 weeks after onset; the 4 sera were tested twice with almost identical results. The only plausible interpretation of the whole pattern of the results on this patient is that infection with a Type 1 poliomyelitis virus stimulated a rapid production of both neutralizing and CF antibodies for Type 2 virus, the former persisting for only about 2 weeks and the latter for 3 months or longer. The same interpretation is probably valid for the data obtained on Fro., but because this patient was only 6 months old at onset it is also necessary to consider the possibility of unusually long persistence of placentally transmitted antibody. Against this possibility are the rather high titer of 1:40 at 6 months of age and the presence of antibody at 9 months of age. It is clear, however, that the Type 2 neutralizing antibody did not persist, since it was completely absent 3 years later when the homotypic neutralizing antibody was still present in high titer (3).

Patients without Type 2 Neutralizing Antibody during Acute Phase.—Six of the 12 patients in this group developed Type 2 neutralizing antibody (neutralization indexes of 50 to 1,000) between 0.3 to 3 days and 2 to 4 weeks after onset. In only 1 of these 6 was this antibody still present at 3 months. The Type 2, CF antibody, tested serially in 4 of these 6 patients, rose fourfold to sixfold during the first 2 weeks in 2 patients. 4 of this group of 6 patients were unquestionably infected with the Type 1 virus recovered from them during the acute phase of their illness, because they developed homotypic neutralizing antibody in increasing titer demonstrable not only at 3 months but also at 3 years in the 3 who were tested at that time (3). In the remaining 6 patients of this group, there was no significant development of Type 2 neutralizing antibody during the first 4 weeks after onset. 3 of them, however, developed Type 2 neutralizing antibody between 4 weeks and 3 months after onset of their clinically diagnosed illness as previously reported (1). In one of these 3 patients there was a fourfold increase in Type 2, CF antibody during the same period, and in the other 2 patients the newly developed Type 2 neutralizing antibody was still present in undiminished titer 3 years later (3); the most plausible interpretation of these data, in the light of the rapid and transitory appearance of the heterotypic antibody in the other patients is that these 3 patients were infected with Type 2 poliomyelitis virus, 4 weeks or more after onset of their clinically apparent infection with Type 1 virus.

The data on this group of 12 patients indicate that a certain proportion (in this case, 50 per cent) of patients, who are infected with Type 1 virus, can develop Type 2 neutralizing antibody during the first 4 weeks after onset, which in most instances is no longer detectable at 3 months after onset. The Type 2, CF antibody appeared in patients who failed to develop significant

amount of Type 2 neutralizing antibody. The Type 2, CF antibody generally appeared earlier and persisted longer than the Type 2 neutralizing antibody.

DISCUSSION

The results of the present study indicate that more than 50 per cent of patients, infected with Type 1 poliomyelitis virus, develop Type 2 neutralizing antibodies within 2 to 4 weeks after onset, which in most instances disappear within 3 months. This rapid disappearance of neutralizing antibody stimulated by infection with heterotypic strains of poliomyelitis virus is to be contrasted with the long persistence of the homotypic neutralizing antibodies. These data provide evidence for the existence of a common antigen between the Type 1 and Type 2 poliomyelitis viruses. They also provide a reasonable explanation for certain observations of others which were previously difficult to understand.

A number of investigators have reported patients whose sera neutralized Lansing virus during the first 2 to 4 weeks after onset but not later on. Turner and Young (11) mentioned 5 patients (of 64 tested) whose sera neutralized Lansing virus 8 to 17 days after onset but not 3 months later. Brown and Francis (12) reported that 17 of 39 patients who had Lansing neutralizing antibody during the acute phase (usually within 9 days after onset) showed a decrease (disappearance?) during convalescence (6 weeks to 1 year, usually 6 months, after onset); among 18 contacts of patients, who had antibody in the first serum specimen, there were 10 whose serum failed to neutralize the virus 1 year later. Both Turner and Young (11) and Brown and Francis (12) used 100 or more LD_{50} of virus in their tests and both the positive and negative results have a greater validity than similar observations of others (13, 14) obtained with approximately 10 LD_{50} of virus. Clark and Rhodes (15) recently reported that 4 of 6 Eskimo patients who had Lansing antibody at 3 to 4 weeks after onset of poliomyelitis failed to neutralize the virus at 12 months, and that another Eskimo patient with antibody 6 days after onset had none at 9 weeks. Although the available evidence indicated that the Eskimo epidemic was caused by non-Lansing strains of virus, these investigators (15) were inclined to interpret their data as indicating that Lansing antibody persists for only a short time after primary infection and that repeated infections are necessary to produce a more persistent immune response.

From the data reported in the present communication, it is clear that the transitory appearance of Lansing antibody reported by the previous investigators (11–15) is most likely the result of infection with Type 1 or other non-Lansing types of poliomyelitis virus.

It is also clear from the data presented here that the appearance of Lansing antibody during the first 4 weeks after onset in patients who had none during the first few days of their illness, as was reported for 9 of 23 patients by Hammon and Izumi (16), does not necessarily constitute evidence that these patients

were infected with Lansing-like strains of virus. Similarly, serologic surveys for Lansing antibody carried out on normal children shortly before and after the summer and autumn months (17) may be expected to yield results which reflect the incidence of inapparent infection not only with Type 2 strains but, to a lesser extent, also with Type 1 or other non-Lansing types of poliomyelitis virus. The data presented in this communication emphasize the fact that it will be necessary to study the antibody response of patients to all 3 types of poliomyelitis virus, before a proper basis can be established for the serologic diagnosis of the immunologic type of poliomyelitis virus responsible for human infections.

SUMMARY AND CONCLUSIONS

Neutralizing antibodies for Type 2 (Lansing) poliomyelitis virus were tested periodically in a group of 18 patients from whom Type 1 poliomyelitis virus was recovered. Data for homotypic neutralizing antibodies and Type 2 complement-fixing antibodies were also available on the majority of these patients. The results indicated that Type 2 neutralizing antibodies first appeared or significantly increased in titer in 11 of the 18 patients during the first 2 to 4 weeks after onset. In most patients the Type 2 neutralizing antibody either completely disappeared at 3 months (in one patient between 2 and 4 weeks) or dropped in titer, while the Type 1 or homotypic antibody persisted in high titer. These results are interpreted as indicating that the Type 1 and Type 2 poliomyelitis viruses share a common antigen, and that the heterotypic antibody response is transitory while the homotypic neutralizing antibody persists for a longer time.

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