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INFECTION OF THE CENTRAL NERVOUS SYSTEM OF MICE BY STANDARD SENDAI VIRUS, DEFECTIVE INTERFERING SENDAI VIRUS AND THE MIXTURE OF BOTH: COMPARISON OF VIRUS MULTIPLICATION AND PATHOGENICITY

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Summary. — The intracerebral (i.c.) infection of newborn mice with standard Sendai virus (SV), defective interfering Sendai virus (DV) and their mixture (SV + DV) has been used as a model for the possible role of defective interfering particles of paramyxoviruses in several chronic degenerative diseases of central nervous system (CNS). The dynamics of Sendai virus multiplication and virus distribution in CNS of mice, as well as the histological changes and the clinical symptoms were evaluated for up to 112 days post-infection (p.i.). The infectious virus was detected in the brains of animals inoculated i.c. either with SV, or DV, or SV + DV as soon as by 5 hr p.i., with maximum infectivity titre at 24 hr p.i. In brains of animals inoculated with SV, the virus was detected until 5th day p.i.; nevertheless in those, inoculated with SV + DV or DV, low infectious titres could be detected even at later intervals. In mice inoculated i.c. with DV, traces of Sendai virus were detected in subpassages, as late as 3 months p.i.

Key words: Sendai virus; intracerebral infection of mice; defective interfering particles

In experimental infection of CNS of mice with vesicular stomatitis virus, Doyle and Holland (1973) demonstrated, that defective interfering particles (DIP) of viruses may alter the usual course of virus infection into a slow degenerative form. In the case of paramyxoviruses, which are most frequently considered for the causative agents of several chronic degenerative diseases of man and animals (e.g. subacute sclerosing panencephalitis, multiple sclerosis, lupus erythematosus, polymyositis, demyelinating encephalomyelitis in dogs, Paget's disease of bone), the role of DIP in virus infection has not yet been studied, to our knowledge, in experimental animals. The CNS of mice, the natural host of the Sendai virus (type 1 parainfluenza virus, which usually causes respiratory infections in mice) is abundant in receptors for this virus (Ito *et al.*, 1983). Therefore, the experimental infection

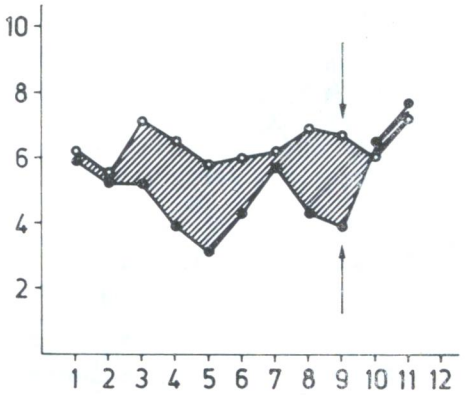

Fig. 1.
Infectivity (log EID_{50}/ml) of Sendai virus-infected allantoic fluids (IAF) from serial passages of undiluted IAF (DV, ●——●) and 10^4 times diluted IAF (SV, ○——○), in chick embryos. The shaded area indicates the infectivity difference between SV and DV from the parallel passages. The arrows point to the log EID_{50}/ml values of SV and DV from the 9th parallel passage, used for i.c. inoculation of the newborn mice. Abscissa: serial number of the passage; ordinate: log EID_{50}/ml.

of mouse CNS with Sendai virus and Sendai DIP and a mixture of both seems a suitable model for such studies (Ruttkay-Nedecká, 1986*b*). In the present paper the results of a long term experiment are briefly described, in which the dynamics of virus multiplication, the virus distribution as revealed by immunofluorescence (IF) and the histological changes in CNS, as well as clinical symptoms in animals after intracerebral (i.c.) inoculation of mice with standard Sendai virus, defective interfering Sendai virus and a mixture of both, have been studied. The results obtained by the histological and IF techniques will be described in a more detail and will be fully documented in the following communication (Rajčáni *et al.*, in preparation). This work had been preceded by the elaboration of the method of Sendai virus DIP purification from the infectious allantoic fluid (IAF) of the chick embryo (CE), complemented with physicochemical and morphological characterization of the purified DIP preparation (Ruttkay-Nedecká, 1984, 1986*a*), as well as by a comparative study of both pathogenetic and immunogenic properties of DIP and standard particles of the Sendai virus after intranasal and intramuscular inoculation in mice (Ruttkay-Nedecká and Libíková, unpublished experiments).

