IMMUNIZATION OF GUINEA PIGS WITH A MODIFIED STRAIN OF LYMPHOCYTIC CHORIOMENINGITIS VIRUS

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Two strains of lymphocytic choriomeningitis virus obtained in 1935 from naturally infected stock mice have been used for experimental work in this laboratory. These strains were highly virulent for guinea pigs when isolated. The present paper deals with the effect on the virus of serial passage through guinea pigs and mice and the use of a modified strain as an immunizing agent for guinea pigs.

The Strains Employed

Strain A, which was used in most of the experiments reported in previous papers (1, 2), showed no marked change in the course of 8 serial passages through guinea pigs by subcutaneous injection[1] or 10 serial transfers in virus-free mice by intracerebral inoculation. The mouse passages were discontinued after the 10th transfer, but the guinea pig passage strain has been maintained.

Strain B was obtained from two guinea pigs, both of which developed severe choriomeningitis after intracerebral inoculation with the brain of a mouse and were killed when moribund on the 12th or 13th day. Virus from their brains has thus far been passed in series through 14 guinea pigs by subcutaneous injection and in another series through 34 virus-free mice by intracerebral inoculation. Brain suspensions were used for subinoculation in guinea pigs and mice.

Effect on Strain B of Serial Passage through Guinea Pigs

The transfers through guinea pigs have maintained the high virulence of strain B for this species. Subcutaneous or intracerebral injections are usually fatal, even when very small amounts of virus are given, such as 1 to 10 minimal infective doses (M.I.D.), and the guinea

[1] Subcutaneous inoculations were made into the metatarsal pads.

pigs that do not die develop a very severe disease with marked emaciation and slow recovery.

All mice injected intracerebrally with this strain become sick, but many of them survive and fail to show the striking convulsions which characterize the disease induced by intracerebral injection with the mouse passage strain. Intravenous or intraperitoneal injection causes illness in the majority of the mice, but the rate of mortality is considerably lower than after intracerebral inoculation. Subcutaneous injection and intranasal instillation produce no symptoms and yet render mice resistant to intracerebral inoculation. The virus can be demonstrated in the blood of infected mice and occasionally persists in it for more than a month after recovery.

Effect on Strain B of Serial Passage through Mice

The results of intracerebral tests show that mouse passage has increased the virulence of strain B for mice. Subclinical infections occasionally occurred in the early passages even when large amounts of virus were inoculated, but injections with more than 10 M.I.D. of virus from the later passages caused typical convulsions and death on the 6th or 7th day in nearly 100 per cent of the mice. Doses near the borderline of infectivity, however, occasionally produced immunity without apparent symptoms. Intravenous, intraperitoneal, subcutaneous, or intranasal inoculations of virus from the later passages have failed to cause any definite signs of illness, but have immunized against intracerebral injection with virus. The blood of three mice injected intracerebrally was infectious when symptoms were manifest, but the virus was not demonstrated in the blood in repeated tests made 1 month after inoculation.

The pathogenicity of the mouse passage strain for guinea pigs has greatly decreased. It is not known exactly when this loss of virulence occurred; brain suspension from the 8th serial transfer was avirulent for guinea pigs. Intracerebral injections of this modified virus are followed by fever lasting several days and no other sign of disease. Some guinea pigs do not gain weight during the febrile period as rapidly as uninjected controls, but to an uninformed observer they do not appear ill. None of the symptoms that characterize the infection with the original virus or the guinea pig passage strain, such

as labored respiration, drowsiness, salivation, vomiting, conjunctivitis, and emaciation, have been noted, and all of the 53 guinea pigs injected either intracerebrally or subcutaneously survived. Subcutaneous inoculations cause fever in the majority of the cases, but it is usually lower and of shorter duration than that induced by intracerebral injection. The amount of virus inoculated either intracerebrally or subcutaneously did not markedly influence the reaction of the animals. The blood contained virus during the febrile reaction on the 7th day after injection in two guinea pigs tested.

