VIROLOGICAL AND IMMUNOLOGICAL ASPECTS OF CORONAVIRUS INDUCED SUBACUTE DEMYELINATING ENCEPHALOMYELITIS IN RATS

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SUMMARY

Infection of rats with the murine coronavirus JHM led to acute or subacute encephalitis. Viral and host factors greatly influenced the outcome of the infection. A number of temperature-sensitive (ts) mutants was obtained which differed widely in their capacity to induce lesions of the central nervous system (CNS) in rats. Under defined conditions a subacute demyelinating encephalomyelitis (SDE) with pronounced clinical signs was observed 14-160 days post infection (p.i.). A number of rats, which showed a remission of SDE later developed a relapse of the disease accompanied by neurological symptoms. Neuropathological examination of such animals revealed lesions of active demyelination and extended remyelinated areas. The presence of viral antigen or infectious virus in the CNS of these rats demonstrated that they were persistently infected. Further investigations indicated that this virus infection triggers a cell mediated immune response against basic myelin protein which may contribute to the development of subacute to chronic encephalomyelitides.

INTRODUCTION

Several important diseases of the human central nervous system (CNS) are associated with inflammatory demyelinating lesions. An impairment of cell functions by a virus infection, an immunopathological reaction or a combination of both are possible mechanisms which lead to myelin destruction (9,10,21). Among the experimental animal models, which allow us to study such events, infections with murine coronaviruses are of particular interest. Most studies have been carried out with the murine coronavirus JHM, a neurotropic

strain which can induce demyelination in the CNS of mice and rats (1,7,20). In mice, acute disease and clinically silent demyelinating lesions have been described which were associated with a persistent infection (3,4,5,6,18). In rats similar CNS lesions can be observed after JHM infection, but in these animals, these changes, particularly demyelination, can lead to clinically recognizable diseases which develop after long incubation times. As has been previously described the infection of rats with wild type JHM virus was followed by different courses of encephalomyelitis (13,14,17). Clinical disease was observed either after a short incubation time of 3 to 11 days (acute encephalomyelitis [AE]) or developed several weeks to months p.i. (subacute demyelinating encephalomyelitis [SDE]). AE represented a rapidly progressing fatal disease course with marked paralysis. Necrotic lesions were found in all parts of the CNS, with involvement of neurons and glial cells. The clinical signs of SDE started with slight hindleg paresis and ataxic gait. Lesions of primary demyelination with relative sparing of axons were restricted to selected areas of the white matter, and in contrast to AE glial cells were preferentially infected. The outcome of SDE was not always fatal, and areas of extended remyelination were detectable after recovery. A high rate of SDE was readily obtained by infection of rats with certain ts mutants (19). These preliminary studies in rats indicate that viral and host factors play an important pathogenic role in the development of the different CNS diseases. In the following communication we summarize our recent findings on these parameters.

RESULTS

Influence of host-age and viral properties on the type of CNS disease

Infection of 21-25 day old rats by wild type JHM virus was followed by both AE and SDE with variable frequencies. Randomly selected clones of tissue culture adapted JHM virus (by plaque passages) caused predominantly AE (Table 1) and not SDE regardless of the dose inoculated. To select virus mutants with altered neurovirulence a collection of ts-mutants was produced by growth in the presence of fluoruracil. These mutants differed widely in their biological properties including neurovirulence (19). Most of these mutants were of low neurovirulence if inoculated into 21-25 days old rats. However, if rats less than 3 weeks old were inoculated with certain mutants a high rate of SDE developed after an incubation time of several weeks to months. Table 2 gives an example of an age dependent development of SDE following infection with ts43. In rats, which showed clinical symptoms, viral antigens were detectable by immune histology and infectious virus could be reisolated. These reisolates were still temperature sensitive, even after months of persistence as shown in Table 3.

