ANALYSIS AND PATHOGENETIC SIGNIFICANCE OF CLASS II MHC (Ia) ANTIGEN INDUCTION ON ASTROCYTES DURING JHM CORONAVIRUS INFECTION IN RATS

Paul T. Massa, Rüdiger Dörries, Helmut Wege and Volker ter Meulen

Institute of Virology and Immunobiology
University of Würzburg
Versbacher Str. 7
D - 8700 Würzburg, F.R.G.

INTRODUCTION

This laboratory has developed over the past several years a rat model for virus-induced [1,2,3] central nervous system (CNS) disease involving inflammatory demyelinating lesions. The JHM strain of murine hepatitis coronavirus causes an array of neurological diseases differing in complexity depending on factors associated with the age and genetic background of the host[4] and the particular JHM virus isolate utilized[5]. Factors related to the age and genetics of the host associated with susceptibility to disease appear to involve immunological function[4], whereas factors related to the virus appear to depend on functional aspects of the E2 envelope glycoprotein[4,6,7] (Wege et al., this volume).

Wild type JHM virus inoculated intracerebrally into suckling rats (1-3 weeks age) invariably produces a rapidly progressing acute encephalitis (AE) showing an apparent pantropic infection of neural cells including various types of neurons and glial cells. The animals rapidly become moribund and die within 5-7 days post-inoculation. Clinical disability and death can be attributed to direct destruction of neural cells by the infection although participation of cellular immune-mediated killing may play a role. However, this disease

M. M. C. Lai et al. (eds.), *Coronaviruses*

pattern occurs across strains and thus would not appear to strongly depend on genetic factors related to immune response.

In contrast to AE, when immune-competent animals, older than 3 weeks of age, are inoculated with JHM virus, a disease pattern emerges showing genetic control of susceptibility in different rat strains, restriction of lesions to white matter, and viral persistence. This disease, termed subacute demyelinating encephalomyelitis (SDE), shows an onset time of at least 2 weeks post-inoculation with clinical signs ranging from mild paresis to complete hindleg paralysis[3] (Wege et al., this volume). Histologically animals have primary demyelinating lesions with little involvement of neurons with mononuclear cell infiltrations at sites of demyelination. Genetic susceptibility to SDE, but not AE, is seen when analyzing the rat strains Lewis, Brown-Norway (BN) and Lewis.BN (rats having a BN RT-1 locus on a Lewis genetic background). Whereas Lewis, BN and Lewis.BN rats are all equally susceptible to AE, only Lewis and Lewis.BN rats are susceptible to SDE[4] clearly showing the involvement of genes located outside the RT-1 locus in susceptibility and distinct pathogenetic mechanisms between monophasic AE and longterm SDE.

Pathogenesis of JHM virus studied in vitro

In order to analyze the complex host and virus genetic factors involved in the development of SDE, virus-neural cell interactions were studied *in vitro*. For this purpose, primary tissue cultures were prepared from newborn rat brain and infected with JHM virus[8].

At the simplest level, the type of disease pattern and specific sites of lesion formation in the brain would depend on the 1) ability of JHM virus to infect specific neural cell populations and 2) the degree to which virus replication progresses in these cells. JHM virus would be expected to replicate to a higher degree in immature neural cells compared to those that are mature to account for AE in young animals and SDE with virus persistence in older animals. Neural cell cultures isolated from newborn animals are well suited for such an analysis because cells in these cultures develop on an in vivo schedule[9,10]. Secondly, JHM virus should show a pantropic infection in younger cultures whereas a more selective infection of glial cells in older cultures. Selective infection of oligodendrocytes would account for restriction of lesions to white matter in SDE. However, as recently shown by us[8], the above two predictions were not observed in vitro. Cultures infected at various times after plating and stages of maturation show no differences in susceptibility to JHM virus infection. At all timepoints of culture, JHM virus produces similar cytopathic effects (CPE) involving large plaque formation and cell lysis. Cells involved in plaque formation are mostly type I astrocytes and brain macrophages (microglia) and to a lesser extent, oligodendrocytes. Thus, factors controlling replication in vivo appear to be not associated with maturation of brain but associated with other extraneous factors, most likely the maturation of the immune system.

The second question, concerning specific neural cell tropism, was also analyzed and found, as well, not to agree with hypothesis. That is, oligodendrocytes were not the primary glial cell targets of JHM virus. Analysis of JHM virus tropism using various in vitro approaches[8] clearly established that the primary targets of JHM virus were type I astrocytes and microglia (Table I).