The virulence of the modified strain for guinea pigs was not restored by a single guinea pig passage, no matter whether blood or brain was used for subinoculation. Intracerebral or subcutaneous injection with a mixture of mouse brain containing modified virus and guinea pig brain in which the virulent strain had been inactivated by heating at 70°C. for ½ hour, likewise failed to increase the virulence of the modified strain. Nor has it been possible to modify the virulent guinea pig passage strain by one or two intracerebral passages through mice. This fact indicates that the modification of the mouse strain probably did not take place during the first two serial transfers in mice.

In the following section of the paper the strain B passed through mice and attenuated for guinea pigs will be called "mouse virus," while the strains A and B passed through guinea pigs and highly virulent for this species will be referred to as "guinea pig virus." For immunity tests with guinea pigs, strains A and B were often mixed to insure a high degree of virulence in the inoculum.

Immunization of Guinea Pigs with Mouse Virus

A single intracerebral or subcutaneous injection with mouse virus was sufficient to induce a high degree of resistance to intracerebral or subcutaneous inoculation with a large amount of guinea pig virus, such as 0.1 or 0.5 cc. of a 10 per cent guinea pig brain suspension, given 3 weeks later. The development of fever in some guinea pigs following subcutaneous inoculation apparently had no relation to the degree of immunity produced.

The immunity arose rapidly as Table I shows. Subcutaneous injection of mouse virus into the left hind pad followed immediately by

a similar injection of guinea pig virus into the right hind pad failed to prevent the disease induced by the latter strain, but there is evi-

TABLE I

Immunity of Guinea Pigs in Relation to Circulating Antivirus

Guinea pig No.	Route of inoculation with mouse virus	Test for antivirus in serum drawn 2–6 hrs. before test of immunity	Test of immunity by injection with guinea pig virus				
			Time after inoculation with mouse virus	Route of inoculation	Reaction	Controls	
						No.	Reaction
1					D 11	17	D 14
2					D 13	18	D 15
3	sc		1 min.	sc	D 27	19	D 9
4					Mild disease, rapid recovery	20	D 11
5					Severe disease, slow recovery	21	D 14
6		–			Fever	22	D 12
	sc		4 days	sc			
7		–			Mild disease, rapid recovery	23	D 12
8		–			Slight fever	24–27	D 11–15
9	sc	–	8 days	ic	None		
10		–			None	28–30	Very severe disease, slow recovery
11		–			Slight fever		
12	sc	+			Slight fever	31	D 11
13		–			None		
			10 days	ic			
14		+			None		
15	ic	–			None	32	D 12
16		–			None		

sc = subcutaneously.
ic = intracerebrally.
D 11 = died on 11th day.
– = no antivirus detected.
+ = antivirus probably present.

dence that its course was modified in two animals (Nos. 4 and 5). On the 4th day a degree of resistance was apparent, and on the 8th or 10th day, 6 of the 9 guinea pigs tested were completely resistant,

while the remainder developed a transient fever. Other guinea pigs not recorded in the table, which were tested for immunity by intracerebral inoculation on the 18th, 21st, or 31st day after subcutaneous or intracerebral injection with mouse virus, were all completely resistant.

The Relation of Circulating Antivirus to the Immunity of Guinea Pigs.—The failure to detect circulating antivirus in mice possessing a high degree of immunity to choriomeningitis was reported in a previous communication (1). Numerous other sera from mice immunized or hyperimmunized by a variety of methods have since been tested but none of them had definite neutralizing power. If antivirus was present, its concentration was too low to account for the high degree of resistance shown by the mice. Extracts of the brains and livers of mice that had become resistant to intracerebral injection by subcutaneous inoculation with mouse virus likewise failed to give any evidence of an antiviral substance. The sera of immune guinea pigs, on the other hand, usually contained antivirus when tested a few weeks after recovery.

Because of this apparent difference in mechanism between the immunity of mice and that of guinea pigs, an attempt was made to determine whether the immunity of the latter was due solely to circulating antivirus or, as in mice, to another factor as yet unknown but closely associated with the tissues affected by the virus.

