CORONAVIRUS INDUCED DEMYELINATING ENCEPHALOMYELITIS IN RATS: IMMUNOPATHOLOGICAL ASPECTS OF VIRAL PERSISTENCY

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INTRODUCTION

Lesions of primary demyelination are a characteristic neuropathological finding for several important neurological diseases of humans and animals. Such diseases can be caused by virus infections like measles, herpes or distemper. In multiple sclerosis, a disease with unknown etiology, a viral agent could be a trigger factor[23]. Coronavirus infections of rodents are studied as interesting experimental models to investigate mechanisms of virus induced demyelination[19].

Lewis rats infected with the murine coronavirus JHM develop different forms of encephalomyelitis[11,12,21]. The host and viral factors which influence the outcome of infection had been described by several previous studies[9,16,17,22]. We have shown, that Lewis rats develop besides an antiviral immune response autoimmune reactions mediated by CD4+ T-cells against myelin basic protein[18]. However, as results from adoptive transfer experiments indicate, this autoimmune cellular response alone does not cause demyelination. We demonstrated previously that antiviral antibodies can be produced locally within the CNS and are detectable within the cerebrospinal fluid of infected Lewis rats[3]. Results from experiments in other virus-host systems such as measles suggested, that the humoral immune response may not lead to the elimination of the virus, but result in an impaired expression of viral glycoproteins at least on the cell surface and promote the establishment of a persistent infection (immune modulation)[5]. Such events may be important for persistent virus infections of the central nervous system, because this compartment is relatively shielded from the peripheral immune system. On the other hand, later in the course of infection an immune response against virus may contribute to demyelination. We present here some evidence which suggest that the antiviral humoral immune response could play a pathogenetic role for primary demyelination and virus persistency after long incubation times.

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RESULTS

Antiviral antibodies and virus infection in glia cell cultures

Primary glial cell cultures were established from brains of newborn Lewis rats[8,9]. The cultures consisted mainly of astrocytes, microglia and oligodentrocytes (e.g. 11 days post plating 48%, 24% and 12%). The cytopathogenicity of different JHM-virus variants is variable[9]. With the JHM-wt virus used in this study, infection leads to the destruction of the culture due to the formation of syncytia. Infectious virus is continuopusly released to the medium (Fig. 1 c). Only 6-10 % of the cells display viral antigen (Fig. 1 a). No significant difference was found in the number of cells positive for nucleocapsid and expressing spike protein on the cell surface. However, the outcome of infection was significantly changed if a mixture of monoclonal antibodies against S-protein was added five days post infection[20]. No visible cytopathology occured and no infectious virus was released to the medium after several days of treatment Fig. 1 d).The number of cells containing N-protein was higher than in untreated cultures. By contrast, relative to N-protein a significantly smaller number of cells displayed S-protein on the cell surface. The S- antigen appeared in a clustered and more polar distribution on the cell surface than in untreated cultures. Such cultures were passaged further in presence of anti- S antibodies. The passaged cultures consist predominantly of astrocytes. Infectious virus was reisolatable in form of a small plaque variant up to 60 days p.i., if the antibody treatment was terminated.

In similar experiments, the infected glia cultures were treated with single anti-S MAb's against defined epitopes. Most interesting was the observation, that some antibodies which do not neutralise infectious virus and are also not impairing cell fusion, suppress the release of infectious virus and promote the establishment of a chronic infection. These results are a first hint, that the antiviral humoral immune response could lead to immunomodulation and support the establishment of a chronic infection in brain tissue.

Neuropathological characterisation of different disease types

For the following study Lewis rats (4-6 weeks old) were infected by the intracerebral route with JHM virus passage designated MP2 SD. Animals at different stages of disease were bleeded after anesthesia and killed by perfusion with cold buffered formaldehyde. The tissue specimens were embedded in paraffin and processed for immunehistology[6]. Serial sections were stained for myelin, axons, different brain cell types, macrophages, T- and B cells, immuneglobulin, complement factor C9 and viral proteins. The disease types described in the following were strictly defined by neuropathological criteria.

Many rats developed an acute encephalomyelitis (**AE**) within 2 weeks p.i.and died rapidly. About 20-30 % of the rats appear to be healthy for several weeks, before neurological signs (ataxic gait, paresis, paralysis) occured. Animals which developed a late onset disease could be classified as either chronic panencephalomyelitis (**CPE**) or subacute demyelinating encephalomyelitis (**SDE**). In CPE,

lesions are localised in grey and white matter (Fig. 2). The lesions are very necrotic, both neurons and glial cell harbor virus antigen. In typical SDE animals however the lesions are localised only in the white matter. Typical lesions of primary demyelination are characterised by preservation of axons and sparing of neurons (Fig. 2).

