BACKGROUND PAPER
ON THE ROLE OF THE IMMUNE RESPONSE IN THE COURSE OF CORONAVIRUS JHM-INDUCED ENCEPHALOMYELITIDES IN MICE AND RATS

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Virus infections of the central nervous system (CNS) may result in the loss of myelin sheaths without axonal destruction. Severe neurological symptoms occur as a consequence of this phenomenon which is called primary demyelination. The sequence of events leading to primary demyelination during the course of viral infections of the CNS is only partially understood. Although it is well established that cytolytic infection of the oligodendroglia cell can cause primary demyelination, mononuclear infiltrates in subacute and chronic courses of the disease indicate that immunological events may take part in this process (reviewed by Johnson (l)). For several reasons, investigations into the dynamic interactions between virus infection of the CNS and the local immune response cannot be conducted in man. (1) Usually clinical specimens are restricted to cerebrospinal fluid which gives limited information about pathological events occuring in the brain parenchyma (2). It is not known at which time point of the preceeding systemic infection, the virus enters the brain and biopsies are taken too rarely and infrequently to understand the kinetics of the events (3). The general absence of genetic homogeneity in man complicates attempts to detect the influence of the immunogenetic background on the course of the disease. In contrast, intracerebral infections of rodents with the murine hepatitis virus JHM (MHV-4) are excellent models to study the relationships between virus-induced demyelination and the local immune response. Based on the work of Cheever (2), animal models of JHM virus-induced demyelinating encephalomyelitis have been developed in mice as well as in rats (3-5). In recent years striking similarities in the course of the infection in both rodents have been detected which might give the impression that these two models can substitute for each other. However, subtle but important differences do exist. Therefore, in the present review attempts are made to summarize and compare the knowledge about demyelinating encephalomyelitides in rats and mice, giving emphasis to the role of the local immune response in the brain.

In general, intracerebral infection of mice and rats may result in either an acute lethal panencephalitis or a subclinical infection (3-9). The acute disease is accompanied by paralytic symptoms and extensive damage in white as well as in grey matter of the brain. In subclinical infections small foci of chronic demyelination can be observed, but no involvement of grey matter is evident. The third type of disease, a subacute demyelinating encephalomyelitis with overt neurological symptoms is seen exclusively in rats (7-9). This subacute disease is characterized by a delayed onset and generally, reduced severity of the paralytic symptoms. Usually animals survive the clinical attack and histopathological changes are mostly confined to white matter with rare involvement of grey matter. Taken together, acute clinical disease is observed in rats and mice where the infection has spread from the periventricular white matter to large areas of the grey matter. In the rat, with decreasing grey matter involvement, clinical symptoms are less severe, although significant primary demyelination is seen. Asymptomatic

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mice or rats have no grey matter destruction and primary demyelination can be detected up to several month of infection. However, the affected areas are very small.

A important factor that determines the outcome of the infection is the age of the animal (5,9,10). As a general rule, infection of suckling animals of all strains of mice and rats with mouse brain passaged wild type JHM will cause an acute fatal disease, whereas with increasing age, inoculation will result in a growing proportion of animals with clinically inapparent infection. Resistant inbred strains differ from susceptible ones in that they acquire disease resistance earlier in life and the proportion of animals without clinical symptoms can approach 100%. Moreover, at least in some inbred strains of susceptible rats, the transition from a fully susceptible to a more resistant stage is characterized by an increasing number of animals with a subacute demyelinating encephalitis. Clearly, factors acting in different inbred strains at different ages govern the susceptibility of mice and rats to clinically overt encephalomyelitis.

In both, rats and mice the age-dependent differentiation of the viral target cell is an endogenous factor which determines the course and the outcome of the infection (11-13). In mice, the role of the differentiating viral target cell was shown by *in vitro* examination of neurons from the resistant SJL strain. These cells are not permissive for JHM virus, whereas neurons of Balb/c mice support the replication of JHM easily (11). Moreover the yield of infectious virus obtained from SJL glial cell cultures is 50-100 fold lower than from glial cell cultures of Balb/c mice (12). *In situ* examination of infected brain from adult animals supported these findings, since viral antigen could not be detected in the grey matter of SJL mice, but was present in the grey matter of Balb/c animals (11). These data explain the resistant state of adult SJL mice to clinically overt neurological disease and the high rate of acute paralytic diseases observed in JHM-inoculated Balb/c mice. In rats of the strain Wistar Furth (WF) there is a correlation between onset of the resistant state and the rise of the enzyme CNPase in oligodendrocytes, which are the main target for JHM in the glial cell fraction of the brain (13). This enzyme is involved in the synthesis of myelin, a major component of oligodendrocytes.

