JHM INFECTIONS IN RATS AS A MODEL FOR ACUTE AND SUBACUTE DEMYELINATING DISEASE

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SUMMARY

An animal model with different central nervous system (CNS) disease processes associated with demyelination is described which provides a basis to analyse the pathogenetic mechanisms leading to these disorders. Intracerebral infection of rats with the murine coronavirus strain JHM can result in an acute encephalomyelitis with a short incubation period or in subacute to chronic encephalomyelitis occurring after prolonged incubation. The most prominent finding of the latter two diseases consists of typical demyelinated lesions distributed in selected areas of the CNS. The induction of high rates of animals with demyelination depends both on properties of the virus used for infection and host factors such as age and immune status. A high number of rats with demyelination was obtained by intracerebral inoculation of temperature sensitive mutants into suckling rats with maternal JHM antibodies.

INTRODUCTION

Subacute and chronic diseases of the central nervous system (CNS) in animal and man are often associated with a persistent virus infection (6). In such disorders a selective demyelination may occur which is a prominent neuropathological finding. The detection of virus particles in brain tissue has led to the assumption that the destruction of oligodendroglia cells by viruses may possibly be the underlying mechanism of demyelination.

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However, available laboratory evidence suggest that the events leading to virus induced demyelination are probably of a more complex nature. Beside the genetic basis of the diseases as well as host reactions to the infections, the specific virus cell interactions which allow the agents to persist are of great interest in the studies to unreveal the pathogenesis of these diseases.

One model for virus induced demyelination is the infection of mice and rats with the neurotropic murine coronavirus strain JHM (1,2,3,4,5,13). In both animals an acute disseminated encephalomyelitis with areas of demyelination in brain and spinal cord can be induced. However, in contrast to mice, where it is difficult to obtain experimentally a subacute CNS disease, rats develop under defined conditions a high rate of marked demyelination without signs of an acute infection after prolonged incubation period (1,7,8,9).

The present communication describes the different CNS disease types observed in rats after JHM virus infection and the virological findings observed in these disorders.

TYPES OF CNS-DISEASES OBSERVED AFTER JHM VIRUS INFECTION

Acute Panencephalitis (APE). Intracerebral inoculation of suckling rats (outbred strain CHBB/Thom) leads after an incubation period of 2 - 8 days to an acute CNS disease which is neuropathologically characterized by widespread necrotic lesions and partly demyelination especially in the cerebral cortex, brainstem and spinal grey matter (7). Virus particles are easily detected in both neurons and glia cells. Inflammatory lesions are also found in the liver.

After intracerebral infection of weanling rats (age 20 -25 days) three different types of CNS-diseases can occur, which we designated as acute encephalomyelitis, subacute demyelinating encephalomyelitis and late demyelinating encephalomyelitis (8,9).

Acute Encephalomyelitis (AE). This disease develops after an incubation period of 7 - 12 days. The animals show incoordination, become motionless and die rapidly. Necrotic lesions are predominantly disseminated in the grey matter of the hippocampus, brainstem and spinal cord. Cell infiltrations are typical for acute inflammations consisting of granulocytes, lymphocytes and macrophages. By immunofluorescence, viral antigen can be found both in neurons and glia cells. Viral particles

are detected only in degenerating oligodendroglia cells.

Subacute Demyelinating Encephalomyelitis (SDE). Diseased animals develop hind leg paralysis after an incubation period of 14 - 30 days (8). Similar observations were recently reported (11). The most prominent finding consists of demyelinating plaques distributed in the white matter of the optic nerve, midbrain, pons, cerebellum and spinal cord. As an example a section from the spinal cord of a rat which developed SDE 30 days p.i. is shown in Fig. 1. Within the demyelinated plaques axons and neurons are well preserved (Fig. 2). By immunofluorescence, no viral antigen can be detected in neurons. Cell infiltrations are consisting of lymphocytes, plasma cells and macrophages.

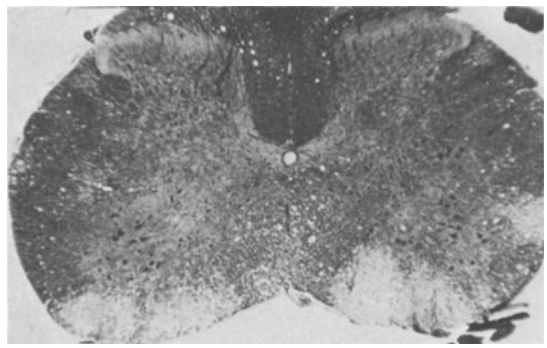

Fig.1: Demyelinated plaques in the spinal cord of a rat diseased with SDE 30 days p.i.

