Replication of the Scrapie Agent in Hamsters Infected Intracerebrally Confirms the Pathogenesis of an Amyloid-inducing Virosis

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SUMMARY

Following intracerebral infection of hamsters with scrapie agent replication started with or without a very short lag phase. Infectivity titres increased exponentially within 35 to 40 days post-infection to a maximum level of \(3 \times 10^9\) LD\(_{50}\) per brain and then remained constant until death. Minimal detectable amounts of scrapie-associated fibrils (SAF) appeared at 42 days and reached high levels 56 days after inoculation. The first clinical symptoms were diagnosed at about 65 days and animals died after 85 to 95 days. These data confirm earlier results in which peripheral infection first revealed agent replication, then SAF formation and finally clinical disease. Unconventional virus diseases, therefore, can best be described as virus-induced, organ-specific amyloidoses.

The scrapie agent is an unconventional slow virus. Such viruses cause subacute spongiform encephalopathies in animals and man leading to death after a comparatively short progressive clinical course of disease. Besides the absence of any measurable immune response and strikingly long incubation periods (years in the natural host, months in experimental animals), infections with unconventional viruses bring about not only degeneration of the brain but also dementia in man (for reviews, see Gajdusek, 1985; Diringer et al., 1986; Fenner et al., 1987). Along with infectivity, scrapie-associated fibrils (SAF) in the brain are characteristic not only for scrapie but for unconventional slow virus diseases in general (Merz et al., 1981, 1987; Diringer et al., 1983; Prusiner et al., 1983). SAF consist mainly of a proteinase K-resistant glycoprotein, the SAF protein [synonyms: PrP for prion protein (Prusiner, 1982) or protease-resistant protein (Bendheim & Bolton, 1986)], which is derived from a normal cellular precursor (Chesebro et al., 1985; Oesch et al., 1985). Intraperitoneal infection of hamsters with the scrapie agent results in formation of SAF protein in the brain as a consequence of virus replication (Czub et al., 1986b). Here we report that intracerebral (i.c.) infection follows the same pattern.

Fifty Syrian hamsters (inbred CLAC) were infected i.c. with 50 µl of a 5% brain homogenate in phosphate-buffered saline containing about 10^7 i.c. infective units of the 263K strain of scrapie (Kimberlin & Walker, 1977). At various times after inoculation randomly selected individual hamsters were scored for clinical symptoms, killed and their brains analysed for scrapie infectivity and SAF protein (Czub et al., 1986b). The results of the experiment are summarized in Fig. 1. After i.c. infection with 10^7 LD\(_{50}\) the scrapie titres of infected whole hamster brains were below the injected dose up to 20 days post-infection. Scrapie titres increased sharply between 20 and 40 days post-infection, and almost all individual hamster brains contained approximately 10^8 to 10^9 LD\(_{50}\)/brain after 35 days of infection. This amount of infectivity remained at a constant level until the end of the disease. This is in agreement with experiments of others (Baringer et al., 1983; Hogan et al., 1986), but it differs from findings of Moreau-Dubois et al. (1982) and Kimberlin & Walker (1986).

The formation of SAF protein and thus of SAF followed the increase of infectivity with a delay of several days. Even using a whole brain sample, negative SAF protein analyses were obtained with almost all individual hamster brains at 30 to 45 days post-infection although those
brains had infectivity titres of $10^8$ LD$_{50}$ and more. The first positive analysis for SAF in 1 brain equivalent, which represents the lowest detectable amount of SAF, was found 42 days post-infection. A 10-fold increase, representing a positive analysis of 0.1 brain equivalent, was found in an animal brain 47 days post-infection. One-hundred and 1000-fold increases of the amount of SAF representing the earliest positive results with 0.01 and 0.001 brain equivalents, respectively, were found in animal brains 51 and 57 days after infection.

The last stage of infection was clinical disease. Early clinical symptoms appeared at about 65 days and full blown disease about 75 days post-infection. Animals died of scrapie between 85 and 95 days post-infection.

These results support and extend our data obtained in an earlier kinetic experiment, in which hamsters were infected intraperitoneally (i.p.) (Czub et al., 1986b). The main difference between the kinetics obtained with the i.c. as compared to the i.p. route of infection is a considerably longer lag phase (with constant low titres of infectivity) of 50 days with the i.p. route to less than 10 days with the i.c. route. Consequently clinical disease is delayed by about 30 days.
to 40 days in the i.p. infected as compared to the i.c. infected animals. We conclude that the
delay in onset of viral replication after an i.p. infection may represent a phase in which virus has
to pass from extraneural tissue into the central nervous system directly. Once this step has
occurred the actual rate of virus replication, the accumulation of the amyloid fibril protein, and
the development of clinical symptoms, are independent of the route of infection in the hamster
system. Virus replication to high titres precedes the formation of the amyloid SAF fibre
independently of the route of infection and this formation again precedes the onset of the
disease.

Continuous replication throughout the incubation and clinical phases has been reported after
i.c. infection in mice (Kimberlin, 1976) and hamsters (Moreau-Dubois et al., 1982; Kimberlin &
Walker, 1986). This is in contrast to our observations. An explanation for this discrepancy may
be that we (looking for the earliest indications of virus replication) scored the infectivity in single
hamster brains, whereas others used pooled materials. Thus from our results we conclude that
independently of the route of infection the pathogenesis of scrapie follows the same pattern once
the scrapie agent has entered the central nervous system. Furthermore we have demonstrated
that in a given system delay in the incubation period after an i.p. as compared to an i.c. infection
is related to processes outside the central nervous system. In the hamster system this observation
merits further discussion. In two independent experiments (Diringer, 1984; Czub et al., 1986b)
low levels of infectivity were shown to be present in the brain for at least 40 days after an i.p.
infection without replication. This low amount of constant infectivity in the brain thus raises the
possibility that the prolonged occurrence of low levels of non-replicating virus after an i.p.
infection reflects a period in which the virus has to penetrate into the central nervous system.
Again, these data emphasize that infectivity and SAFs are not identical (Czub et al., 1986a, b).
The latter exhibit all the typical properties of amyloid fibrils. Therefore, we regard the
pathogenesis of scrapie as a virus-induced, organ-specific amyloidosis (Braig & Diringer, 1985;
Diringer et al., 1986).

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