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Resistance of Convalescent *Macaca mulatta* to Challenge with Homologous and Heterologous Strains of Poliomyelitis Virus.*

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Reports concerning the immunity that occurs in monkeys following recovery from infection with poliomyelitis virus have been variable,[1-16] the incidence of such immunity in some instances being high and in others low; *e.g.*, Flexner and Lewis[1] and Leiner and Von Wiesner[2] reported negative results while Burnet and McNamara[3] and Flexner[7] reported positive results. Often the same laboratory, *e.g.*, Paul and Trask,[5] Toomey[8] and this laboratory[12,15] reported both positive and negative findings. The amount of immunity that develops would appear to depend upon several possible variables such as the strains of virus involved, the length of time between the attack and challenge, the challenge dose, and the route of inoculation. In general, a higher degree of immunity occurs when recovered animals have been challenged with an homologous strain of virus rather than with heterologous strains.

The current report was designed to determine, under as uniform and standardized conditions as possible, with an adequate number of monkeys, the comparative resistance of convalescent *Macca mulatta* to challenge with both an homologous and with heterologous viruses.

Procedures. The BK strain which in 1941[12] in this laboratory had shown promise of producing a high degree of immunity to both homologous and heterologous challenge was selected as the base strain for this comparison.

1. *Preparation of Pool.* Twenty monkeys were inoculated with BK cord suspension from the seventeenth passage. Twelve developed symptoms within the first two weeks and from these the cord and brain stems were harvested during the acute phase of the disease. A pool was made by grinding the entire collection, suspending in saline at 10% concentration by weight, and centrifuging to remove the coarser particles. The supernatant was then placed in phials containing 5-25 cc each and stored in a deep freeze box where they were kept at a maximum temperature of minus 50°C until used.

2. *Titration of Pool.* This pool was titrated in 10-fold dilutions with the following results:

10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
10/10	10/10	7/10	6/10	0/4

PD_{50} 10^{-4}

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3. *Selection of Dosage to Insure High Attack Rate.* Since all animals inoculated at 10^{-2} developed symptoms and 7 out of 10 at 10^{-3}, we selected an intermediate dosage, *i.e.*, 1:500 dilution or 20 M.I.D. for the initial infection experiment.

4. *Standardized Inoculation Procedure.* One cc of inoculum containing the selected number of M.I.D. of virus was inoculated intracerebrally into the left pre-motor area.

5. *Daily Temperatures.* Rectal temperatures were recorded daily beginning the day following inoculation and being continued either until the acute phase of the disease was passed or for 30 days in non-attack animals.

6. *Spinal Fluid Cell Count.* A cell count was made on the spinal fluid of each animal prior to inoculation and again at the time of onset of symptoms or in non-paralyzed animals between the tenth and fourteenth day.

7. *Technic of Daily Muscle Checks.* Muscle weakness or paralysis was recorded daily in 4 degrees of severity, 1 representing the least amount of weakness and 4 the greatest degree.

8. *Homologous Challenge.* Reinoculation with homologous virus of 65 recovered animals 2 to 3½ months after first inoculation. (Points 3-7 above were repeated on animals at the time of reinoculation tests).

9. *Heterologous Challenge.* Reinoculation of 60 BK immune animals with 6 heterologous strains of virus one to 2 months following homologous challenge. (Points 3 to 7 also repeated).

Results. Attack Rate. One hundred and one animals were injected by the intracerebral route in the left pre-motor area with 1 ml dose of cord suspension containing 20 M.I.D. of BK virus. Ninety-five of these animals developed visible symptoms, the attack rate therefore being about 95%. Apart from natural deaths and the control animals sacrificed during the experiment, 50 of the animals recovered sufficiently to be used in the following test.

Challenge of Recovered Animals with Homologous Virus. Fifteen monkeys recovered from the titration experiment under Point 2, and the 50 recovered from the above attack rate study, were allowed to convalesce for periods ranging from 2 to 3½ months and then subsequently were inoculated, some with 100 M.I.D. and some with 1000 M.I.D. of BK virus. Two of the 65 animals, monkeys 4837 and 5038, developed visible increased symptoms, motion picture records being kept of both. These animals were sacrificed and attempts made to transmit the virus to other monkeys. One of the attempts, with the cord of monkey 4837, was successful. Monkey 4837 gave the following history:-

First inoculation: February 14, 1947, intracerebral inoculation, with 1.0 cc of BK pool No. 3, 1-1000 dilution, *i.e.* 10 M.I.D. of virus.