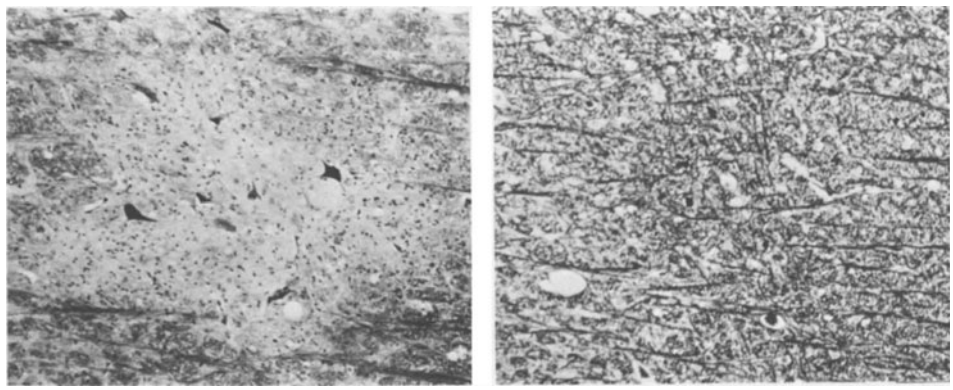

Fig.2: Preservation of neurons and axons within a demyelinated plaque (pons, 30 days, p.i.) a) stained with KB for myelin b) stained with GM for axons

Late Demyelinating Encephalomyelitis (LDE). After an incubation period of 2 - 8 months animals develop paralysis. The neuropathological lesions are typical plaques of primary demyelination as described for SDE. In addition, remyelination is detectable by electron microscopy (9). Infectious virus can be reisolated from diseased rats by conventional methods. Virus particles are detectable by electron microscopy in degenerating oligodendroglia cells.

VIROLOGICAL ASPECTS.

The above described disease types were only observed after intracerebral inoculation of JHM virus. Other murine coronaviruses are much less neurovirulent for weanling rats (12). Both weanling and suckling rats are relatively resistant against intraperitoneal infection. Furthermore, the type of clinical disease was not dependent on the amount of JHM virus injected but strongly influenced by the properties of the virus preparation inoculated as will be shown by the following experiments.

Comparison of uncloned and cloned JHM virus. Uncloned JHM-virus propagated in suckling mice induces in weanling rats the different types of disease as described above. From a virological point of view, the observation suggests that uncloned virus consists of a heterologous virus population. In order to investigate the course of AE and SDE, a time kinetic study was performed (Fig. 3). Beginning three days after infection with uncloned virus, groups of four clinically healthy rats were dissected for neuropathology, virus isolation and antibody determination at intervals of three days. Almost all clinically healthy animals revealed 6 - 14 days p.i. a latent AE. Infectious virus was easily recovered from brain and spinal cord during this acute stage. Later after infection, no infectious virus could be isolated, the latent AE disappeared, and in parallel to the raise of neutralizing antibodies clinically silent demyelinating lesions were found.

With the intention to obtain viruses with different neurovirulence, the JHM virus was cloned in tissue-culture and tested for its capacity to induce demyelinating diseases. As shown in table 1, the cloned tissue