A number of guinea pigs injected subcutaneously or intracerebrally with mouse virus were bled by heart puncture under deep ether anesthesia a few hours before they were tested for immunity as indicated in Table I. The sera obtained were avirulent for mice, except that of guinea pig 11 which produced the disease in one of the two mice inoculated. Neutralization tests were performed with the sera according to the method already described (1).

The results of the tests recorded in Table I indicate that the high degree of immunity present on the 8th and the 10th day after injection was not associated with demonstrable antivirus in the majority of the animals. Other guinea pig sera drawn 1 month after injection with mouse virus had definite neutralizing properties.

DISCUSSION

Strains of choriomeningitis virus isolated in earlier experiments from mice of the infected stock (2) often differed in virulence for guinea

pigs although similar in every other respect. Some caused merely a febrile reaction in the majority of the animals, while others induced a very severe, fatal disease. However, not one of the strains of low virulence was quite consistent in its effect, a fatal disease sometimes resulting with those that were relatively non-pathogenic. The variations in severity of the disease were thought to be mainly due to differences in the susceptibility of guinea pigs, and this assumption was supported by the fact that the virus obtained from the same mouse at different times was differently pathogenic for guinea pigs (1, Table III). However, the rapid and striking change of strain B brought about by a few serial passages through mice now suggests that variations of the virus may be in part responsible for those differences. It has been shown that guinea pigs from the same stock as those used before react uniformly both to the guinea pig passage strain, which invariably causes very severe choriomeningitis, fatal in 80 to 90 per cent of the cases, and to the modified mouse strain, which has failed to produce symptoms in any of the 53 guinea pigs injected.

The modified virus is a very good immunizing agent for guinea pigs, since it is harmless to the animals and produces a solid immunity after a single injection.

The immunity to choriomeningitis of guinea pigs and mice differs in that immune guinea pigs as a rule develop circulating antivirus, whereas the sera from immune mice thus far tested have had no definite neutralizing properties. The comparatively late appearance of the antivirus in the guinea pig indicates, however, that it does not solely account for their immunity, which seems to be closely associated with the tissues affected by the virus. Similar observations have been reported for other viruses, for example, foot-and-mouth disease (3) and poliomyelitis (4–6). The possibility of infecting immune animal tissues *in vitro* with a variety of viruses, *e.g.*, virus III (7), vaccinia (8), herpes simplex (9), the salivary gland virus of guinea pigs (10), and pseudorabies (11), does not necessarily weigh against this conception of the mechanism of antiviral immunity. The washing and soaking of the tissue fragments, which usually preceded the infection in such experiments, may have removed an antiviral substance that was fixed in the tissues or rendered it ineffec-

tive by dilution. The evidence thus far obtained with tissue extracts from immune mice does not favor the assumption that such a substance is present, but further tests are necessary to disprove its existence. It is also possible that the removal of the tissue from its physiological environment and its maintenance under extremely unfavorable conditions alter the permeability to viruses of the cellular membrane. This permeability may provide the basis of susceptibility and immunity.

SUMMARY

A strain of choriomeningitis virus which was highly virulent for guinea pigs, as isolated from a naturally infected white mouse, has been markedly attenuated for guinea pigs by serial intracerebral passage through white mice. The change of virulence occurred before the 8th serial passage. The modified virus as a rule produces fever in guinea pigs but no other symptoms, and the infection is followed by a very solid immunity. Parallel passages of the same strain through guinea pigs have maintained its high virulence for this species but slightly reduced its pathogenicity for mice. These observations indicate that the differences in virulence for guinea pigs noted before (1, 2) with different strains of choriomeningitis virus obtained from infected stock mice may be due not only to differences in the susceptibility of guinea pigs but also to variations in the virulence of the virus.

A marked degree of resistance was demonstrable in several guinea pigs on the 4th, 8th, and 10th day after injection with modified virus, when antivirus could not yet be detected in the serum. Circulating antivirus appears therefore to play a secondary part in their immunity, which seems to be closely associated with the tissues as in mice.

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