RELATIONSHIP BETWEEN SERUM ANTIBODIES AND SUBCLINICAL INFECTIONS WITH POLIOMYELITIS VIRUS*, ‡

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Subclinical infection with poliomyelitis virus among familial associates of cases has been adequately demonstrated (1–15). Although there is serological evidence of the development of antibodies in the patient, there is little information with regard to the antibody status of his familial associates. Early neutralization tests with the sera of cases (16–21) and of contacts (22, 23) utilized stock passage strains of virus which were not the specific strains responsible for the disease of the patients although Brodie *et al.* (24) also used a strain of virus isolated from the outbreak during which the bloods were collected. Wenner and Tanner (25) studied neutralizing antibodies in the sera of five cases and one contact; the virus used was a pool representing three fatal cases from the same epidemic but was not specifically isolated from the individuals under study. More recently, Hammon and Roberts (26) and Steigman and Sabin (27), using strains of virus isolated from poliomyelitis patients, found that these viruses were neutralized in low titer by the patients' serum obtained from 1 to 5 days after onset and that a definite increase in specific antibodies occurred during the 3 months of convalescence. However, their studies were confined to cases and did not include any other members of the family. The present study was undertaken to determine whether there was any relation between the occurrence of poliomyelitis virus in the intestinal tract of members of families in which cases of frank poliomyelitis were observed and the presence in the sera of these persons of antibodies to the actual strain of virus recovered from the family. Since the demonstration of antibodies to the Lansing strain of virus is readily accomplished the sera were also tested against this strain of virus.

Materials and Methods

Families.—Three families residing in Detroit, in the summer of 1948 were selected for study on the condition that specimens could be obtained within a short time after onset of clinically recognizable poliomyelitis in one member of the family group.

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Family A consisted of the parents, 42 and 34 years old, and seven children, 18, 8, 7, 5, 4, 3, and 1 years. The child of 5 had complained of headache and had a temperature of 102°F. on Aug. 10. He seemed well until Aug. 15 when he had a slight fever and complained of headache and nuchal pain. Early in the morning of Aug. 16 he experienced nausea and weakness of the legs. On admission to the hospital that afternoon he had a stiff neck and back, paralysis of the hip flexors and quadriceps of both legs, and was diagnosed as having poliomyelitis. Two siblings, 4 and 3, had experienced slight fever on Aug. 10, but seemed well thereafter. Another child, 8, had complained of headache and fever on August 16. She was made to stay in bed for 2 days although free of pain. Weakness and malaise continued and on Aug. 22, she was admitted to the hospital with paralysis of the left hips and thigh and weakness of the left gastrocnemius. All other members of the family had remained well.

Blood specimens were taken from the first case, 5, on Aug. 17, 21, 24, 29, and Oct. 24; from the second case, 8, on Aug. 19, 23, 30, and Sept. 13; from the remainder of the family on Aug. 19 and Sept. 10. Stool specimens were obtained from the case 5 on Aug. 19, 9 days after his first symptoms, and on Aug. 27; from case 8 on Aug. 23 and 29, and from the rest of the family on Aug. 20 and Sept. 10.

Family B consisted of a mother 44 years old, children 22, 16, 15, and 6 years, and the wife, 24, and infant of 3 months, of the 22 year old son. The child, 6, had contracted poliomyelitis and was admitted to the hospital on July 6 where she remained throughout the period of the study. Her brother, 22, suffered fever and headache on July 17, and tremors the next day. On July 21, he was admitted to the hospital with paralysis of both legs and partial paralysis of the arms and abdominal muscles.

All other members of the family had been well.

Blood specimens were taken from the case, 22, on July 21, Aug. 3, 14, 21, and 28, and from the other members of the family, except the infant, on July 21 and Aug. 13. Thus, the first specimens were obtained 4 days after the first symptoms in the case, 22, but 15 days after the onset in his sister. Stool specimens were obtained from the case on July 28 and Aug. 28, and from the family on July 22. A second specimen was obtained on Aug. 13 from all the family except the mother, 44, and the original case, 6.

Family O consisted of a grandmother, aged 74 years, the parents, 42 and 39 years, and children 11, 10, 6, and 2 years. The child 11 had suffered with rheumatic fever for 6 years and had complained of his eyes hurting for several months. On July 31, he complained of pain in his arm and leg, vomited, and had a fever. However, he slept with his sister, 10, as usual. The next 2 days he stayed in bed and at night slept with his mother in the same room with his other sister, 6. He vomited again on Aug. 2, had difficulty in swallowing, and a change in his voice was noticeable. On Aug. 3, the pain in his legs and back returned and the following day, Aug. 4, he was admitted to the hospital where a diagnosis of bulbar poliomyelitis was made. With the exception of his sister, 6, who vomited and complained of stomach ache on Aug. 21, the family had been well.