Table I - Infection of cultured glial cell subpopulations

Treatment	Cells removed or not present	Principle cells remaining	Infectability (Infected cell type)
Orbital shaking	Oligodendrocytes[1]	Astrocytes Macrophages	+ (Astrocytes and Macrophages)
Anti-Sulfatide and Galactocerebroside plus complement	Oligodendrocytes	Astrocytes Macrophages	+ (Astrocytes and Macrophages)
Five-day primary glial cultures	Oligodendrocytes	Astrocytes Macrophages	+ (Astrocytes and Macrophages)
Panning and replating non-adherent cells	Macrophages[2]	Oligodendrocytes Astrocytes	+ (Astrocytes)
Normal rabbit IgG fraction	No cells removed	Oligodendrocytes Astrocytes Macrophages	++ (Macrophages)
Replating of oligodendrocytes after orbital shaking	Macrophages Astrocytes	Oligodendrocytes	-

1 - cells replated and incubated with JHM virus: cells are not infectable
2 - cells replated and incubated with JHM virus: cells are infectable

Infection of oligodendrocytes was rare and distinct in that infection occurred by occasional fusion with previously infected astrocytes. The resistence of oligodendrocytes to infection at various developmental stages was remarkable in that these cells often survived, uninfected, during the lytic infection of astrocytes and microglia. These findings suggest that oligodendrocytes are not the primary targets of JHM virus and that oligodendrocyte destruction by JHM virus may be limited and perhaps not sufficient to explain the extent of demyelination that is seen in the animal. This may explain why SDE lesions do not resemble demyelinating lesions seen in the human disease PML caused by JC papovaviruses, known to selectively replicate within and destroy oligodendrocytes during immunosuppression [11]. SDE demyelinating lesions are inflammatory in nature containing mononuclear cell infiltrates consisting of macrophages and T-lymphocytes whereas demyelinating lesions of PML show no cellular infiltrates. The above observations leave unanswered the question as to how JHM virus induces extensive widespread demyelination in a rat strain specific manner. We have further attempted to answer this question using neural cell cultures.

The mechanism of demyelination resulting from JHM virus infection of susceptible animals now appears to be a complex interaction between the virus, brain cells and the immune system. Lesions of primary demyelination are invariably associated with infiltations of macrophages and T-lymphocytes[3]. The question therefore arose, whether demyelination occurred through mechanisms similar to experimental autoimmune encephalomyelitis (EAE). EAE is a delayed type hypersensitivity immune reaction (DTH) to myelin basic protein (MBP) characterized by perivascular cuffing of helper T-lymphocytes and macrophages in the white matter[12]. Watanabe et al. have shown that T-lymphocytes from animals with SDE show a proliferative response to MBP not seen in control animals[13]. That T-lymphocytes sensitized to MBP in SDE animals play a role in clinical disease was indicated by the ability to adoptively transfer the disease to recipients[13]. These lymphocytes were of the helper phenotype, which are restricted by class II MHC (Ia) molecules. Interestingly, lymphocytes derived from clinically healthy persis-

tently infected BN rats never showed a proliferative response to MBP perhaps accounting for resistance to SDE (Watanabe et al., unpublished observations). Similar results were obtained with JHM virus antigen specific T-lymphocyte responses indicating a general susceptibility of Lewis rats to Ia-restricted DTH reactions, be it toward MBP or JHM virus.

A possible clue to the mechanism in which JHM virus elicits pathological DTH reactions in brain may be the recently uncovered role of type I astrocytes to act as potent immune assessory cells[14] and the ability of JHM virus to specifically activate an astrocyte assessory cell phenotype as described below.

Recently, gamma interferon (IFN-γ) has been shown to induce class II MHC antigens (Ia) on astrocytes in vitro which then become potent antigen presenting cells (APC) and are able to present MBP to encephalitogenic MBP-specific T cell lines[14]. For the induction of EAE and possibly DTH to other antigens, the importance of Ia^+ APC is pointed out by the fact that 1) these diseases are mediated by class II restricted T-lymphocytes[15], 2) Ia antigen positive macrophages and astrocytes become detectable in brain tissue during development of EAE[16] and 3) the induction of EAE can be prevented by treatment of experimental animals with monoclonal anti-Ia antibodies[17].

Since induction of Ia on astrocytes is likely to play a crucial role in mediating DTH in brain, the recent discovery by us that JHM virus could induce Ia on astrocytes independently of IFN-γ was an important finding[18].