TABLE 1 CORONAVIRUS JHM INFECTION IN RATS

VIRUS INOCULATED	AGE OF ANIMALS AT INFECTION	
	SUCKLING (< 20 DAYS)	WEANLING (> 20 DAYS)
WILD TYPE VIRUS PASSAGED IN MICE	AE*	AE AND SDE**
PLAQUE PURIFIED VIRUS (CELL ADAPTED)	AE	AE
TS-MUTANTS	DEPENDING ON MUTANT AND AGE OF RATS AE AND/OR SDE	SDE

* AE = ACUTE ENCEPHALOMYELITIS

** SDE = SUBACUTE DEMYELINATING ENCEPHALOMYELITIS

TABLE 2 COMPARISON OF NEUROVIRULENCE OF JHM wt and JHM ts 43

AGE AT TIME OF INFECTION (+ 1 DAY)	VIRUS*	DISEASED TOTAL	INCUBATION TIME (DAYS)	RECOVERY FROM DISEASE	TYPE OF DISEASE
4	wt **	7/10	3-5	None	AE
	ts 43	26/26	10-17	4/26	AE > SDE
10	wt	8/10	3-6	None	AE
	ts 43	23/43	14-158	11/23	SDE
15	wt	7/12	4-11	None	AE
	ts 43	1/12	92	-	SDE

* 4×10^3 PFU/RAT were inoculated into outbred rats, strain CHBB/Thom.

** JHM wild type virus cloned by plaque passages in Sac(-) cells

TABLE 3 TEMPERATURE SENSITIVITY OF REISOLATED VIRUS FROM RATS INFECTED WITH ts43*

DESIGNATION OF ISOLATE	ONSET OF CLINICAL DISEASE DAYS p.i.	TEMPERATURE SENSITIVITY ^{**}) OF REISOLATED VIRUS
ts43-1	45	1.3×10^{-4}
ts43-2	48	1.5×10^{-4}
ts43-3	50	6.7×10^{-3}
ts43-4	50	5.0×10^{-4}
ts43-5	65	5.0×10^{-3}
ts43-6	76	2.0×10^{-2}
ts43-7	90	4.0×10^{-3}

- * 4×10^3 PFU WERE INOCULATED INTO RATS AT AN AGE OF 9-11 DAYS
 ** EFFICIENCY OF PLAQUING (EOP) = PFU AT 39.5°C : PFU AT 34°C .
 EOP OF ts43 USED FOR INOCULATION = 1.3×10^{-5}

Relapse of SDE

The course of the CNS disease after infection with ts-mutants was highly variable. Many rats with clinical symptoms of SDE showed a partial or complete remission. Among 43 rats with complete remission 9 rats developed a second attack of SDE 2-18 weeks after recovery from the first attack (Table 4). These rats revealed hindleg paresis and severe ataxic gait 14-33 days after virus inoculation. These symptoms gradually disappeared, but returned with the second attack. The neuropathological changes clearly indicated a chronic disease process as documented in Fig. 1. In all animals fresh demyelinating lesions with infiltrations of mononuclear cells were detectable in pons, thalamus or spinal cord, and remyelination of axons by Schwann cells (PNS-type) or oligodendroglia (CNS-type) was also observed. The spinal cord section shown in Fig. 1 exemplifies active demyelination (Fig. 1a and c), remyelination of the CNS-type (Fig. 1d) and remyelination by Schwann cells (Fig. 1b, e). All rats investigated revealed viral antigens in glia cells in and near to the demyelinating plaques and infectious virus could be isolated from brain tissue or spinal cord.

TABLE 4 CORONAVIRUS JHM INDUCED RELAPSING OF SDE

CASE NO.	MUTANT INOCULATED*	RAT STRAIN	ONSET OF SYMPTOMS DAYS P.I.	DURATION OF SYMPTOMS DAYS	RELAPSE OF SDE DAYS P.I.
1	ts6	Thomae	14	9	70
2	ts6	Thomae	14	15	47
3	ts6	Lewis	14	8	41
4	ts42	Thomae	19	18	56
5	ts43	Thomae	21	23	71
6	ts43	Lewis	22	15	50
7	ts43	Lewis	33	22	95
8	ts43	Lewis	33	12	138
9	ts43	Thomae	20	25	173

* Rats were inoculated at an age of 10-15 days with 4×10^3 PFU of virus (i.c.)

Cell-mediated immune reactions against myelin basic protein

The observation of a chronic relapsing disease course after JHM infection of Lewis rats with lymphocyte infiltrations has certain similarities with experimental allergic encephalomyelitis (EAE). This disease results from sensitisation of animals with mixtures of basic myelin protein (BMP) or CNS-tissue extracts in combination with complete Freund's adjuvants (CFA). It is known that this massive immunisation leads to sensitisation of lymphocytes which are mainly responsible for the course of disease. Animals which recover from EAE are no longer susceptible to repeated sensitisations with CFA and BMP.

