Pathogenesis of Scrapie: Study of the Temporal Development of Clinical Symptoms, of Infectivity Titres and Scrapie-associated Fibrils in Brains of Hamsters Infected Intraperitoneally

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SUMMARY

After an intraperitoneal infection of hamsters with scrapie agent, early low and constant titres of about 100 LD$_{50}$/brain between days 10 to 50 were followed by a dramatic increase to maximum levels of $3 \times 10^9$ LD$_{50}$/brain within about 15 days. The plateau of maximum infectivity remained unchanged from day 70 to the time of the first and final signs of disease at 95 and 123 days post-infection, respectively. Scrapie-associated fibrils (SAF) as measured by immunoblotting of SAF protein could not be detected before 79 days post-infection even when a total brain was used for analysis. Subsequently, the concentration of SAF increased gradually by about 10000-fold until the time of clinical disease. The kinetics suggest a virus-induced amyloidosis of the brain as the cause of disease.

Unconventional slow viruses cause slow, progressive, degenerative diseases of the central nervous system (for review, see Gajdusek, 1985). Kinetic studies of the appearance and distribution of infectivity in mice after a peripheral (subcutaneous) infection with the scrapie agent revealed (i) that infectivity spreads rapidly to various organs in the periphery, reaching the earliest and highest titres in spleen and lymph nodes without histopathology and (ii) that measurable titres in the target organ, the brain, appear late after infection, reaching higher titres than in any other organ prior to the onset of clinical symptoms (Eklund et al., 1967).

Here we report data on virus replication and the production of disease-specific scrapie-associated fibrils (SAF) (Merz et al., 1981, 1983; Diringer et al., 1983; Prusiner et al., 1983) in brains of hamsters after a peripheral infection. Under these conditions the virus replicates to high titres in the brain in a short period of time, inducing the production of SAF.

We performed an experiment in which 40 AURA hamsters were each infected intraperitoneally (i.p.) with 0.1 ml of a 5% brain homogenate in phosphate-buffered saline containing about $10^7$ intracerebral (i.c.) infective units of the 263K strain of scrapie (Kimberlin & Walker, 1977). (In an independent experiment with 15 hamsters this dose gave an incubation period of 123 days with a S.D. of ± 9 days.) The animals were scored for clinical symptoms until they were used for infectivity and SAF protein analyses.

At various times after infection animals selected randomly were killed, 10% brain homogenates (10 ml) of the individual brains were prepared and small portions (50 μl) of each homogenate were injected i.c. into five recipient hamsters to assay infectivity present in the individual donor hamster brains by incubation period measurements (Dickinson & Meikle, 1969).

Subsequently, the individual homogenates were subjected to a differential centrifugation and extraction procedure resulting in the concentration of SAF and thus of SAF protein in a formic acid extract (Multhaup et al., 1985) in which SAF protein was determined by immunoblotting (Bode et al., 1985).
Fig. 1. Temporal development of infectivity (○, ± standard deviation), SAF formation (■ positive, □ negative) in hamster brains, and the appearance of the beginning (a) and final (b) clinical symptoms after an i.p. infection with scrapie agent. The outer left ordinate represents incubation periods in recipient hamsters which were converted into i.c. titres in donor brains (inner left ordinate). The highest titres of infectivity, i.e. $3 \times 10^9$ LD$_{50}$/brain (determined in three independent experiments by endpoint titration), were related to the shortest incubation period ($72 \pm 3$ days). Intracerebral infection with 50 μl of a 10% brain homogenate limited the assay of infectivity to a minimum value of roughly 100 LD$_{50}$/brain. These were related to incubation periods longer than 150 days (Diringer et al., 1983b).

Between these values, tenfold differences in the amount of infectivity relate to decreases in incubation periods of about 10 to 12 days (Prusiner et al., 1981; Marsh & Hanson, 1978; Kimberlin & Walker, 1977, 1986; Diringer et al., 1983b). For the semi-quantitative assay of SAF protein, formic acid extracts obtained from preparations of individual hamster brains before drying were divided into fractions representing 0.9, 0.09, 0.009 and 0.001 brain equivalents. The limitations of the experimental system allows that we relate these to roughly 1, 0.1, 0.01 and 0.001 brain equivalents. By immunoblotting the lowest dilution was determined at which the test for SAF protein was positive. On the right ordinate, negative and positive results of SAF protein determinations in 1 brain equivalent have been plotted as a 1 log$_{10}$ difference. At days 10 to 50 the scrapie incidence (diseased/total infected hamsters) is given.

The results of the experiment are summarized in Fig. 1. Ten to 50 days after an i.p. infection, very low and constant levels of infectivity, i.e. about 100 LD$_{50}$/brain, were detectable in the hamster brains (Fig. 1). This is indicated by the fact that during this early period not all i.c.-infected recipient hamsters developed the disease within a 350 day scoring period. Furthermore, recipient animals that developed the disease needed incubation periods of more than 150 days. Similar constant low titres in brain after an i.p. infection during the early infection period have been observed before (Diringer, 1984).
A very sharp decrease in incubation period was observed between 60 and 70 days post-infection in the i.c.-infected recipient hamsters, indicating that in all the donor brains (except one at day 60) infectivity titres must have risen suddenly to surprisingly high levels, varying between $10^7$ LD$_{50}$ and $10^9$ LD$_{50}$. Almost no variation in incubation period and hence in infectivity titres was observed between 80 and 130 days post-infection. During this period the first animals with early clinical symptoms were observed at 95 days. Full scrapie cases in the remaining group of animals occurred between 115 and 130 days.