First Symptoms:- February 21/47, right leg and left leg weak.

Maximum Paralysis, first attack right arm 2, left arm 2, right leg 4, left leg 4.

Residual Paralysis, June 4/47, right arm 0, left arm 1, right leg 4, left leg 4.

Challenge Inoculation:- June 4/47, with 100 M.I.D. of homologous BK virus from Pool 3.

June 18/48, Temperature 104°, also 70 cells in spinal fluid.

Maximum paralysis, second attack, right arm 2, left arm 3. Animal photographed and sacrificed on June 23/48.

Sections were made for histologic study. The pathologists reported changes compatible with poliomyelitis. Cord material was inoculated into monkeys 5076 and 5077, the latter developing characteristic paralytic symptoms on the tenth day.

From these observations it is projected that at least one recovered animal, in the series of 65 monkeys, upon challenge with homologous BK virus, developed a second attack. Sixty-three of these 65, however, definitely were resistant and it is concluded that they were immune to the challenge dose of virus used.

Challenge with Heterologous Virus. Sixty of the 63 monkeys definitely resistant to homologous challenge were divided into 6 groups of 10 each and each group subsequently challenged with a heterologous virus, 10% suspension of infected monkey cord, the fol-

TABLE I.

Virus strains in challenge	BK	McK	Ca	Fr	MV	Le	La
Recovered from BK virus	63/65	10/10	10/10	9/10	7/10	0/10	0/10

lowing 6 strains being used: McK and Le from the Los Angeles area isolated in our own laboratory; Ca from Dr. H. K. Faber in San Francisco; Fr from Dr. David Bodian in Baltimore; M. V., the much used strain from the Rockefeller Institute, procured by us from Dr. E. W. Schultz of Stanford University; and La, the Lansing strain received from Dr. W. McD. Hammon of the University of California. Table I gives the summary of the results of both homologous and heterologous challenge, the denominator representing the number challenged in each group and the numerator the number definitely immune in each group.

These results indicate a close antigenic relationship between BK, McK, Ca and Fr strains, partial relationship between BK and MV and no close relationship between BK and Le or La strains. It should be pointed out, however, that 7 of the La-challenged group and 5 of the Le-challenged group recovered from the second attack. Such a recovery rate is much higher than we have observed in this laboratory in animals that develop first attacks with La and Le viruses, the death rate from these two strains being near 100%. A partial immunity of BK recovered animals to these two strains therefore was apparent.

Sections from the cords of the second attack animals challenged with heterologous virus exhibited histopathologic changes compatible with poliomyelitis. Virus was recovered from one each of the MV, Le and La second-attack animals from which attempts were made to transmit virus.

Studies are now in progress to confirm the antigenic relationship observed in this report by the additional methods of (a) challenging vaccinated animals with both homologous and heterologous viruses and (b) by testing the sera of the immune animals for neutralizing antibodies against both homologous and heterologous viruses.

Conclusions and summary. 1. Sixty-five *Macacca mulatta* recovered from a primary attack of poliomyelitis, induced by intracerebral inoculation of BK strain of virus, were subsequently challenged either with 100 or 1000 M.I.D. of the same pool of virus. Sixty-three exhibited complete resistance to challenge, only 2 showing increased paralysis following challenge. From one of these 2 animals, virus was recovered by passage 4 days following the onset of second attack symptoms which was 4 months following the first inoculation.

2. Sixty of the immune monkeys were divided into groups of 10 each and the monkeys of each group rechallenged with one of the following heterologous viruses, McK, Ca, Fr, MV, La and Le. All animals inoculated with McK and Ca viruses were immune and 9 of the 10 challenged with Fr were immune. These results indicate that BK, McK, Ca and Fr viruses are closely related and may be placed in a single immunogenic group. For the time being, we suggest that these and others which may be added to the group be designated as Group A or Group I of monkey-adapted strains from man.

3. The MV strain shows partial relationship since 7 of the 10 BK-recovered monkeys, challenged with MV, were immune.

4. The La and Le strains are not closely related to BK since all BK immune animals challenged with both of these two viruses developed second attacks. Whether these two belong to an identical group or whether each represents an independent strain must be determined by future study.