The Immune Adjuvant Properties of JHM virus

Astrocytes are induced to express Ia after exposure to IFN-γ released by activated T-lymphocytes. However, the lack of lymphatic drainage in brain and the presence of the so-called blood-brain barrier restricting traffic of cells and macromolecules, suggests that IFN-γ may not be readily available, at least during the initial phases of viral infections.

Table II

Induction of Ia antigen expression on glial cell cultures

Treatment of primary glial cell cultures	Ia induction
Control (DMEM) with 15 % FCS)	-
Control conditioned media	-
Rat gamma interferon (10 units/ml)	+
Rat gamma interferon + anti-rat gamma interferon (1000 NU/ml)	-
Infectious JHM virus (10^3 PFU/ml)	+
UV inactivated JHM virus (10^3 PFU/ml)	+
JHM virus + anti-rat gamma interferon	+
JHM virus + a non-neutralizing anti-JHM antibody	+
JHM virus + a neutralizing anti-JHM antibody	-

\+ detectable by immunofluorescence microscopy

\- undetectable by immunofluorescence microscopy

* All experiments performed with both glial and DBT cell derived JHM virus

Therefore, the ability of components derived from infectious agents to act directly as immune adjuvants in tissues, especially in the brain, may be important in mounting an immune response to infection.

The induction of astrocyte Ia by JHM virus occurs in a dose dependent manner in that the induction capacity can be titered in plaque forming units/ml (PFU/ml). Peak induction is observed using 10^3 PFU/ml of JHM virus, (5-10 % of the cells) either infectious or U.V. inactivated, showing that inactivated viral particles were sufficient to elicit a response by astrocytes. However, expression of class I MHC antigens on astrocytes and oligodendrocytes is not appreciably increased by JHM virus over its normally high expression on 50-60 % of the cells in vitro, also seen in oligodendrocytes and astrocytes freshly isolated from brain. The ability of JHM viral particles to induce Ia was a direct activation of astrocytes, independent of virus-elicited secondary soluble factor, because a neutralizing antibody directed toward the E2 glycoprotein can totally abolish the induction capacity of glial-derived virus supernatants to naive recipient cultures (Table II). In agreement with these findings was the total absence of various types of interferons in infected cultures which might otherwise induce Ia. In particular, IFN-γ appeared to play no role in virus Ia induction because a potent polyclonal antisera to IFN-γ did not block JHM virus induced Ia (Table II). As presented below, we have evidence that JHM virus may exert its effect on astrocytes through specific virus receptor linked transmembrane signalling.

Possible mechanisms of astrocyte Ia induction by JHM virus

Recent studies indicate that IFN-γ acts on cells through receptors linked to transmembrane signalling processes associated with activation of protein kinase C[19,20]. In agreement with such studies, is the induction of Ia antigens on B-lymphocytes by agents known to stimulate protein kinase C (lipopolysaccharides and phorbol diesters)[21] and activate macrophages, similar to the effects of IFN-γ [20]. We therefore tested these agents on astrocytes derived from Lewis rats and found that lipopolysaccharide, muramyl dipeptide (adjuvant peptide), phorbol myristate acetate (PMA) and Ca^{++} ionophore A23187 induce Ia on astrocytes in a dose dependent fashion and kinetics fitting well with the induction by JHM virus and distinct from Ia induction by IFN-γ (Table III).

Table III Flow Cytometric Analysis of Ia Induction

Dose giving maximal induction	Percentage of cells induced 2 days	5 days
10 units/ml recombinant rat gamma interferon	18 %	28 %
10^3 PFU/ml JHM virus	<1 %	10 %
1.0 ug/ml LPS	<1 %	14 %
0.1 ug/ml adjuvant peptide	<1 %	11 %
10 ng/ml PMA	<1 %	15 %
0.3 uM A23187	<1 %	19 %
Medium alone	<1 %	<1 %

This indicates that JHM virus particles induce Ia on astrocytes by virus receptor-linked activation of intracellular protein kinase C because phorbol diesters are specific activators of this enzyme in these cells. In agreement with this was the induction of Ia on brain macrophages (microglia) as well, however, only in the presence of indomethacin, which blocks production of prostaglandins of the E series (PGE), potent suppressors of Ia induction. In contrast, astrocytes appear resistent to PGE suppression.