The defective interfering Sendai virus has been obtained as described in the previous communication (Ruttkay-Nedecká, 1986*a*), by serial passages of undiluted IAF, and the standard Sendai virus by parallel serial passages of 10^4 times diluted IAF in 9 to 11-day-old CE. The total number of parallel passages was 11. The number of haemagglutination units in 1 ml of IAF (HAU/ml) was determined by the Salk (pattern) method in test tubes using 0.5 % suspension of rooster red blood cells in phosphate buffered saline pH 7.2 (PBS), at about 4 °C. For each sample of IAF three parallel determinations were made and their geometric means were calculated. The infectivity of IAF was determined in CE, and expressed as EID_{50} in 1 ml IAF. From each ten-fold dilution of IAF, 0.2 ml portions were inoculated in 8 CE, the later were incubated for 2 days at 35 °C, chilled at approx. 4 °C, and their allantoic fluid examined for HA activity. EID_{50}/ml was calculated according to Reed and Muench. As diluent for IAF, in course of EID_{50} determinations, as well as virus passaging, served PBS supplemented with 2 % of heat inactivated bovine serum and antibiotics (500 units per ml of penicillin and 500 μg/ml streptomycin). The interfering activities of IAF from undiluted passages with low infectivity titres were assayed

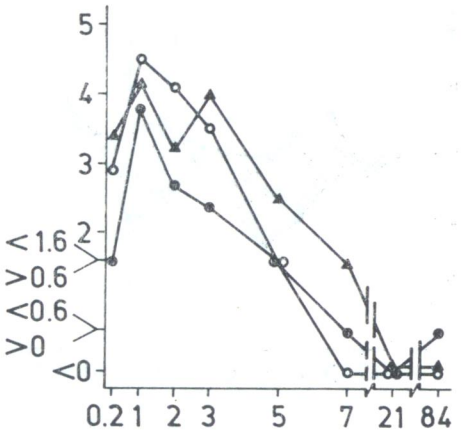

Fig. 2.
Variation of the infectivity (log EID_{50}/ml) of 10 % mice-brain suspensions with time elapsed after i.c. inoculation of mice with standard Sendai virus (SV, ○——○), defective interfering Sendai virus (DV, ●——●), and their mixture (SV + DV. ▲——▲).
Abscissa: days after inoculation; ordinate: log EID_{50}/ml.

by yield reduction test. They reduced the virus yield in mixed infection with standard virus in CE by more than 98 %. The IAF from diluted passages had a mean value of log HAU/ml equal to 2.92 (geometric mean from 11 serial passages) with standard deviation 0.27. The mean value of log HAU/ml of IAF from the 11 parallel serial undiluted passages was almost the same, namely 2.91, but the standard deviation was considerably larger, namely 0.63. The values of log EID_{50}/ml of IAF from the parallel diluted and undiluted serial passages are given in Fig. 1.