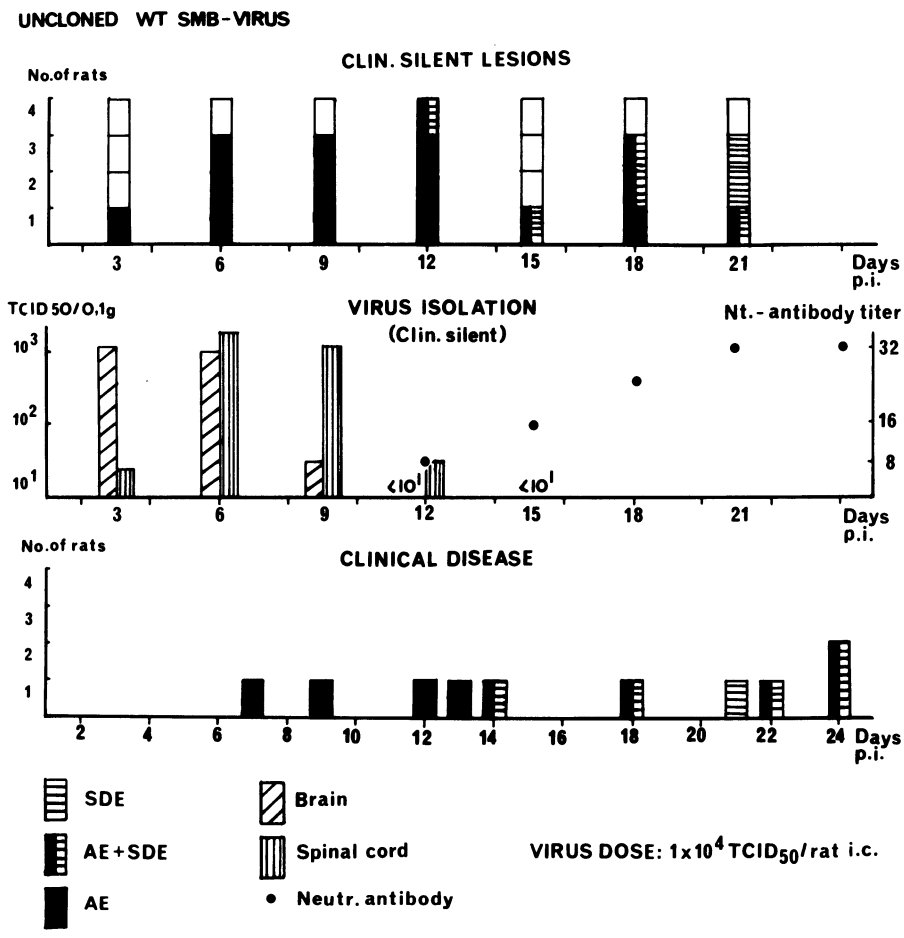

Fig.3: Time kinetics of clinically silent and overt encephalomyelitis, virus growth and development of neutralizing antibodies after infection of weanling rats with uncloned JHM virus.

culture adapted virus has lost its capacity to induce SDE or LDE in rats. Inoculation of uncloned virus resulted in about 15 % AE, 11 % of survivors developed SDE and 5 % of the remaining animals came down with LDE. Rats inoculated with the cloned, tissue culture adapted virus however, developed in 65 % AE. Survivors remained healthy without showing any clinical signs of a CNS disease.

Table 1. CNS-Diseases Induced by Uncloned and Clone Cloned JHM-Virus in Weanling Rats

VIRUS (I.C.) 4×10^3 $TCID_{50}$/RAT	CLINICAL DISEASE AE*	SDE**	LDE***
UNCLONED VIRUS (MOUSE BRAIN)	11/74	5/46	2/41
CLONED VIRUS (SAC(-)-CELLS)	29/45	0/16	0/16

* DISEASED / TOTAL

** DISEASED / UNDER FURTHER OBSERVATION

*** DISEASED / SURVIVORS OF SDE

As illustrated by Fig. 4, the survival rate of animals inoculated with cloned virus is dose dependent, whereas the type of clinical diseases is dose independent. All animals which died revealed neuropathologically a typical AE. Infectious virus could easily be isolated from diseased brain material with standard techniques or within two weeks p.i. from clinically healthy animals which revealed silent lesions.

Infection with temperature sensitive mutants. The failure to isolate from diseased animals a virus clone by tissue culture adaptation which induced SDE or LDE only, led to experiments in which the neurovirulence of cloned virus by selection of temperature sensitive mutants was changed. Mutants were induced by growth in presence of fluoruracil at 34,5 °C and selection of temperature sensitive mutants from the surviving fraction were carried out at 39,5 °C. A collection of mutants was obtained and biologically characterized by efficiency of plating, thermolability and leakiness.

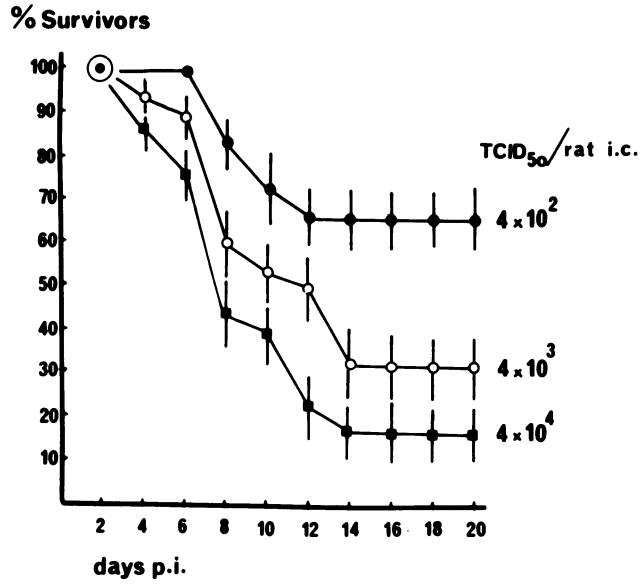

Fig.4: Dose dependent survival of weanling rats after i.c. injection of cloned JHM virus

