Archives of Virology 82, 105—109 (1984)

Archives of Virology

Sustained Viremia in Experimental Hamster Scrapie

Brief Report

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Accepted April 8, 1984

Summary

After an intraperitoneal injection of scrapie agent into hamsters, a viremic phase is maintained from day 10 to at least day 40 post infection. Infectivity can be detected in the brain as early as 5—10 days after inoculation.

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After peripheral infection, spread of virus along axons is a possible route for herpes and polio virus, and for rabies virus it is the only pathway to reach the central nervous system (CNS). Most conventional viruses, however, spread and infect the brain via a viremic phase in the blood (see 8).

For the unconventional slow agents causing subacute spongiform encephalopathies (6) the entrance of scrapie agent 139A into the CNS and its spread via sympathic nerves has been demonstrated in mice (9—12). Viremia in these diseases, however, is a matter of debate. A few years ago viremia has been shown to occur in guinea pigs (14) or more recently in mice (13) infected with Creutzfeldt-Jakob agent although earlier experiments failed to detect infectivity of this disease and Kuru in the blood of man (6). The rapid distribution of infectivity in various organs after infection with scrapie (4, 16) would argue for a viremic phase and thus spread of agent via the blood stream. However, several trials to detect scrapie infectivity in the blood of goats (7, 17) or mice (4) or to detect the slow agent causing transmissible mink encephalopathy (15) in blood of mink have failed. Very early after intracerebral infection of mice (5) or in some cases of terminal disease, presence of the agent in blood has been reported (1, 2), although levels of infectivity were very low and might have resulted from tissue contamination (1).

To determine low levels of infectivity in the blood or an organ, however, is a technical problem with these diseases as it can only be done by an intracerebral (i.c.) injection into a recipient animal thereby severely limiting the amount of material to be tested. With an isolation procedure developed to purify scrapie infectivity from hamster brain (3), one can now overcome this problem and therefore, it was evaluated i) whether one can find viremia in hamsters and ii) how early scrapie infectivity can be detected in brain after an intraperitoneal infection.

The following experiemtal protocol was used: Twenty five inbred CLAC hamsters were infected intraperitoneally with 100 µl of a 10^{-2} dilution of hamster scrapie brain containing about 10^6 infectious i.c. units (3). Five, 10, 20, 30 and 40 days later (about one third of the total incubation period) 5 animals at a time were anaesthetized with Ketanest and Rampun, the thorax was opened, and blood was withdrawn by cardiac puncture. A total of 10—12 ml was obtained. Subsequently the brains were removed. These materials were used for partial purification and hence concentration of the suspected scrapie agent (3). As low levels of infectivity were to be expected and to invalidate the argument that this low level after the isolation and concentration procedure of the blood might have resulted from tissue contamination (1) we introduced the following control: The hearts of the animals killed at 20, 30 and 40 days post infection, from which the blood had been withdrawn were punctured a second time and about 50 µl of blood were taken. Using this same puncture needle the material was directly injected i.c. into recipient hamsters. If infectivity was indeed derived from tissue damage the animals receiving the material from this second heart puncture should be expected to develope scrapie.

The 10—12 ml of whole blood as well as the brains (5 brains/time point) were run through a differential centrifugation procedure which resulted in a fraction of enriched scrapie infectivity designated P_{215S} (3). Dispersing this material in only 250 µl of phosphate buffered saline with ultrasonication and injecting 50 µl i.c. for the assay of infectivity, we were able to apply the equivalent of 2 ml blood or one complete hamster brain to a single animal. The animals were than scored for the development of scrapie by incubation period measurement and the results are summarized in the table.

No animal which received the material from heart puncture directly developed scrapie. Thus scrapie infectivity detected in the blood concentrate was probably not derived from heart muscle destruction. In the group of animals having received concentrated blood, taken 5 days post infection, only one out of six animals in two independent experiments came down with the disease. Without exception all animals injected with material sampled 10, 20, 30 and 40 days post infection developed scrapie. Thus a viremia as a prerequisite for hematogenous spread of the agent is fulfilled and explains the early distribution of infectivity to many organs in mice (4,16).

Table 1

Sampling of material (days post intraperitoneal infection)	Scrapie incidence and incubation period (days ± standard error)		
	Blood directly from 2nd cardiac puncture	Blood concentrate	Brain concentrate
5	—	a 1/4 214 b 0/2	a 2/2 127 ± 9 b 2/3 182 ± 48
10	—	a 4/4 133 ± 15 b 4/4 169 ± 27	2/2 105 ± 0 —
20	0/3	3/3 186 ± 35	3/3 113 ± 6
30	0/3	4/4 191 ± 48	4/4 119 ± 12
40	0/3	2/2 150 ± 35	4/4 129 ± 25

Scrapie incidence and incubation period of hamsters which received material sampled at indicated times from animals after an intraperitoneal infection. Concentrated blood and brain were generally injected into four animals. Some hamsters died within 24 hours infection because of extensive bleeding caused by the detergent in the sample. a and b are independent experiments; — not determined

The level of infectivity in blood is low though and when calculated from the range of incubation period (130—200 days), assuming some loss during the isolation procedure (3), is in the order of 5—50 infectious i.c. units per ml of blood.

All animals except one receiving the equivalent of one brain of an intraperitoneally infected animal developed the disease even when the brains were taken 5 days post infection. Incubation periods in all but one (5 days exp. b) group were shorter with brain material than with blood concentrates. Thus after an intraperitoneal injection of about 10^6 i.c. infectious units some agent (about 100 infectious i.c. units per brain) must have entered the central nervous system already very early, but according to the incubation period assay no increase of infectivity could be measured during the 40 days after infection. The infectivity isolated from brain is unlikely to be derived from residual blood being present in the material: i) the brains were removed after cardiac puncture and the volume of blood in one brain weighing 0.9—1 gram is low compared to 2 ml of tested blood, ii) the content of infectivity in 2 ml of blood is lower than that in 1 g brain according to measurement of incubation periods.

In conformity with experiments on Creutzfeldt Jakob disease in guinea pigs (14) and mice (13) a sustained viremia has now been shown to occur with scrapie agent 263K in hamsters. Furthermore infectivity has been detected in brains of hamsters 5—10 days after an intraperitoneal infection. This early presence of agent in the brain again can only be explained by hematogenous spread.

The low level of viremia lasting for at least 40 days is surprising. Perhaps it may be seen in a connection with a rapid increase of infectivity in spleen and lymph nodes to a certain level which then remains almost constant or slightly drops throughout the incubation period (4). Cell-bound infectivity as found in Creutzfeldt-Jakob disease (13, 14) could be released continously into the blood stream from these tissues thereby enabling a steady flow of agent from these to other organs.

The early low level of infectivity in brain may not be important in the pathogenesis of the disease, as no increase of infectivity could be measured during forty days. Thus agent may have reached a compartment where it persists but does not replicate. If, however, agent replication in the brain requires a long latent period, then the early presence of infectivity in this organ could have relevance for the pathogenesis of the disease.

Acknowledgements

The technical assistance of C. Berndt is gratefully acknowledged. The experiments were supported by the Deutsche Forschungsgemeinschaft.

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Received March 26, 1984