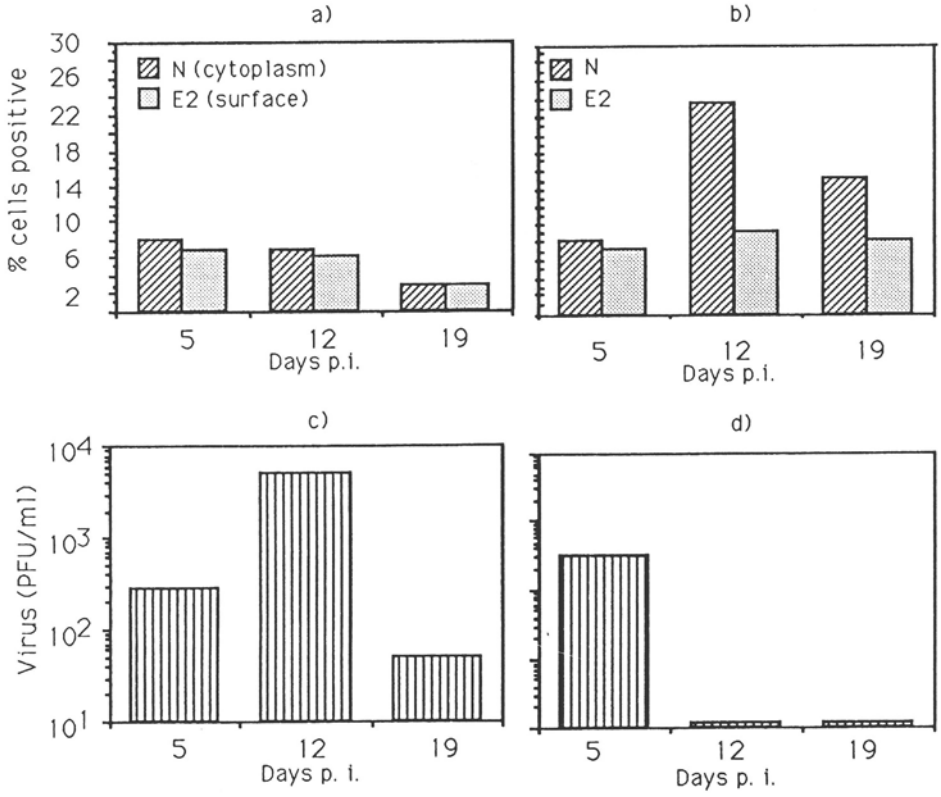

Fig. 1 Influence of anti- S Mab's on JHM infected glia cell cultures. The infected cultures were maintained in parallel with or without anti- S MAb's. At 5, 12 and 19 days p.i. the amount of infectious virus was determined by plaque tests on L-cells. The number of cells containing nucleocapsid protein was counted after immunostaining with an anti- N MAb. Cells displaying spike protein on the surface were quantitated with a mixture of anti- S MAb's. Monolayers were disintegrated by trypsinisation and the cells fixed on adhesion-slides. **a)** Amount of cells containing N protein in the cytoplasm and S protein on the cell surface in JHM infected cultures maintained without Anti- S MAb's. **b)** Amount of virus proteins in cultures maintained in presence of anti- S MAb's. **c)** Infectious virus released from cultures maintained without anti- S MAb's (a). **d)** Infectious virus released from cultures maintained with anti- S MAb's (b).