Blood specimens were obtained from the case, 11, on Aug. 5, 13, 20, 27, and Oct. 8, and from the rest of the family, except the infant, 2, on Aug. 5 and 27. Stool specimens were taken from all on Aug. 6 and 7, the 6th and 7th days after onset in the case, and again, except for the baby, on Aug. 27 and 28.

Storage of Specimens.—Blood specimens were allowed to stand overnight in the refrigerator and the sera removed the next day following centrifugation. They were then stored at 4°C. in rubber-stoppered tubes until tested.

Stool specimens were frozen under solid CO_2 refrigeration until tested.

Isolation of Virus.—Individual stool specimens were thawed, a portion ground with alundum, and resuspended in physiological salt solution to approximately 10 per cent by weight. The material was agitated for 30 minutes on a shaking machine and refrigerated at 4°C.

overnight. It was again shaken, then centrifuged for 30 minutes at 2000 R.P.M. in a horizontal centrifuge. The sediment was saved for intranasal inoculations. The supernatant was centrifuged at 4000 R.P.M. for 1 hour in the cold and ether was added to approximate 20 per cent by volume. This material was shaken daily for 30 minutes for 5 days and except for the period of shaking was maintained at 4°C. It was then centrifuged at 2000 R.P.M. for 30 minutes and the ether was removed by evaporation under low pressure. Both aerobic and anaerobic sterility tests were applied to each preparation and if no bacterial growth was observed, the specimen was inoculated into a *rhesus* monkey. An initial intracerebral injection of 0.5 cc. into the vicinity of the thalamus was followed every 2 or 3 days by inoculations of 10 to 15 cc. intraperitoneally and 2 cc. intranasally until the specimen was exhausted. Daily temperatures were recorded and the animals were observed for a maximum of 30 days. Monkeys which developed paralysis were subjected to autopsy promptly; all others, at the end of the period of observation. A diagnosis of poliomyelitis was made if sections of the spinal cord revealed typical changes including perivascular cuffing, leucocytic infiltration, neuronolysis, and neuronophagia.

Virus-Neutralizing Antibodies.—Each serum was tested for its ability to neutralize two viruses, the mouse-adapted Lansing strain of poliomyelitis, and the specific "family" virus isolated from an individual suffering clinical disease in the same family.

The Lansing antibodies were studied with the thought that if the infecting virus represented a type other than Lansing, the information obtained might furnish evidence bearing on the significance of antibodies to different types of poliomyelitis virus. The antibody content of the sera to the family specific virus was studied to ascertain if its presence affected subclinical infection with the identical agent.

(*a*) The Lansing strain of virus had been passed 79 times in this laboratory and when titrated in mice as a control for each test consistently had a PD_{50} between $10^{-3.8}$ and $10^{-4.0}$. Appropriate dilutions of serum and virus were mixed to give final dilutions of 1/5 and 1/20 of each serum with 50 PD_{50} of virus. After an incubation of 45 minutes in the water bath at 37°C., 0.03 cc. of a mixture was inoculated intracerebrally into each of eight mice. A virus titration and controls incorporating known positive and negative human sera accompanied each test. Mice were observed daily for 30 days. The minimum criterion for a positive serum was survival of four mice receiving the 1/5 dilution of serum which thus represented a neutralization index of 250.

(*b*) Each specific *family* virus was obtained from the nervous tissue of a monkey paralyzed following inoculation of the stool specimen from a paralytic case in each family. It was passed to another monkey and then inoculated into three or four monkeys. The cords and brain stems of these animals were ground and diluted in salt solution to make a 20 per cent suspension. Each pool was frozen in sealed glass ampoules until used.

Since these viruses had been found to titer approximately 10^{-3} in monkeys, the final dilutions of virus employed for neutralization tests with each serum were 10^{-1} and 10^{-2} so as to represent 100 and 10 PD_{50} of virus. Early tests employed undiluted serum but following certain inconsistencies in some of those results necessitating repetition of the tests, final dilutions of 1/10 and occasionally 1/50 were used. A virus titration employing final dilutions of 10^{-1}, 10^{-2}, and 10^{-3} of virus in normal monkey serum accompanied each test. Each virus-serum mixture was incubated in the water bath at 37°C. for 45 minutes, then maintained at 4°C. during the course of the inoculations. Two *rhesus* monkeys were inoculated intracerebrally with 0.5 cc. of each mixture. Rectal temperatures were taken daily and the animals observed for signs of paralysis.

Autopsies were performed on the 1st day of paralysis and sections of nervous tissue removed for histological examination. Neutralization indices were calculated by multiplying the PD_{50} of virus inoculated by the final dilution of serum protecting the animals. In clas-

sifying the sera those with indices of less than 10 were considered negative, between 10 and 100 were considered questionable or plus-minus, and those with indices greater than 100 were classified as definitely positive.

