HOMOTYPIC COMPLEMENT-FIXING ANTIBODY IN MONKEYS INFECTED WITH TYPE 2 POLIOMYELITIS VIRUS BY THE ORAL ROUTE

By JORDI CASALS, M.D., PETER K. OLITSKY, M.D., and ALBERT B. SABIN,* M.D.

(From the Laboratories of The Rockefeller Institute for Medical Research, New York, and The Children's Hospital Research Foundation, University of Cincinnati, Cincinnati‡)

(Received for publication, March 28, 1952)

The present investigation was undertaken to determine the frequency, rapidity, and level of the homotypic complement-fixing (CF) antibody response in monkeys infected with a Type 2 (Lansing-like) poliomyelitis virus by the oral route. This information was sought particularly for the purpose of a comparison with the Type 2, CF antibody response in patients infected with heterotypic Type 1 poliomyelitis virus (Brunhilde-like strains) (1). Previous observations (2) on 5 *rhesus* monkeys convalescent from infection produced by intracerebral injection of Type 2 strains, had revealed no CF antibody in one, bled 2 and 26 days after onset, and significant titers, not higher than 1:16, were present in 3 of 4 monkeys bled 41 to 162 days after onset. Although this does not permit any conclusion with regard to the time of appearance of CF antibody after infection resulting from intracerebral inoculation of virus, the results are in keeping with repeated observations (3, 4) that the neutralizing antibody in such animals usually does not develop until 2 to 3 months after inoculation. However, neutralizing antibody has been shown to develop very early after infection in monkeys to which virus had been given by mouth (5, 6), a finding comparable to the early appearance of this antibody in patients (7, 8).

The present report presents data on the development of homotypic CF and neutralizing antibodies in 20 *cynomolgus* monkeys presenting evidence of paralytic, non-paralytic, or inapparent infections after oral administration of Type 2 poliomyelitis virus. All the data on these animals, except the performance of the CF tests, had been obtained by one of the authors (A.B.S.) during the course of another investigation on various problems relating to infection by the oral route.

* The writers acknowledge the technical assistance and cooperation of Dr. Lenora Brown of The Rockefeller Institute.

‡ The studies at The Children's Hospital Research Foundation, University of Cincinnati, were aided by a grant from The National Foundation for Infantile Paralysis, Inc.

Materials and Methods

Infection of Monkeys by Oral Route.—The Type 2 virus used was the Y-SK strain obtained from Dr. J. L. Melnick as 11th mouse passage material. The spinal cords and brain stems of paralyzed mice, representing the 12th to 14th passage, were prepared as 10 per cent suspension in 0.9 per cent solution of NaCl and stored in the frozen state in a chest containing solid CO_2. Intracerebral titrations in mice yielded LD_{50} titers of $10^{-3.8}$ to $10^{-4.4}$ for an inoculum of 0.03 ml.

The *cynomolgus* monkeys used in these tests weighed between 1.5 and 2 kilos. They were allowed to drink 2 ml. of the uncentrifuged, 10 per cent virus suspension from a rubber-tipped pipette or syringe resting between their teeth, in the morning and afternoon of 3 consecutive days; the total was 12 ml. or approximately 400,000 mouse intracerebral LD_{50} of virus. As part of another experiment, all but 3 of these monkeys (15–58, 15–61, and 15–62) were given 20 ml. of human or cow's milk by mouth from a rubber-tipped syringe shortly before the administration of each dose of virus. The monkeys were bled before the virus feedings and at various times thereafter as indicated in Table I. Some were bled immediately after they died. The diagnosis of paralytic poliomyelitis was confirmed by histological examination. The monkeys, which exhibited no discernible paralysis or weakness in the gross, as well as the surviving paralyzed monkeys were sacrificed 28 days after the first dose of virus and the following portions of the central nervous system were examined histologically: 15 to 30 levels of the spinal cord, 6 levels of the medulla, 2 levels of midbrain through the colliculi, one level each through the thalamus, hypothalamus, and anterior perforated substance, and partial serial sections of the olfactory bulbs and Gasserian ganglia. None of these monkeys exhibited poliomyelitis lesions in the olfactory bulbs or Gasserian ganglia. The lesions present in the monkeys with non-paralytic poliomyelitis were similar to those previously described in *rhesus* monkeys infected by the intracerebral route (9, 10).

Neutralization Tests.—These tests were performed in mice, using mixtures consisting of serial 5-fold dilutions of mouse-passaged Lansing virus and undiluted serum. The neutralization index is the ratio between the LD_{50} titer of the virus in saline solution and in the serum.

Complement Fixation Tests.—All 40 sera were tested in a single run. The preparation of the antigen and the technique of the test were essentially as previously described (2), except for the following: (*a*) the MEF1 antigen was more potent (up to 32 units) and was therefore used diluted to contain 8 to 16 units; (*b*) since 31 of the 40 sera inactivated at 60°C. for 20 minutes were anticomplementary in the 1:2 dilution and occasionally in higher dilutions, the result in the 1:2 dilution was disregarded; (*c*) some of the sera, retested after heating at 65°C. for 20 minutes, lost their anticomplementary properties and also exhibited a 2- to 4-fold lower titer of the specific antibody.