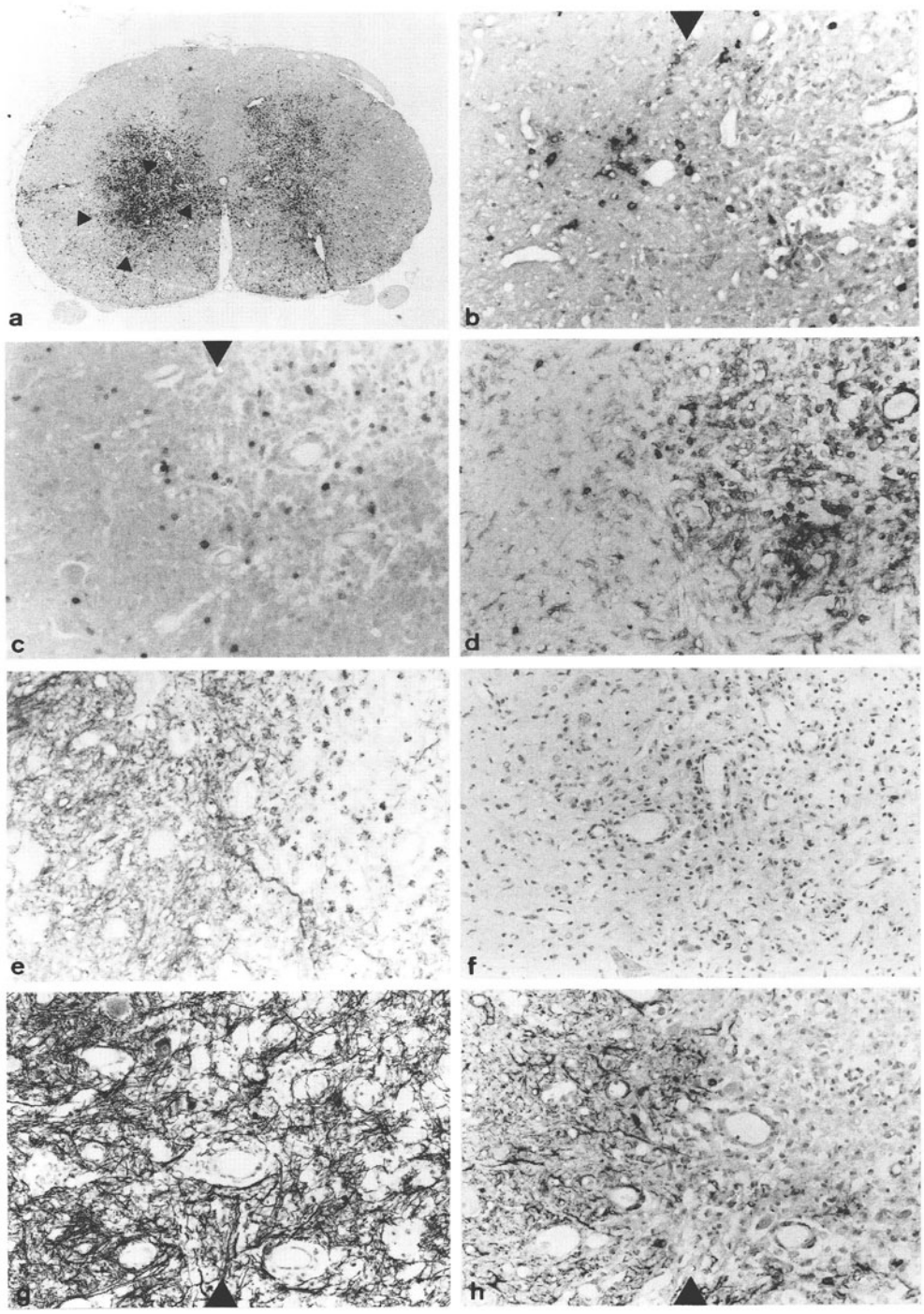

Fig. 2 Chronic Panencephalitis (CPE). Rat with clinical signs 50 days p.i., dissection 2 days later. **a)** Symmetrical macrophage infiltrations in grey and white matter. Arrows show positions of pictures b-h (serial sections). ED 1, 23x **b)** Many viral antigen positive cells surround necrotic lesion. Rabbit anti JHM serum. 140x **c)** Pronounced T-cell infiltration. W3/13. 140x **d)** Ia antigen

on macrophages and dendritic (microglial) cells in the surrounding.Ox6. 140x **e)** Demyelination, degraded myelin in macrophages. Anti-MBP MAb. 140x **f)** Perivascular infiltration. Hematoxilin-eosin, 140x **g)** Reduced axonal density, loss of nerve cells. Bielschovsky silver impregnation. 140x **h)** Loss of astrocytes, gliosis in surrounding tissue. GFAP. 140x