Combining the points of highest infectivity at any given time during the period of great variation (to answer the question what is the shortest time for development of high titres in the brain?), the curve in fact demonstrated that scrapie agent replicated in about 10 to 15 days from $10^2$ to $10^9$ LD$_{50}$/brain, a titre which subsequently did not change. When a complete hamster brain equivalent was analysed for SAF, the earliest time post-infection at which we were able to detect SAF protein was 79 days. A total of 27 animals were tested and scored negative between 12 and 77 days post-infection. Increasing amounts of SAF protein were found after 79 days. At 87, 95 and 119 days we detected this protein in samples representing 0.1, 0.01 and 0.001 brain equivalents, respectively. Thus, SAF protein became measurable only after infectivity had increased to the highest levels. SAF protein accumulated subsequently, reaching 1000-fold higher levels at a time when the first clinical symptoms became apparent.

The highest concentration of SAF protein, a 10000-fold increase, was reached at about the time of full clinical disease.

A peripheral infection of hamsters with scrapie according to our experiment clearly results in maximum infectivity titres in the brain arising very suddenly and before the onset of clinical signs of scrapie. Although such a rapid increase of infectivity has never been recognized before, subcutaneous (Eklund et al., 1967) or i.c. infection (Collis & Kimberlin, 1985) of mice as well as i.c. infection of hamsters (Baringer et al., 1983) has been shown to result in a plateau of infectivity much earlier than clinical signs emerge.

An instant increase of infectivity in the central nervous system after an i.p. infection can be seen in studies dealing with neuronal spread of scrapie agent. In these studies too, the rate of agent replication in the brain was greater after an i.p. than an i.c. infection (Kimberlin & Walker, 1979, 1986).

Nevertheless, replication of scrapie agent has been reported to occur continuously from i.c. infection to the onset of clinical disease in mice (Kimberlin, 1976) and hamsters (Moreau-Dubois et al., 1982; Kimberlin & Walker, 1986). Indeed, continuous and slow growth is believed to be a characteristic property of unconventional viruses (see Fenner, 1984; Fenner et al., 1974; Gajdusek, 1985).

Considering the artificiality of the i.c. route of infection and the discrepancy in the results obtained by various groups using this route (two, however, support our i.p. results), we question the concept of slow agent replication in brain and would like to suggest the following concept for scrapie pathogenesis in hamsters. The agent reaches the brain relatively early after infection, perhaps infecting only a limited number of target cells (Dickinson & Outram, 1979). For unknown reasons, replication is delayed for a considerable time, but then proceeds rapidly, and may be in a single burst resulting in maximum titres shortly thereafter. Subsequently, clinical disease is induced.

The induced disease could be an amyloidosis, because with the beginning of high infectivity titres the accumulation of SAF begins and it terminates around the time when clinical symptoms are firmly established.

After an i.c. infection, SAF have been detected by electron microscopy well in advance of the onset of clinical scrapie, increasing steadily in number and following rather closely early scrapie-associated changes such as infectivity titres and behavioural changes (Merz et al., 1983b). Our data reported here and earlier (Braig & Diringer, 1985) confirm the observation of SAF formation prior to clinical disease. Beginning with a certain threshold, this formation of SAF seems to relate to clinical symptoms. However, in contrast to the electron microscopy studies the kinetics we obtained document the termination of virus replication before the onset of measurable amounts of SAF.
SAF have been discussed as being a form of the infectious scrapie agent (Diringer et al., 1983b; Merz et al., 1983a; Prusiner et al., 1983). However, all these groups also considered the possibility that these disease-specific fibrils could represent a pathological product of the disease process. If this is the case, as was recognized in the earliest contribution, the morphological appearance of this amyloid-like structure distinguishes it from amyloids known so far (Merz et al., 1981). Moreover, the structural properties of the fibrils (Merz et al., 1981), their staining with congo red (Prusiner et al., 1983), chemical analysis (Multhaup et al., 1983), gene-fishing experiments (Oesch et al., 1985; Chesebro et al., 1985), and our kinetic studies reported here and earlier (Braig & Diringer, 1985) strongly favour the concept that agent replication in the brain causes an amyloidosis. Interestingly, one of the groups doing molecular genetic studies reported that the genetic information for SAF protein is translated into mRNA only in brain and tissue culture neurons. In spleen, however, where high titres of infectivity also develop, or in other tissues no SAF protein-specific mRNA was detectable (Chesebro et al., 1985). This would explain why unconventional viruses induce an amyloidosis only in the central nervous system.

Extracellular deposits of amyloid are known to occur in unconventional slow virus diseases (see Bruce et al., 1976) and similarities between scrapie and Alzheimer's disease are obvious (Bruce, 1984). Scapie models have, therefore, been proposed to be useful systems in which to study cerebral amyloidoses (Wisniewski et al., 1975; Bruce et al., 1976). Kinetic studies on the development of amyloid plaques in mouse models of scrapie (Bruce, 1981) and the results reported here on the temporal development of infectivity, SAF and clinical symptoms in hamsters support this view.

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REFERENCES


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