As the sources of the defective interfering virus and the standard virus for i.c. inoculation of mice, served the IAF from the 9th undiluted passage (DV) and the IAF from the parallel 9th diluted passage (SV), respectively. Except SV and DV, also their 1 : 1 mixture (SV + DV) and the uninfected (normal) allantoic fluid (NAF) from 12-days old CE were used as inoculum. The groups of 50 newborn laboratory white mice (1 to 2-days old, specific pathogen free) were inoculated i.c. with 20 μl amount of SV ($10^{5.0}EID_{50}$/mice), DV($10^{2.2}$ EID_{50}/mice) and SV + DV($10^{4.7}$ EID_{50}/mice), respectively, into the right cerebral hemisphere. The half of the control group was inoculated i.c. with 20 μl of NAF per mice, and the second half was not inoculated. The virus multiplied in the mouse brain was determined in different intervals p.i. (from 5 hours to 3 month p.i.) by infectivity titrations of the brain suspensions in CE. In different intervals p.i. three arbitrary choosen mice were sacrificed from each group and from their left cerebral hemispheres 10 % suspensions (w/v) in Eagle's basal medium (BEM) supplemented with 10 % of heat inactivated calf serum and 100 μl/ml kanamycin, were prepared. The brain suspensions were clarified by centrifugation (15 min. at 2000 rev/min) and stored at approx. −70 °C until used. The infectivities of the brain suspensions were determined as described for IAF, except that the suspensions were diluted in BEM instead in PBS, each dilution inoculated in 6 CE and the later were incubated for 3 days. Those brain suspensions, which after four-fold dilution and inoculation in CE did not cause any detectable HA activity in the allantoic fluid, were inoculated again, but undiluted, in CE. In absence of HA activity after 4 days of incubation of these CE, a further blind passage in CE was made. Mice were checked daily for clinical symptoms during the period of three month p.i. and weighted weekly until the end of the fourth week p.i.

For histological and IF examinations two mice from each inoculated group (SV, DV, SV + + DV) were sacrificed on the 3rd, 5th, 7th, 14th, 21st, 56th, 84th and 112th day p.i., and their brains removed. Except these animals, mice from the control group and those from the inoculated groups which showed marked hydrocephalus or moribund appearance were examined too. The details of the histological examinations and IF will be given in the following communication (Rajčáni et al., in preparation).

Fig. 2 shows the variation of the infectivity of the Sendai virus in 10 % brain suspension prepared from the left cerebral hemispheres, with time

elapsed after inoculation of newborn mice into the right cerebral hemisphere with SV, DV and SV + DV, respectively. In all virus-inoculated groups (SV, DV, SV + DV), the infectious virus could be detected starting from 5 hours p.i., with maximum infectivity attained on the first day p.i. Between the first and second day p.i., the highest and the lowest infectivity titres were found in the groups SV and DV, respectively. In the left cerebral hemisphere of mice inoculated with SV into the right cerebral hemisphere, the virus could be detected only until the 5th day p.i., but in mice inoculated with SV + DV or DV, low titres were detected also in the later intervals p.i. Nevertheless, only in the group of mice inoculated i.c. with DV, traces of Sendai virus in the brain suspension could be found as late as 3 months p.i., when blind passages in CE were examined. The IAF obtained from the passage subsequent to the blind passages revealed typical Sendai virus particles in the electron microscope, when concentrated by differential centrifugations.

Between the groups of animals inoculated with SV, DV, SV + DV, NAF and those not inoculated, statistically significant differences in the increase of the body weight could not be detected. Some animals developed neurological symptoms or died in the groups of mice inoculated with SV or DV, but not in the other groups.

In the brains of mice infected with SV or SV + DV, positive IF of the Sendai virus antigen was seen on days 3—7 p.i. in ependyma of the third ventricle and lateral ventricles and in a few neurons of cerebral cortex. In mice inoculated with DV, positive IF was detected in a few ependymal cells of the lateral ventricles and in single neurons on the 3rd day p.i. In animals from all infected groups (SV, DV and SV + DV), specific IF of Sendai virus antigen was detected also outside the CNS, in the columnar epithelium cells of nasopharyngeal mucosa.

As revealed by histological examination, meningitis and choriomeningitis developed from days 5 to 21 p.i. in mice infected with SV and in a lesser extent in those infected with SV + DV. A typical hydrocephalus developed in mice inoculated with SV, starting 5 weeks p.i. and in those inoculated with SV + DV, starting from 16 weeks p.i. In animals inoculated with DV minimal mononuclear infiltration was seen below the ependyma cells of the lateral ventricles, in choroid plexi and in the meninges, on days 3—7 p.i. In this group of animals, the development of hydrocephalus was not observed. Long term experiments with quantitative evaluation of pathogenetic differences after inoculation of mice with SV, DV and SV + DV, respectively, are currently in progress.

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