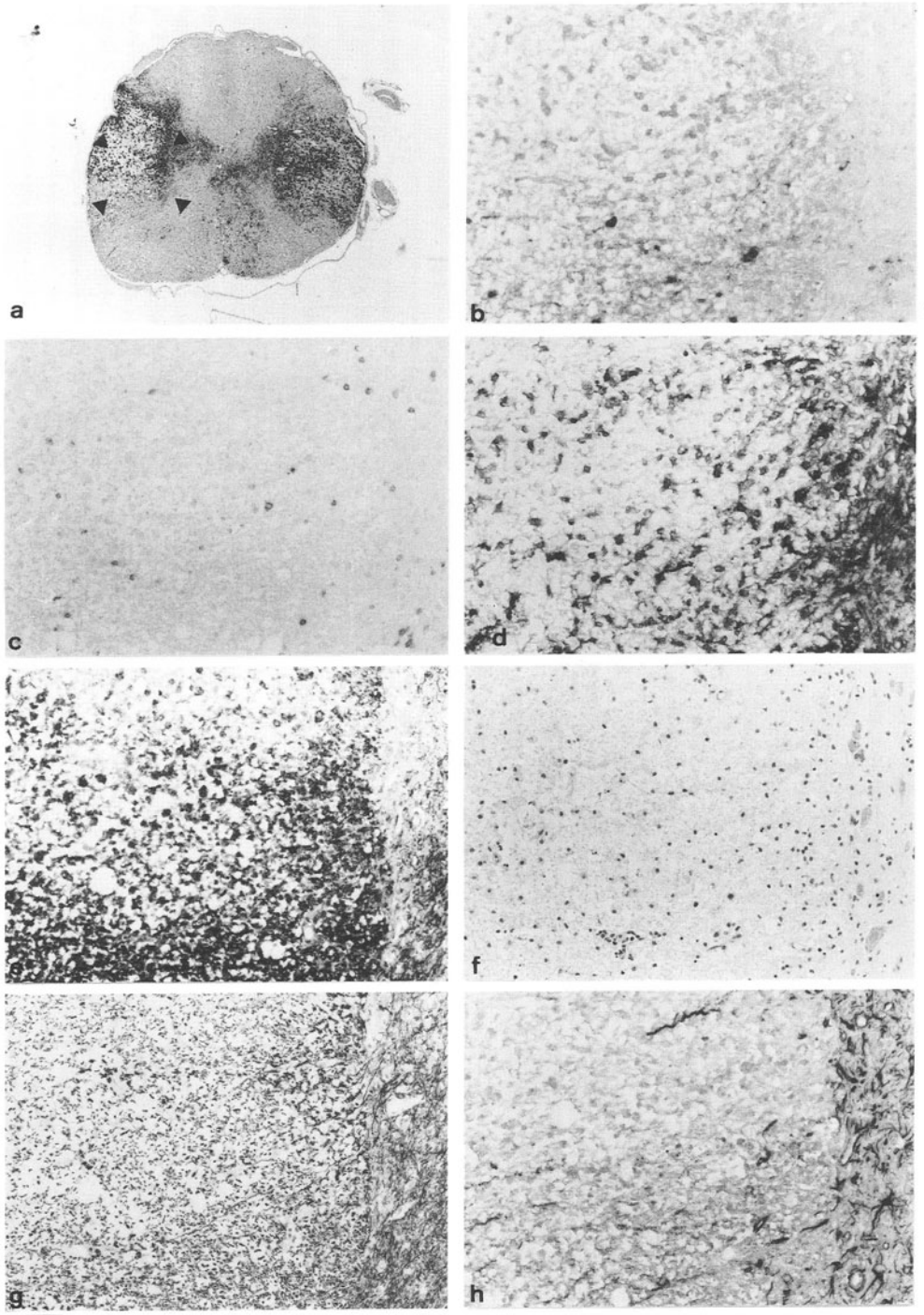

Fig. 3 (Legend on next page)

Type and quantitative composition of inflammatory infiltrates

Quantitative evaluations including statistical analysis were performed by counting cells positive for the marker in question from several lesions and from at least six animals for each disease type In all cases, macrophages and T-cells dominate within the lesion. About 3.5-5 times more macrophages were counted than T-cells (about 500 cells/mm^2). With regard to macrophages and T-cells, no essential differences were found between CPE and SDE. By contrast, in SDE 3x more plasma cells (about 55 cells/mm^2) were counted than in CPE. Moreover, a pronounced immuneglobulins staining was a typical finding for SDE- lesions. In addition to that, within the lesions complement factor C9 was detectable by immunehistology.

Distribution of viral nucleocapsid and spike protein in vivo

In parallel to the quantitative evaluation of cellular infiltrates, the number of cells positive for virus proteins and viral RNA were determined by immune histology and in situ hybridisation. Independent of the stage and kind of disease, identical numbers cells were found positive for either viral antigen or RNA. The amount of cells containing nucleocapsid and spike protein was quantitiated by immunostaining with monoclonal antibodies. Between AE and CPE, no essential difference was found between the relative proportions of cells positive for nucleocapsid and spike protein. If the number of cells positive for N-protein is set to 100%, about 45-60% of cells are positive for S-protein.Such a difference has to be expected, because the amount of N-protein exceeds that of S-protein. An interesting shift in the relative proportions was found for SDE. In these animals, the proportion of cells positive for S-protein was only 15-25 % of the values obtained for N-protein.