Astrocytes could be especially effective antigen presenting cells in the brain owing to their ubiquity and their ability to phagocytose, process and present antigen to class II restricted T-lymphocytes. In addition to the role of astrocyte Ia induction in mounting an immune response to infection, Ia expression by astrocytes might carry the risk of inappropriate presentation of brain antigens as is thought to occur in autoimmune processes directed against the thyroid gland. This would especially apply to individuals with genetic background genes allowing excessively high constitutive expression of Ia on tissue specific cells.

In considering the hypothesis that hyperinduction of astrocyte Ia may be a phenotypic marker determining genetically controlled susceptibility to DTH reactions in the brain, in this case, experimental autoimmune encephalomyelitis (EAE), we recently compared the IFN-γ induction of Ia molecules on astrocytes and macrophages from rat strains that are susceptible or resistant to this disease. Our data demonstate that Lewis (fully susceptible) and Brown-Norway (BN) (fully resistent) rats are very different in that Lewis astrocytes express much higher levels of Ia than BN astrocytes. At least one gene responsible for Ia-hyperinduction is located outside the rat RT-1 locus. Animals congenic at the RT-1 locus of BN rats but with background genes of the Lewis rat exhibit intermediate levels of Ia compared to BN and Lewis rats, which fits well with the reduced EAE-susceptibility of these congenic animals[22]. Further, hyperinduction of Ia is astrocyte specific, since peritoneal macrophages or microglial cells of susceptible

and resistant strains exhibit identical profiles of Ia induction. Thus, astrocyte Ia hyperinducibility may be a major strain and tissue specific factor that contributes to Ia restricted DTH reactions in the brain. We can now extend these observations to strain-specific Ia induction by JHM virus, since a parallel situation is seen as that observed with IFN-γ . That is, Lewis and congenic Lewis.BN rat astrocytes show induction of Ia by JHM virus whereas BN astrocytes are totally insensitive to JHM virus Ia induction despite the ability of the virus to replicate in these cells (Table IV).

Table IV

Induction of Ia antigen expression on glial cell cultures by JHM coronavirus.

Rat Strain	Astrocyte Ia induction by JHM virus	Control DBT cell conditioned media
Lewis	+	-
BN	-	-
Lewis.BN	+	-

Table I legend

+, Detectable by immunofluorescence microscopy (induction of Ia on at least 2,500 - 5,000 cells per cm^2); -, undetectable by immunofluorescence microscopy (expression of Ia on 0-10 cells per cm^2). JHM virus stock was obtained from tissue culture supernatants of the DBT cell line infected with wild-type JHM murine coronavirus. Stock virus from the DBT cell line contained $2x10^5$ PFU/ml when cytopathic effect reached 90 %. Conditioned control supernatants from uninfected, DBT cells served as the control for the virus supernatant preparations. Glial cultures were prepared as previously described [8] and plated onto polyornithine coated glass coverslips. The total number of cells in the cultures was, on average, 10^5 cells/cm^2. Ten days post-plating the stock infectious JHM virus was allowed to adsorb to the glial cell monolayer for 1 hour after which in-

oculum was removed and cultures were refed. Five days post-infection the cultures were stained for Ia using the OX6 monoclonal antibody (from hybridoma supernatant), then examined by FITC fluorescence microscopy.

This observation correlates with susceptibility to SDE, Lewis and congenic Lewis.BN rats being fully susceptible and BN rats fully resistant. Thus, both JHM virus induction of Ia molecules on astrocytes and susceptibility to SDE is controlled entirely by genes located outside the RT-1 locus of rats.

In conclusion, the strain-specific differential induction of class II Ia molecules on astrocytes by either endogenous immunoregulatory factors or viral particles correlates well with strain-specific susceptibility to autoimmune demyelinating disease. That EAE and SDE appear to involve DTH reactions to brain tissue mediated by Ia-restricted encephalitogenic T-lymphocytes fits with this correlation. In recent studies by us, a correlation between increased numbers of Ia^+ astrocytes, totally lacking in normal brain, with increasing clinical symptoms of SDE, suggests that Ia^+ astrocytes are potentially capable in mediating the pathogenetic immune processes ensuing within the brain during JHM virus infection.

ACKNOWLEDGEMENT

The experiments described in this publication were supported by Hertie-Foundation and Deutsche Forschungsgemeinschaft.

REFERENCES

1. K. Nagashima, H. Wege, R. Meyermann, and V. ter Meulen, Corona virus induced subacute demyelinating encephalomyelitis in rat: a morphological analysis. Acta Neuropathol 44: 63 (1978).

2. K. Nagashima, H. Wege, R. Meyermann, and V. ter Meulen, Demyelinating encephalomyelitis induced by a long-term coronavirus infection in rats. Acta Neuropathol 45: 205 (1979).

