EXPRESSION OF MHC CLASS I GENES IN MOUSE HEPATITIS VIRUS (MHV-A59) INFECTION AND IN MULTIPLE SCLEROSIS

Ehud Lavi[1,4], Akio Suzumura[2], Lois A. Lampson[3], Richard M. Siegel[1], Donna M. Murasko[1*], Donald H. Silberberg[2], and Susan R. Weiss[1]
Department of Microbiology[1] and Neurology[2], University of Pennsylvania School of Medicine; Children's Cancer Research Center of The Children's Hospital of Philadelphia[3]; and the Wistar Institute of Anatomy and Biology[4], Philadelphia, Pennsylvania
*On sabbatical leave from the Department of Microbiology and Immunology, Medical College of Pennsylvania, Philadelphia, Pennsylvania

The alteration in expression of major histocompatibility complex (MHC) class I genes on neural cells in association with central nervous system (CNS) demyelination was studied. Infection of mice or glial cell cultures with a neurotropic demyelinating coronavirus, mouse hepatitis virus (MHV) strain A59, was used as a model system. While uninfected neural cells do not express detectable amounts of MHC class I surface antigens, after infection with MHV-A59 *in vitro* or *in vivo*, these antigens can be detected by reaction with a monoclonal antibody against H-2D and H-2K. Induction of MHC class I antigens by MHV-A59 infection in mice was associated with increased levels of H-2 mRNA. Upregulation of MHC class I antigens involved the production of soluble factor/s produced by infected astrocytes. The identity of the factor, which is not interferon, is at present unknown. Brain specimens from autopsies of patients with multiple sclerosis, a human inflammatory demyelinating diseases, were also examined for alteration of MHC antigens. Both class I and class II surface antigens were found in active plaques but not in chronic plaques or in normal brain. These results are consistent with the hypothesis that MHC class I antigen induction may play a role in inflammatory CNS demyelination in both experimental animals and in humans.

Persistent infection of MHV-A59 in mouse glial cultures

MHV-A59 causes a chronic demyelination disease in mice, associated with persistence of the viral RNA in CNS white matter.[1,2,3] To investigate the mechanism of demyelination, the interaction between MHV-A59 and its putative target cells, CNS glial cells, was studied. Primary mixed glial cultures derived from newborn mouse brains were fractionated into enriched oligodendrocyte and astrocyte cultures.[4,5,6,7] Three days after plating of enriched cultures, astrocyte cultures contained more than 90% glial fibrillary acidic protein (GFAP) positive cells (astrocytes[8]) and oligodendrocyte cultures contained approximately 70% galactocerebroside (GalC) positive cells (oligodendrocytes[9]). Three days after infection with MHV-A59, approximately 10% of oligodendrocytes and 30% of astrocytes

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

expressed viral antigens as assessed by double immunofluorescent staining using antisera directed against viral proteins, and either anti-GalC or anti-GFAP. All three culture types released a burst of virus in the few days after infection, followed by release of 10^3-10^5 tissue culture infectious dose ($TCID_{50}$/ml) of infectious virus for at least 45 days. During these infections there was no obvious cytopathic effect although the cells releasing virus may have been dying.

Similar enriched cultures have been derived from five day old mice that were previously infected with MHV-A59. These cultures were similar to those infected in vitro in both antigen expression and virus release. Thus astrocytes and oligodendrocytes are early targets for virus infection in vivo as well as in vitro. Moreover, these glial cell cultures maintained persistent infection of MHV-A59 as long as the cultures were viable. Infected glial cell cultures were subsequently used for studies on expression of MHC antigens.

Expression of H-2 class I antigens in MHV-A59 infected glial cultures

Infection of mixed glial cells with MHV-A59 in vitro induced expression of MHC class I, but not Ia antigens, on the surface of oligodendrocytes and astrocytes.[7] The same was true of glial cells derived from previously infected mice.[7] Mock-infected cells did not express these antigens. Antigen expression was assessed by indirect immunofluorescence and radioimmunoassay using monoclonal antibodies against H-2D and H-2K. Direct infection of astrocyte-enriched cultures resulted in induction of H-2 class I antigens on the surface of over 90% of the cells. However, infection of oligodendrocyte-enriched cultures did not induce H-2 antigens. When supernatant from infected astrocyte cultures was treated with ultraviolet light to inactivate the virus and transferred to an oligodendrocyte-enriched culture, induction of H-2 class I antigens occurred in over 90% of the cells. The use of UV inactivated supernatant from infected 17Cl-1 cells did not induce H-2 antigens. These experiments suggested that a soluble factor (or factors) was involved in the induction.[7] To determine which cells were responsible for production of the "H-2 inducing factor" cultures enriched for different CNS and spleen cells were prepared, infected with MHV-A59, and the supernatants were tested for inducibility of H-2 class I on the surface of astrocytes and oligodendrocytes. The cultures included astrocytes, oligodendrocytes, meningeal fibroblasts, macrophages-microglia, and spleen cells. Of these, only astrocytes, oligodendrocytes and meningeal fibroblasts were productively infected. Only supernatant from astrocyte-enriched cultures contained the "H-2 inducing factor".[7] The level of interferons in a supernatant from astrocyte infected culture containing the "H-2 inducing factor" was undetectable (<60 units/ml). When the supernatant was incubated with antibodies against alpha-beta or against gamma interferons, in at least 10X excess of neutralizing activity, these antibodies failed to block the H-2 inducing effect. Thus interferons were ruled out as the H-2 inducing factor, and the identity of the factor is at present unknown.