Fig. 1 Relapse of SDE 138 days p.i.

- a) Fresh demyelination in the anterior and lateral area of white matter (upper half) and remyelinating area in the dorsal column (arrow heads) of the spinal cord. The rat was perfused with buffered glutaraldehyde-paraformaldehyde solution. Hematoxilin-eosin and luxol-fast blue stain, x76.
- b) Higher magnification (x312) of remyelinating area. Increased number of Schwann cells and darker staining (thin arrows) compared to CNS type myelin (thick arrow) indicated PNS-type remyelination.
- c) Naked axons (arrow heads) and macrophage infiltration (arrow) in fresh demyelination. 1 μ m section from same area as a) embedded in epon and stained with toluidin blue, x662.
- d) Remyelination of the CNS type in same spinal cord. Note very thin myelin sheaths around many axons. Stained with toluidine blue, x800.
- e) Electron microscopy from same level as a) showing remyelination of the PNS type. Basement membrane and cytoplasm of Schwann cell around axon, x32000.

TABLE 5 SUSCEPTIBILITY TO EAE IN RATS RECOVERING FROM SDE

GROUP OF ANIMALS		INCUBATION TIME FOR EAE (DAYS)	CLINICAL SCORE AVERAGE
I	CONTROL	11.7 + 1.2	4.7
II	RECOVERED FROM SDE	12.8 ± 2.2	1.6
III	ACTIVELY SUPPRESSED (BMP/IFA)	11	0.5

The following experiments were performed to determine whether an immunopathological reaction could play a pathogenic role as shown for EAE during the course of SDE. Firstly, rats which recovered from SDE were challenged with BMP plus CFA. As shown in Table 5 normal rats developed a clinical EAE within 12 days, whereas SDE rats revealed a reduced susceptibility to BMP. A further control consisted of rats, which were repeatedly pretreated with BMP and incomplete Freund's adjuvants, a procedure which is known to result in suppression of susceptibility to induction of EAE. Rats from this group rarely developed EAE upon sensitization.

Secondly, we measured the stimulation of lymphocytes derived from SDE animals with BMP and JHM virus antigen (purified, inactivated virus). Lymphocytes were collected from spleen, thymus and peripheral blood and cultured with or without antigens (Table 6). The stimulation index was determined by incorporation of 3-H-thymidine. As can be seen in Table 6, lymphocytes from diseased rats were not only stimulated by virus antigen but also by BMP. Similar stimulation values were observed for lymphocytes derived from rats with EAE. No stimulation was found if histone, a protein with similar physicochemical properties as BMP, was used as antigen.

Thirdly, we transferred these BMP restimulated cells into healthy rats by intravenous inoculation. These lymphocytes induced slight clinical symptoms and perivascular cell infiltrations in the recipients within 5-10 days (Table 7). These perivascular infiltrations were located mainly in the dorsal area of the white matter of the spinal cord, the pons, the cerebellar white matter and thalamus. No extended demyelination was found near to the perivascular cuffs. These lesions are unlikely to be the result of transferred virus. No virus was detectable by immunohistology in transferred rats. Intravenous infection with high doses of JHM-virus or virus-lymphocyte mixtures did not lead to any CNS changes in recipients. Furthermore, no virus could be isolated from lymphocytes of rats with SDE. These experiments suggest that during JHM virus infection in Lewis rats a cellular immune response against neuroantigens develops which can influence the course of disease.

TABLE 6 STIMULATION OF LYMPHOCYTE CULTURES BY ANTIGENS*

GROUP OF RATS (strain Lewis)	ANTIGEN ADDED TO CULTURE MEDIUM		
	BASIC MYELIN PROTEIN	JHM VIRUS	HISTONE
SDE	x 4.3 + 2.7	x 6.6 + 4.8	x 1.0 + 0.1
Uninfected Controls	x 0.9 ± 0.2	x 1.0 ± 0.1	x 0.8 —

*Figures indicate the stimulation index measured by incorporation of 3-H-thymidine relative to cultures without antigen. Lymphoid cells from spleen, thymus and peripheral blood were cultured for 72 hours in microplates as described by Richert et al. (1979). Before incubation, 1 µg/well of the required antigen was added. After 48 hours, 1 µCi/well 3-H-thymidine was added and the incorporated radioactivity was measured 24 hours later.