3. H. Wege, R. Watanabe, and V. ter Meulen, Relapsing subacute demyelinating encephalomyelitis in rats during the course of coronavirus JHM infection. J. Neuroimmunol 6: 325 (1984).

4. H. Wege, P.T. Massa, R. Dörries, R. Watanabe, and V. ter Meulen, Coronavirus infection of rats: a model for virus induced demyelinating disease associated with autoimmunity, in: Modern Trends in Virology (Bauer et al., eds., Springer Verlag (1986) (in press).

5. H. Wege, J.R. Stephenson, M. Koga, H. Wege, and V. ter Meulen, Genetic variation of neurotropic and non-neurotropic murine coronaviruses. J Gen Virol 54: 67 (1981).

6. H.M. Baybutt, H. Wege, M.J. Carter, and V. ter Meulen, Adaptation of coronavirus JHM to persistent infection of murine Sac(-) cells. J Gen Virol 65: 915 (1984).

7. H. Wege, R. Dörries, and H. Wege, Hybridoma antibodies to the murine coronavirus JHM: characterization of epitopes on the peplomer protein (E2). J Gen Virol 65: 1931 (1984).

8. P.T. Massa, H. Wege, and V. ter Meulen, Analysis of murine hepatitis virus (JHM strain) tropism toward Lewis rat glial cells in vitro: Type I astrocytes and brain macrophages (microglia) as primary glial cell targets. Lab Invest 55: 318 (1986).

9. E. Barbarese and S.E. Pfeiffer, Developmental regulation of myelin basic protein in dispersed cultures. Proc Natl Acad Sci USA 78: 1953 (1983).

10. A.G. Walker, J. Chapman, C.B. Bruce, and M.G. Rumsby, Immunocytochemical characterization of cell cultures grown from dissociated 1-2 day post-natal rat cerebral tissue: a developmental study. J Neuroimmunol 7: 1 (1985).

11. B.L. Padgett and D.L. Walker, New human papovaviruses. Prog Med Virol 22: 1 (1976).

12. W.F. Hickey, N.K. Gonatas, H. Kimura, and D.B. Wilson, Identification and quantitation of T-lymphocyte subsets found in the spinal cord of the Lewis rat during acute experimental allergic encephalomyelitis. J Immunol 131: 2805 (1983).

13. R. Watanabe, H. Wege, and V. ter Meulen, Adoptive transfer of EAE-like lesions from rats with coronavirus-induced demyelinating encephalomyelitis. Nature 305: 150 (1983).

14. A. Fontana, W. Fierz, and H. Wekerle, Astrocytes present myelin basic protein to encephalitogenic T-cell lines. Nature 307: 273 (1984).

15. A. Ben-Nun, H. Wekerle, and I.R. Cohen, The rapid isolation of clonable antigen-specific T-lymphocyte lines capable of mediating autoimmune encephalomyelitis. Eur J Immunol 11: 195 (1981).

16. K. Sakai, T. Tabira, M. Endoh, and L. Steinman, Ia expression in chronic relapsing experimental allergic encephalomyelitis induced by long-term cultured T cell lines in mice. Lab Invest 54: 345 (1986).

17. S. Sriram, and L. Steinman, Anti I-A antibody suppresses active encephalomyelitis: Treatment model for diseases linked to IR genes. J Exp Med 158: 1362 (1983).

18. P.T. Massa, R. Dörries, and V. ter Meulen, Viral particles induce Ia antigen expression on astrocytes. Nature 320: 543 (1986).

19. W.H. Yap, T.S. Teo, Y.H. Tan, An early event in the interferon-induced transmembrane signaling process. Science 234: 355 (1986).

20. T.A. Hamilton, J.E. Rigsbee, W.A. Scott, and D.O. Adams, Gamma-interferon enhances the secretion of arachidonic acid metabolites from murine peritoneal macrophages stimulated with phorbol diesters. J Immunol 134: 2631 (1985).

21. G.G.B. Klaus, A. O'Garra, M.K. Bijsterbosch, and M. Holman, Activation and proliferation signals in mouse B-cells. VIII. Induction of DNA synthesis in B-cells by a combination of calcium ionophores and phorbol myristate acetate. Eur J Immunol 16: 92 (1986).

22. E. Günther, H. Odenthal, and W. Wechsler, Association between susceptibility to experimental allergic encephalomyelitis and the major histocompatibility system in congenic rat strains. Clin. exp. Immunol. 32: 429 (1978).