RESULTS

Isolation of Virus.—In family A poliomyelitis virus was recovered from the stools of the mother and six of the seven children including the two cases. Only two of these individuals were still carrying virus 3 weeks later.

In family B virus was recovered from the two cases and the 3 months old infant but not from the four other associates and from none of the specimens collected 3 weeks later.

In family O the stools of the case and of two other children yielded virus at the time of the first collection but all were negative 3 weeks later.

The results are presented in Table I which shows that all 5 cases were excreting virus and 8 of 17 familial associates had subclinical infections at the time of the first collection of specimens but that in only 2 of these did virus persist for as long as 3 weeks. With two exceptions the positive isolations were obtained with specimens from children of 11 years of age or less. One of the exceptions was the 22 year old case in family B and the other was the mother of family A which included two cases and four inapparent infections. All individuals from whom virus was not recovered were 15 years or older.

Virus-Neutralizing Antibodies.—The neutralization test with the Lansing strain of virus in mice proved to be essentially an all-or-none phenomenon. Most sera appeared to neutralize the virus completely or not at all. Since the lowest dilution used was 1/5, the possibility exists that some sera contained slight amounts of antibody which would not be considered significant. In spite of the fact that the neutralization index must be recorded as *less than* the lowest amount tested it is more likely that such sera were completely negative (Table I).

In family A, the sera of the two parents (VA and SA) and oldest child (ViA) completely neutralized the Lansing strain in all dilutions tested while the sera of all the others were negative except for the second specimen of SaA, which barely satisfied the criterion for a positive serum. In family B all sera except that from CB were found to be positive. Family O contained two individuals, MsO and MaO, whose sera failed to neutralize the virus and all the others were positive.

It will be seen in Table I that there is a striking relation between the presence or absence of virus in the stools of the individual and the absence or presence of Lansing antibodies in his serum. At the time of the first collection this relationship obtained for 18 of the 21 individuals. The same relationship is shown with 12 of 16 specimens from the second collection; the four individuals falling out of the pattern were those from whom virus was isolated at first but not 3 weeks later and who had no significant circulating Lansing antibodies at the time of either collection.

Neutralization tests with the family viruses and these sera revealed the same relationship between the presence of antibody and the absence of virus in the stools although neutralization indices of some of the sera were only 10 to 100. A clear-cut relation was found in 12 of 20 individuals at the time of the

TABLE I

Occurrence of Subclinical Infection and Serum Antibodies in Poliomyelitic Families

Family	Age	Virus in stool		Neutralization index of sera			
		1st specimen	2nd specimen	Lansing		"Family"	
				1st	2nd	1st	2nd
	yrs.						
A							
JA (case)	5	+		<145	<145	0	>1750
SA	34	+	−	>580	>580	175	>1750
VA	42	−	−	>580	>580	>61	<61
ViA	18	−	−	>580	>580	>632	632
SaA (case)	8	+		<145	252	1750	>1750
BA	7	+	+	<145	<145	61	61
WA	4	+	−	<145	<145	>632	>632
AA	3	+	−	<145	<145	61	>61
FA	1	+	+	<145	<145		>1750
B							
RB (case)	22	+	−	>725	>725	>350	>3160
JB	24	−	−	>725	>725	110	>3160
KB	44	−		>725	>725	>350	>3160
JeB	16	−	−	>725	>725	>350	>3160
DB	15	−	−	>725	>725	>350	31
CB (case)	6	+		<185		>350	
AB	<1	+	−				
O							
JO (case)	11	+		>2000	>2000	0	40
BO	39	−	−	>2000	350	200	200
HO	42	−	−	>2000	650	100	>100
MsO	10	+	−	<250	<250	100	>100
MaO	6	+	−	<250	<250	<10	>100
MuO	74	−	−	>725	>2000	200	>100

first collection. Analysis of the results obtained with stools and sera of the second collection shows that 12 of 14 individuals from whom virus could not be isolated had serum antibodies to the specific strain.

Only one individual showed a rise in antibody titer to Lansing virus while over half were found to develop an increase in antibodies to the virus isolated from their family. This is highly indicative that the viruses involved in the

infection were not of the Lansing type. Furthermore, in instances in which tests were complete, six of those whose stools were originally positive failed to yield virus on the second test; of these, 4 and probably 5 (SA, AA, MsO, MaO, RB) showed a rise in antibody titer of the serum to the specific virus. This suggests that the cessation of virus excretion is related to the development of antibody.