SUMMARY AND DISCUSSION

It was shown, that antibodies against the spike protein promoted the establishment of a chronic infection in primary glial cultures. By neuropathological criteria, three different types of disease were defined (AE, CPE and SDE). The relative amount of viral nucleocapsid and spike protein was quantitated in different types of demyelinating lesions. In rats, where primary demyelination was restricted to the white matter (SDE), a

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Fig. 3 Subacute Demyelinating Encephalomyelitis (SDE). The rat was dissected 96 days p.i., 11 days after onset of disease. **a)** Symmetrical macrophage infiltration in the lateral white matter. Arrows show positions of pictures b-h (serial sections) ED1, 23x **b)** Viral antigen in the surrounding white matter. Rabbit- anti JHM serum, 140x **c)** Distinct T-cell infiltration in and around the lesion. W3/13. 140x **d)** Ia expression in regions of ongoing demyelination, rim of the lesion. Ox6. 140x **e)** Complete loss of myelin, degradation products in macrophages. Anti-MBP MAb, 140x **f)** Perivascular cuffing, cellular infiltration, small edema. Hematoxilin-eosin, 140x **g)** Axons within the lesion are well preserved. Bielschovsky silver impregnation, 140x **h)** Loss of astrocytes in the lesion, surrounded by marked gliosis. GFAP. 140x

significantly lower amount of cells was found to contain spike protein than nucleocapsid. Furthermore, in SDE lesions a higher amount of plasma cells than in AE and CPE was counted. Within demyelinated areas, immunoglobulins and the complement component C9 were detected in addition to intensive infiltrates of macrophages and T-cells.

Several observations indicate, that virus persistency could be promoted by the immune response, if infectious virus is not eliminated during the acute phase of disease. Treatment of JHM virus infected mice with monoclonal antibodies against S-protein modulated the disease from an acute fatal infection to a disease with more pronounced demyelination[1]. Furthermore, mice wich were infected in presence of maternal antibodies against JHM virus developed a late onset demyelinating disease[13]. At the present stage not much is known on the molecular mechanisms of coronavirus persistency in the brain tissue. By combining in situ hybridisation and immune histology, we could not obtain evidence for the existence of cells containing only viral RNA without expression of viral structural proteins. For measles virus, immune modulation results not only in a reduced surface expression of viral glycoproteins but also in a reduced transcription and translation rate of viral proteins[5]. Due to the coronavirus gene organisation (positive strandedness, nested set structure) a detailed analysis on presence of genome, expression of various mRNA species and viral proteins in brain tissue was not yet possible. It is conceivable, that the interaction between antibody induced modulation of S-protein expression on the cell surface and selection for variants with antigenic changes of the S-protein interact during establishment of persistency. It is known, that variants which escape neutralisation are less neurovirulent and reveal molecular changes of the S-protein[2,4,22]. In addition, the role of antibodies against other viral structural proteins (M, N and HE) should be further evaluated by in vivo. Furthermore, independently of immunity a selective replication of variants occurs in rat neural cells during the acute stage of infection[10,16].

The predominant mechanism of demyelination may differ depending on the virus-host system and time cinetics in individual animals. In acute disease the cytolytic destruction of oligodendroglia by virus infection, activated macrophages and the T-cell response may be the major mechanism (see Dörries et al., this vol.). Our data suggest that in SDE antibody mediated cytotoxicity against infected cells could lead to virus induced primary demyelination. This does not disclose the possibility, that a combination of a cellular autoimmune response and antiviral immunity lead to demyelination. It had been shown, that in chronic relapsing allergic encephalomyelitis the intensity and type of demyelination can be influenced by the combination of encephalitogenic T-cells and demyelinating antibodies[6]. The pathogenesis of SDE may start with an early phase of sensitation against virus- and neuroantigens during the acute phase. Besides antigen presentation by perivascular macrophages and microglia, astrocytes could provide an additional amplification loop for activation of T-cells[7,10] (see Mößner et al., this vol.). The early cellular immune response could help to survive the acute stage of disease without complete virus clearance[14] (Dörries et al. this vol.). During the incubation time, a smoldering chronic infection may be favoured by the local humoral immune response. As a consequence of a disturbance of the blood- brain barrier or if viral variants emerge, a SDE or CPE with pronounced inflammatory

demyelination and clinical symptoms could be incuced. The neuropathological differences between CPE and SPE may be related to the specific cellular tropism of variant viruses for neuronal cells.

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