TABLE 7 ADOPTIVE TRANSFER OF LYMPH CELLS RESTIMULATED BY BMP*

SOURCE OF TRANSFERRED CELLS		EAE-LIKE LESIONS IN RECIPIENTS	
DONOR RATS (Lewis)	ANTIGEN	CLINICAL	HISTOLOGICAL
SDE	BMP	6/26	13/26
SDE	None	0/5	0/5
Control	BMP	0/8	0/8

*Lymphocytes from spleen, thymus and peripheral blood were cultured for 3 days with or without antigen (Richert et al., 1979). 1×10^6 viable lymph cells were then inoculated intravenously into Lewis-rats. Recipient rats without clinical symptoms were dissected about 10 days after transfer for histology.

COMMENTS

The CNS disease processes observed in rats in the course of JHM infections provide a model to study the pathogenic mechanisms which are responsible for the different CNS changes. In particular, the observation of lymphocyte stimulation by basic myelin protein in SDE rats represents an interesting finding since generally brain damage involving the release of myelin does not evoke an autoimmune response. Moreover, experimental studies have proven that EAE can only be induced if brain antigen in combination with Freund's complete adjuvants is given. Obviously, in SDE rats the viral infection of the

central nervous system substitutes for the Freund's complete adjuvants effect which is followed by a specific lymphocyte response against myelin basic protein.

It has been shown in JHM infections of mice and rats that the virus replicated preferentially in oligodendroglia cells (5,13). Replication in these cells may either lead to cell destruction resulting in an acute disease or to a persistent infection of glial cells. This infection could then result in an interference with cell functions followed by myelin breakdown. Cellular dysfunctions associated with persistent viral infections have been recently demonstrated in studies of neuronal cells in culture. It has been shown that interference with special neural cell functions such as a production of acetylcholine, acetyltransferase or acetylcholine esterase occurs in persistent virus infections with lymphocytic choriomeningitis, measles or rabies and do not always effect cell morphology, growth rate or protein synthesis (8,11,12,15). In the case of measles virus persistence in a neural cell line the presence of antiviral antibodies could overcome a specific virus-induced cellular defect (2). In C6 rat glioma cells, measles virus persistence interferes with β -adrenergic receptor stimulated cAMP synthesis. This function was restored when viral proteins were removed from the cell membrane by the action of antiviral antibodies. It is possible that immune responses could limit the damage caused by JHM virus infection of rats until a disease with clinical signs develops after a long incubation period. On the other hand the occurrence of lymphoid cells which are sensitized against basic myelin protein during the course of JHM-infection in rats suggests that viral replication in oligodendroglia cells triggers an autoimmune reaction. It is possible that either JHM virus structural proteins which are formed in CNS cells cross-react with myelin or the infection leads to changes of the oligodendroglia membrane. This may then result in the development of immunogenic structures which are also recognized on uninfected glia cells. Furthermore, infected oligodendroglia cells could release myelin material which is itself immunogenic.

At the present time none of these different mechanisms have been proven but the rat model allows us to analyze these suggested different pathogenic mechanisms. Moreover, further experiments may provide valuable information which could be of help in the study of human demyelinating disease processes associated with viral infections.