TABLE II

Effect of Age on Presence of Serum Antibodies and Subclinical Infection

Age		Lansing antibodies		Virus in stool		Homologous "family" antibodies	
yrs.							
74	MuO	L	L	O	O	F	F
44	KB	L	L	O		F	F
42	VA	L	L	O	O	F	F
42	HO	L	L	O	O	F	F
39	BO	L	L	O	O	F	F
34	SA	L	L	V	O	F	F
24	JB	L	L	O	O	F	F
22	RB (case)	L	L	V	O	F	F
18	ViA	L	L	O	O	F	F
16	JeB	L	L	O	O	F	F
15	DB	L	L	O	O	F	±
11	JO (case)	L	L	V		O	±
10	MsO	O	O	V	O	F	F
8	SaA (case)	O	L	V		F	F
7	BA	O	O	V	V	±	±
6	MaO	O	O	V	O	O	F
6	CB (case)	O		V		F	
5	JA (case)	O	O	V		O	F
4	WA	O	O	V	O	F	F
3	AA	O	O	V	O	±	F
1	FA	O	O	V	V		F

F, neutralization index = >100.
±, " " = 10-100.
O, " " = <10.

Antibodies for the Lansing strain of virus were demonstrated in the sera of each of the twelve persons of 11 years or more but virus was recovered from only three, two of them actual cases. It is striking that with one exception, all those who possessed Lansing antibodies also had antibody to the homologous virus recovered from the family. None of the nine persons under 11 years had circulating Lansing antibodies and virus was recovered from the stools of all nine of them; only four of them, however, had antibodies to the family virus at the time of the first examination. (Table II).

DISCUSSION

The numerous subclinical infections detected in the present families are further demonstrations of the rapid spread of virus through a family group (7, 13–15). The presence in a few of these individuals of neutralizing antibodies for the specific or family virus at the time of the first collection of serum is not therefore unexpected. That they were of recent origin is proved by the fact that the antibody titers were still rising in many individuals during the 3 weeks between collections.

Two facts would indicate that the family viruses were not of the Lansing type. First, as mentioned above, many sera of the second collection showed an increase in antibody titer to the family virus without a comparable increase in antibodies to the Lansing virus; and secondly, all attempts to adapt these agents to rodents were unsuccessful.

The demonstration that antibodies to both Lansing and family viruses occurred in the sera of all those whose stools did not contain virus initially could mean that these individuals had been exposed to two immunological types of virus in the past and had acquired resistance to subclinical infection prior to the current episode in their families.

The present data show a striking inverse relationship of both types of serum antibodies and subclinical infection with the current virus. It is less prominent when considered in terms of homologous antibody than in terms of Lansing antibody. A tempting suggestion is that Lansing type of virus may have a broad antigenic pattern demonstrable in active immunity, but not evident by routine immunological tests. This is in agreement with a previous finding (28) that although the presence of neutralizing antibodies to Lansing virus does not necessarily afford protection against the occurrence of clinical poliomyelitis, fewer acute cases between the ages of 1 and 15 years had such antibodies than did healthy children of the same age group. The importance of cross-immunity in protection against poliomyelitis still must be considered.

The demonstration of antibodies for the Lansing strain of virus in all persons over 10 years is also in agreement with the results of most investigators in this field (28–30). These antibodies are less frequently found in children although occasionally high percentages of the younger age groups are shown to be positive, reflecting the recent prevalence of this type of virus in the area. Since the presence of antibody was largely related to the age of the subjects, it is not entirely clear whether the occurrence of virus in the stool is a function of age, with respect to lack of exposure; of the absence of antibody; or results from a combination of the two factors. If the absence of Lansing antibodies is the significant one in determining the presence of virus in the stool, the question is immediately raised as to the breadth of resistance in man recovering from infection with poliomyelitis virus.

SUMMARY

Three families in which cases of poliomyelitis had recently occurred were studied for the extent of subclinical infection and the presence of serum antibodies to both the mouse-adapted Lansing strain and the specific strain of poliomyelitis isolated from members of the family.

Virus was recovered from the stools of all 5 cases and from 8 of 17 familial associates at the time of the first collection of specimens. Only two of the associates were still carrying virus 3 weeks later.

The individuals from whom virus was recovered were younger than 11 years of age with two exceptions, one of them a frank case.

Antibodies to the mouse-adapted Lansing strain of poliomyelitis virus were demonstrated in the sera of every person 11 years of age or older but in none from individuals under this age with the possible exception of one whose second specimen was weakly positive.

Antibodies for the specific agent isolated from each family were likewise found at the beginning of the study in every person over 11 years of age but also in 4 of 9 under that age. The neutralization indices of sera of the second collection were generally higher than those of the sera collected soon after the disease occurred in the family, reflecting the antigenic stimulus of the "family" viruses.

The inverse relationship of demonstrable antibodies to virus isolations strongly suggests that the presence of serum antibodies is effective in limiting subclinical infection with poliomyelitis virus.

The question of cross-immunity in protection against infection with poliomyelitis virus is discussed.

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