Intracerebral inoculation of these mutants into weanling rats resulted either in no detectable disease at all or in SDE and LDE. A representative experiment with some of our mutants is summarized in table 2. The dose used for inoculation depended on the virus titre which was obtained in tissue culture. As can be seen in table 2 no AE was induced with this temperature sensitive mutants. Only different low rates of SDE and LDE developed. In addition, 10 - 30 % of clinical healthy animals revealed neuropathologically demyelinating lesions, an observation which demonstrates the property of these mutants to induce demyelination. However, inoculation of these mutants into suckling rats leads always to an acute encephalitis, suggesting that host factors such as the immune system in weanling rats contributes to the development of SDE.

Table 2. CNS-Diseases Induced by TS-Mutants Derived From Cloned JHM-Virus

	TCID50 DOSE/RAT	CLINICAL DISEASE AE*	SDE**	LDE***
TS1	$1,4 \times 10^6$	0/37	0/28	1/28
TS6	$8,0 \times 10^3$	0/57	4/34	2/30
TS42	$1,0 \times 10^4$	0/31	0/26	2/26
TS43	$4,0 \times 10^4$	0/33	3/18	2/15

* DISEASED / TOTAL
** DISEASED / UNDER FURTHER OBSERVATION
*** DISEASED / SURVIVORS OF SDE

Infection of rats immunized by maternal antibodies. Adult, female rats were immunized by four intraperitoneal injections of cloned JHM-virus and thereafter mated. Litters of suckling rats born from these animals revealed neutralizing antibodies which were still present 30 days after birth. These litters were challenged by intracerebral injection of either cloned virus or temperature sensitive mutants at an age of 4 -6 days. The results obtained after intracerebral injection with ts mutants are shown in table 3 and summarized in table 4. Intracerebral infection of non immune suckling rats led to high rates of APE within 8 days p.i., whereas immune animals stayed healthy for 20 - 32 days after infection.Thereafter, these immune rats developed at a high rate paralysis and neuropathologically signs of SDE. In contrast, maternal JHM antibodies did not change the outcome of the disease in animals inoculated with cloned JHM virus.

Table 3. JHM Infection of Suckling Rats Possessing Maternal JHM Antibodies

CONTROL ANIMALS

DOSE	INCUBATION PERIOD (DAYS)				
PFU/RAT	8	16	24	32	40
2×10^4	10/11*	10/11	10/11	10/11	10/11
2×10^3	9/11	10/11	10/11	10/11	10/11
2×10^2	2/10	2/10	2/10	2/10	2/10

PREIMMUNIZED ANIMALS
(MATERNAL ANTIBODIES)

DOSE	INCUBATION PERIOD (DAYS)				
PFU/RAT	8	16	24	32	40
2×10^4	0/11*	0/11	3/11	5/11	•(CLIN.SIL.4/6)
2×10^3	0/12	0/12	5/12	5/12	5/12
2×10^2	0/9	0/9	2/9	2/9	2/9

NEUTRALIZING ANTIBODY TITRE 4 - 6 DAYS AFTER BIRTH: 1 : 32

PREIMMUNIZATION OF MOTHERS: CLONED VIRUS

CHALLENGE VIRUS (I.C.) : TS 6

*DISEASED ANIMALS/TOTAL

Table 4. JHM Infection of Suckling Rats Possessing Maternal JHM (wt) Antibodies

CHALLENGE VIRUS	SUCKLING RATS (AGE 4 - 6 DAYS)	OCCURRENCE OF CLINICAL DISEASE	TYPE OF CLINICAL DISEASE
CLONED VIRUS	MINUS JHM MATERNAL ANTIBODIES	4 - 8 DAYS	APE
	PLUS JHM MATERNAL ANTIBODIES	4 - 8 DAYS	APE
TS 6	MINUS JHM MATERNAL ANTIBODIES	4 - 8 DAYS	APE
	PLUS JHM MATERNAL ANTIBODIES	23 - 40 DAYS	SDE WITH SCAR OF APE

DISCUSSION

The experiments carried out in this study reveal that after intracerebral inoculation of rats with JHM virus different CNS disorders of acute to chronic nature can be induced. Obviously, the development of these diseases depend on many parameters including biological properties of the virus preparation, age of the animals at the time of infection as well as the status of immunity of the host as summarized in table 5. In general, seronegative suckling rats always come down with an acute fatal CNS disease shortly after infection irrespective of the virus preparation used. Obviously, at this age virus spread cannot be limited either by the unmaturated immune system or by non-immune defence mechanisms.