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REFERENCES

- 1) Bailey, O.T., Pappenheimer, A.M., Cheever, F.S. and Daniels, J.B. (1949). A murine virus (JHM) causing disseminated encephalomyelitis with extensive destruction of myelin. II. Pathology. *Journal of Experimental Medicine* 90, 195-212.
- 2) Barrett, P.N. and Koschel, K. (1983). Effect of antibody-induced modulation of measles (SSPE) virus membrane proteins on β -adrenergic receptor-mediated adenylate cyclase activity. *Virology* 127, in press.
- 3) Haspel, M.V., Lampert, P.W. and Oldstone, M.B.A. (1978). Temperature-sensitive mutants of mouse hepatitis virus produce a high incidence of demyelination. *Proceedings of the National Academy of Sciences USA* 75, 4033-4036.
- 4) Herndon, R.M., Griffin, D.E., McCormick, U. and Weiner, L.P., (1975). Mouse hepatitis virus-induced recurrent demyelination. *Archives of Neurology* 32, 32-35.
- 5) Knobler, R.L., Dubois-Dalcq, M., Haspel, M.V., Claysmith, A.P., Lampert, P.W. and Oldstone, M.B.A. (1981). Selective localization of wild type and mutant mouse hepatitis virus (JHM strain) antigens in CNS tissue by fluorescence, light and electron microscopy. *Journal of Neuroimmunology* 1, 81-92.
- 6) Knobler, R.L., Tunison, L.A., Lampert, P.W. and Oldstone, M.B.A. (1982). Selected mutants of mouse hepatitis virus type 4 (JHM strain) induce different CNS diseases. *American Journal of Pathology* 109, 157-168.
- 7) Lampert, P.W., Sims, J.K. and Kniazeff, A.J. (1973). Mechanism of demyelination in JHM virus encephalomyelitis. Electron microscopic studies. *Acta Neuropathologica (Berlin)* 24, 76-85.
- 8) Lunden, R., Vahlne, A. and Lycke, E. (1980). Measles virus infection of mouse neuroblastoma (C1300) cells. *Proceedings of the Society of Experimental Biology and Medicine* 165, 55-62.
- 9) McFarlin, D.E. and McFarland, H.F. (1982). Multiple Sclerosis. *New England Journal of Medicine* 307, 1246-1251.
- 10) ter Meulen, V., Kreth, H.W., Carter, M.J. (1982). Immunological aspects of slow virus infections of the nervous system. *Clinics in Immunology and Allergy* 2, 425-455.

- 11) Miller, C.A., Erlech, S. and Raine, C.S. (1981). Persistent measles virus infection of a clonal line of neuroblastoma: effect on neural differentiation. *Life Sciences* 29, 2473-2480.
- 12) Münzel, P. and Koschel, K. (1981). Rabies virus decreases agonist binding to opiate receptors of mouse neuroblastoma-rat glioma hybrid cells 108-CC-15. *Biochemical and Biophysical Research Communications* 101, 1241-1252.
- 13) Nagashima, K., Wege, H., Meyermann, R. and ter Meulen, V. (1978). Coronavirus induced subacute demyelinating encephalomyelitis in rats. A morphological analysis. *Acta Neuropathologica* 44, 63-70.
- 14) Nagashima, K., Wege, H., Meyermann, R. and ter Meulen, V. (1979). Demyelinating encephalomyelitis induced by a long-term coronavirus infection in rats. *Acta Neuropathologica* 45, 205-213.
- 15) Oldstone, M.B.A., Welsh, R.M. and Joseph, B.S. (1975). Pathogenic mechanisms of tissue injury in persistent viral infections. *Annals of the New York Academy of Sciences* 256, 65-72.
- 16) Richert, J.R., Driscoll, B.F., Kies, M.W. and Alvord, E.C. (1979). Adoptive transfer of experimental allergic encephalomyelitis: incubation of rat spleen cells with specific antigen. *Journal of Immunology* 122, 494-496.
- 17) Sörensen, O., Percy, D. and Dales, S. (1980). In vivo and in vitro models of demyelinating diseases. III. JHM virus infection of rats. *Archives of Neurology* 37, 478-484.
- 18) Stohlman, S.A. and Weiner, L.P. (1981). Chronic central nervous system demyelination in mice after JHMV virus infection. *Neurology* 31, 38-44.
- 19) Wege, H., Koga, M., Watanabe, R., Nagashima, K. and ter Meulen, V. (1983). Neurovirulence of murine coronavirus JHM temperature sensitive mutants in rats. *Infection and Immunity* 39, 1316-1324.
- 20) Weiner, L.P., Johnson, R.T. and Herndon, R.M. (1973). Pathogenesis of demyelination induced by a mouse hepatitis virus (JHM virus). *Archives of Neurology*, 28, 293-303.
- 21) Wisniewski, H. (1977). Immunopathology of demyelination in autoimmune diseases and virus infections. *British Medical Bulletin* 33, 54-59.