RESULTS

The results are summarized in Table I. Although only monkeys which developed neutralizing antibody were included in this investigation, it should be noted that by the procedure used only 10 per cent of *cynomolgus* monkeys fed this amount of virus fail to develop neutralizing antibodies (11). All 13 surviving monkeys developed CF antibodies by the end of 4 weeks after the first dose of virus, regardless of whether their infection was paralytic, non-paralytic, or clinically and pathologically inapparent. The titers of CF antibody were also of the same order of magnitude in the 3 groups of monkeys. These titers, interestingly enough, are not higher than those encountered in patients infected with heterotypic Type 1 virus (1). 4 of the 7 paralyzed monkeys, which

were bled shortly before or after death, developed CF antibodies, 2 of them as early as 2 days after onset of paralysis. The 3 monkeys which failed to develop

TABLE I

Development of Homotypic Complement-Fixing Antibody in Cynomolgus Monkeys Infected with Y-SK Virus by Oral Route

Group	*Cynomolgus* No.	Postinfection serum		Neutralization index of serum		CF titer of serum	
		Time after onset of paralysis	Time after 1st dose of virus	Before infection	After infection	Before infection	After infection
		days	*days*				
Paralytic, Died	15–76	2	12	4	160+	0	1:4
Bled just before or	16–01	2	14	2	50	0	1:16
shortly after death	16–02	4	18	2	50	AC (1:4)	1:16
	16–19	6	15	2	200+	0	0
	15–77	6	16	2	160+	0	1:64
	15–98	6	17	2	160+	0	0
	15–73	8	18	8	160+	0	0
Paralytic, Survived	16–08	16	28	2	50	0	1:8
	16–17x	18	28	1	50	0	1:16
	16–24x	18	28	1	40	AC (1:4)	1:8
	15–62	18	28	1	160+	AC (1:4)	1:16
	16–00x	20	28	50*	200+*	AC (1:4)	1:32
Non-Paralytic	15–58	—	28	1	100	0	1:4‡
Poliomyelitis le-	15–66	—	28	1	63	0	1:64
sions present in	15–91	—	29	4	130+	AC (1:8)	1:128
CNS							
No poliomyelitis le-	15–61	—	28	1	130+	0	(1:4)?‡
sions	15–68	—	28	2	80	AC (1:16)	1:8
	15–69	—	28	2	63	0	1:8
	15–83	—	29	8	130+	0	1:8
	15–90	—	29	1	160+	AC (1:4)	1:64

* *Cynomolgus* 16–00x had a serum-dilution neutralizing antibody titer against 50 LD_{50} of virus of 1:5 in the preinfection serum and of 1:180+ in the postinfection serum.

‡ These are the titers of the sera heated at 65°C.

AC = anticomplementary.

0 = negative in 1:4 dilution.

CF antibody died 6 to 8 days after onset of paralysis. The absence of CF antibody in these 3 moribund or dead monkeys may be due to a slower development of CF than of neutralizing antibody in some severely affected animals or to more extensive combination with CF antigen in the tissues of such animals, although it is possible that some animals, regardless of the severity of infection,

may not develop CF antibody within 6 to 8 days after onset. It is noteworthy that the titer of CF antibody 2 to 6 days after onset of paralysis in the other moribund or dead monkeys was as high as that found in the surviving monkeys 16 to 20 days after onset of paralysis. This early development of CF antibody in maximum titer in monkeys infected with homotypic Type 2 virus is similar to that found in human beings developing Type 2, CF antibody following infection with heterotypic Type 1 virus (1). These results suggest that the usual 4-fold or greater rise in titer required for diagnostic purposes might be achieved only infrequently for the CF antibody in poliomyelitis.

SUMMARY AND CONCLUSIONS

CF tests with Type 2 poliomyelitis antigen (MEF1) were performed on the pre- and postinfection sera of 20 *cynomolgus* monkeys which developed paralytic, non-paralytic, or inapparent infection following oral administration of a Type 2 strain of virus (Y-SK). All the monkeys developed neutralizing antibody, and 17 developed CF antibody in an original serum dilution titer of 1:4 or greater. The 3 monkeys which did not develop this level of CF antibody were in a group of 7 which died within 8 days after onset of paralysis. The CF titers were as high at 2 to 6 days after onset of paralysis in the other 4 moribund or dead monkeys as in the surviving animals tested 16 to 20 days after onset of paralysis. All the surviving monkeys were tested 4 weeks after the first dose of virus and the CF titers were of the same order of magnitude in the groups with paralytic, non-paralytic, or inapparent infection. The Type 2 poliomyelitis CF titers developed in monkeys as a result of infection with homotypic virus were not greater than those found in human beings infected with heterotypic Type 1 poliomyelitis strains.

BIBLIOGRAPHY

1. Casals, J., Olitsky, P. K., and Sabin, A. B., *J. Exp. Med.*, 1952, **96**, 35.
2. Casals, J., Olitsky, P. K., and Anslow, R. O., *J. Exp. Med.*, 1951, **94**, 123.
3. Sabin, A. B., and Olitsky, P. K., *J. Exp. Med.*, 1936, **64**, 739.
4. Morgan, I. M., *Am. J. Hyg.*, 1947, **45**, 390.
5. von Magnus, H., and Melnick, J. L., *J. Immunol.*, 1948, **60**, 583.
6. von Magnus, H., *Acta path. et microbiol. scand.* 1950, **27**, 222.
7. Steigman, A. J., and Sabin, A. B., *J. Exp. Med.*, 1949, **90**, 349.
8. Hammon, W. M., and Roberts, E. C., *Proc. Soc. Exp. Biol. and Med.*, 1948, **69**, 256.
9. Sabin, A. B., and Ward, R., *J. Exp. Med.*, 1941, **73**, 757.
10. Bodian, D., and Howe, H. A., *Bull. Johns Hopkins Hosp.*, 1941, **68**, 58.
11. Sabin, A. B., *Am. J. Pub. Health*, 1951, **41**, 1215.