However, already at the age of 20 days the disease type is determined by the virus preparation inoculated. Wild JHM virus induces acute, subacute or late diseases suggesting that different populations within this virus preparation are responsible for these diseases. In contrast, cloned JHM virus causes only an acute encephalomyelitis, whereas ts mutants predominantly lead to subacute or late disorders. In this case,

Table 5. Coronavirus JHM Infection in Rats

VIRUS	AGE OF ANIMALS UPON INFECTION: SUCKLING	AGE OF ANIMALS UPON INFECTION: WEANLING
UNCLONED WILDTYPE PASSAGED IN SUCKLING MICE (WT SMB)	• ACUTE PANENCEPHALITIS (4 - 8 DAYS) *	• ACUTE ENCEPHALOMYELITIS (7 - 12 DAYS) • SUBACUTE DEMYELINATING ENCEPHALOMYELITIS (14 - 30 DAYS) • LATE DEMYELINATING ENCEPHALOMYELITIS (2 - 8 MONTHS)
CLONED CELL ADAPTED (WT SAC CLONED)	• ACUTE PANENCEPHALITIS (4 - 8 DAYS)	• ACUTE ENCEPHALOMYELITIS (7 - 12 DAYS)
TS-MUTANTS	• ACUTE PANENCEPHALITIS (4 - 8 DAYS)	• SUBACUTE DEMYELINATING ENCEPHALOMYELITIS (14 - 30 DAYS) • LATE DEMYELINATING ENCEPHALOMYELITIS (2 - 8 MONTHS)
TS-MUTANTS (INOCULATED IN IMMUNIZED ANIMALS)	→ SUBACUTE DEMYELINATING ENCEPHALOMYELITIS (20 - 40 DAYS)	N.T.

* Incubation time p.i

the majority of animals do not develop clinical signs of illness, but reveal silent neuropathological lesions. It is of interest to note, that the neuropathological changes observed in animals with a subacute encephalomyelitis are proceeded by small clinically silent lesions of acute nature, consisting of focal necrosis and massive infiltration. Obviously in these instances virus spread is confined and an acute stage of the disease is prevented. However, the host fails to eliminate this infection which gradually proceeds developing into a subacute or late disorder with a prolonged incubation period of weeks or months.

The virus host relationship is certainly very complex in these diseases and at the present time poorly understood. Virological investigations have shown that after the initial infection in weanling rats infectious virus is easily isolatable from brain or spinal cord material even in those animals which look clinically healthy. However, after 12 - 14 days p. i., when JHM antibodies are present, no infectious virus can be recovered with standard techniques. Yet, in animals with clinical SDE or LDE infectious JHM virus is present. These findings indicate that a persistent JHM infection is established after inoculation which is probably activated at a later time before onset of clinical disease.

It is well established that humoral and cell-mediated immune reactions play a major role in a successful encounter with the virus infection. This defence system is still immature in newborn rats and it is therefore not surprising that in general these animals cannot overcome the JHM infection. However, maternal JHM antibodies influence the development of the disease processes. Whereas an acute fatal encephalomyelitis of unprotected control animals was observed in suckling rats, presence of JHM antibodies lead to a high rate of SDE after a prolonged incubation period when ts mutants of JHM virus were inoculated. Obviously, JHM antibody interferred with a spread of virus, prevented the acute disease and may have influenced replication of JHM virus as has been suggested for measles virus in the presence of antibodies (10). On the other hand, this protection was not sufficient in the case of infection with cloned JHM virus since these animals came down with an acute disease.

The described animal model of virus induced acute or subacute demyelinating encephalomyelitis reveals the complex virus host interaction underlying the different CNS diseases. Moreover, it offers the possibility to in-

vestigate the pathogenetic mechanisms of these diseases and provides the basis to understand the cell -virus interactions which lead to these disease processes. Especially the subacute and late demyelinating encephalomyelitis, caused by JHM virus in rats is of great interest in analogy to chronic CNS diseases of man associated with a virus infection.

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