The important role of the immune response during intracerebral infection with JHM was first recognized following immunosupressive treatment of rats and mice with cyclophosphamide or cyclosporin A (3,14,15). Wistar Furth (WF) rats, although highly susceptible to acute paralytic disease as suckling animals can be infected subclinically as adult animal. However, cyclophosphamide treatment after subclinical infection causes exacerbation of neurological symptoms (14). Wistar Lewis rats which acquire a resistant state to paralytic disease after a few days of life become very susceptible at the age of 35 days post partum (dpp) when immunosupressed by cyclosporin A (15). Adult swiss outbred mice usually reveal a considerable number of animals with clinically inapparent demyelinating encephalitis after infection with JHM virus. Immunosupression by cyclophosphamide prior to the infection causes a drift from inapparent demyelinating disease to acute lethal encephalomyelitis (3). In all immunosuppressed animals, involvement of grey matter is increased as compared to the untreated ones. This observation suggests that the immune response most likely interferes with the spread of the virus from the periventricular white matter to wide areas of the grey matter, which is a characteristic of the viral infection in mice and rats succumbing to acute encephalitis.

A virus-specific immune response is based on two effector mechanisms:

1. the synthesis of neutralizing antibodies and
2. the differentiation of cytotoxic T-lymphocytes which are able to kill virus-infected target cells.

Both axes of the response are dependent on the induction of helper T-lymphocytes providing help for differentiation of the contributing cells. Antibodies can prevent or delay neurological disease if passively given before infection. Suckling mice nursed by mothers preimmunized with JHM develop the subacute type of disease which is usually seen in weanling rats (16), and suckling rats protected by maternal antibodies develop a subacute disease instead of the fatal acute encephalomyelitis (17). In both cases, viral antigen is not eliminated from the brain, but is detected more often in the white- than in the grey matter. This suggests that virus-specific antibody selects for non-neurotropic variants, an assumption which is further strengthened by the fact that virus grown in the presence of neutralizing monoclonal antibodies with specificity for the S-protein is less neurotropic and preferentially infects glial cells (18-20). Such antibodies administered passively to susceptible mice protect the recipients from disease and

grey matter infection but do not prevent chronic demyelinating disease (21). High titres of neutralizing antibodies may favor the rise of escape mutants which preferentially infect glial cells leading to persistent infection and chronic demyelination. This idea is supported by findings that temperature sensitive mutants of JHM generated by chemical treatment of the wild type JHM strain reveal an altered cell tropism *in vivo* (22-24). In rats as well as in mice such ts-mutants are detected preferentially in glia and rarely infect neurons. This property corresponds to the fact that these viruses do not induce acute lethal encephalitis but rather a subacute- or clinically inapparent encephalomyelitis.

There is only limited data available on the function of the cellular immune response following JHM virus infection. Transfer experiments in mice with either T-cell clones or polyclonal T-cells into recipients which have been infected reveal the following picture (25-29). Nylon wool non-adherent CD4+ positive helper T-cells can protect syngeneic recipients from disease provided that they are specific for JHM and are compatible with class II antigens of the recipient. However, they do not eliminate the virus. This can only be achieved by transfer of a small nylon wool adherent T-cell fraction which consists of CD4+ helper and CD8+ cytotoxic T-cells into irradiated recipients. Depletion of the recepients of either CD4 cells or CD8 cells results in failure to eliminate the virus. This finding in combination with the fact, that only transfer into class I compatible recipients will result in clearance of the virus, clearly indicates that elimination of virus from the infected host is dependent on CD8 positive virus-specific T-cells, which probably require help from CD4+ T cells for sucessful action. Compared to these data, little is known in the rat about the function of individual T lymphocyte subsets. However it is clear, that either absence of a functional T-cell compartment as in athymic nu/nu rats (30) or immunosuppression by cyclosporin A results in prolonged susceptibility of animals to paralytic disease (15). Detailed functional data on individual lymphocyte subsets are lacking.

In summary, the local immune response of rats and mice infected intracerebrally with coronavirus JHM prevents disease and helps to overcome the infection of the CNS by clearing the virus from the body. However, many details about the local immunological effector mechanisms in the brains of these animals are still unknown. Two of the most important questions which remain to be answered are:

1. Does the action of cytotoxic T-lymphocytes contribute to primary demyelination *in vivo* and as a consequence, to the severity of the neurological symptoms?
2. Is the virus-specific antibody response generated within the CNS capable of selecting for variants of JHM which preferentially infect glial cells, thus driving the matter?

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