Expression of H-2 class I mRNA in MHV-A59 infected mice

To determine whether the induction of MHC class I antigens results from increased levels of the messenger RNA for H-2 protein, 4-6 week old C57BL/6 mice were intracerebrally infected with 1000 PFU of MHV-A59. This low dose produces only mild encephalitis followed by demyelination. At daily intervals after inoculation, mice were sacrificed and RNA was isolated from brain samples, electrophoresed on 1% agarose-formaldehyde denaturing gels and blotted onto nitrocellulose filters. Blots were probed with ^{32}P labelled cloned DNA representing the conserved region of $H\text{-}2L^d$ (MHC class I).[10] Autoradiograms showed a single band with

electrophoretic mobility slightly faster than 18S RNA, consistent with the size of authentic H-2 mRNA. H-2 RNA first appeared in the brain of infected mice 2 days after inoculation (before the lymphocytic infiltration) and increased in intensity to day 4. RNA from mock-infected mice showed no hybridization with the probe. This suggests that induction of H-2 in MHV-A59 infection is at the transcriptional level. However, the possibility that induction involves alteration of mRNA half life rather than synthesis has not been ruled out. The mechanism of upregulation of H-2 class I expression and the question whether it is part of a more general induction of other genes still awaits further investigation.

Expression of HLA-class I antingens in multiple sclerosis

The increasing evidence of the importance of MHC expression in experimental virus-induced demyelination[7] combined with the theory that multiple sclerosis (MS), the most common human demyelinating disease, may have an immune-mediated pathogenesis[11], has prompted the search for altered expression of MHC class I antigens in MS.

Specimens from different areas of the brain including active and chronic plaques were obtained from three MS patients. Frozen sections were fixed in methanol and reacted with different monoclonal antibodies using avidin-biotin-immunoperoxidase as described.[12] The monoclonal antibodies to class I and class II MHC antigens included mouse monoclonal antibodies to native HLA A,B,C molecules, an anti human β2-microglobulin, and two monoclonal antibodies to class II antigens. Positive controls were provided by monoclonal antibodies which recognize a determinant in all intermediate filament units including neurofilaments and glial filaments. A myeloma cell medium containing the appropriate class and amount of immunoglobulin, provided a negative control. The class I monoclonal antibodies did not react with brain cells from normal individuals except for endothelial cells. Class II antigens were expressed in normal brains in rare endothelial cells and occasionally in microglia or reactive astrocytes.[12]

Specimens of MS tissue were classified according to three categories based on the amount of lymphocytic infiltration. Active areas had severe lymphocytic infiltration in both perivascular and parenchymal areas, intermediately active areas showed mild lymphocytic infiltration, and non-active areas had no lymphocytic infiltrates.

Based on this classification of activity, MHC class I antigen expression was found in three immunologically active areas in the majority of the cells, including cells of the immune system and glial parenchymal cells with processes. In nine intermediate active areas, MHC class I antigens were present on lymphocytes and to a variable extent on parenchymal cells, ranging from sparse to intense density, but not as much as in active areas. In six non-active areas there was no detection of class I antigens except on endothelial cells as previously shown.[12] Expression of class II antigens was detected in active areas in the same distribution and intensity of the expression of class I antigens. However three different intermediately active areas expressed class II antigens in the absence of class I.

Summary

Our studies revealed that virus induced demyelination as well as human inflammatory demyelination involves upregulation of class I MHC genes and surface expression of antigenes encoded by these genes. Induction involves the action of an intermediate soluble factor/s which is at present unknown. These findings suggest that MHC class I restricted, cytotoxic T lymphocyte (CTL) reactions, against self or foreign antigens may play a role in these conditions. These findings may help to elucidate

the mechanism of coronavirus-induced demyelination as well as the pathogenesis of multiple sclerosis.

Acknowledgments:

The authors thank Louella Davis for preparation of the manuscript.

This study was supported by grant RG-1421 from the National Multiple Sclerosis Society and grants A1 17418, NS 21954 and NS 10037 from the National Institutes of Health.

References

1. E. Lavi, D.H. Gilden, Z. Wroblewska, L.B. Rorke, S.R. Weiss, "Experimental demyelination produced by the A59 strain of mouse hepatitis virus," Neurology, Cleveland (1984) 34:597-603.
2. E. Lavi, D.H. Gilden, M.K. Highkin, S.R. Weiss, "The organ tropism of mouse hepatitis virus A59 is dependent on dose and route of inoculation", Lab. Anim. Sci. (1984) 36:130-135.
3. E. Lavi, D.H. Gilden, M.K. Highkin, S.R. Weiss, "Persistene of mouse hepatitis virus A59 in a slow virus demyelinating infection in mice as detected by in situ hybridization", J. Virol. (1984) 51:563-566.
4. K.D. McCarthy, J. de Vellis, "Preparation of separate astroglial and oligodendroglial cell cultures from rat cerebral tissue", J. Cell Biol. (1980) 85:890-902.
5. A. Suzumura, S. Bhat, A. Ecclestone, R.P. Lisak, D.H. Silberberg, "The isolation and long-term culture of oligodendrocytes from newborn mouse brain", Brain Research (1984) 324:379-383.
6. M. Hirayama, D.H. Silberberg, R.P. Lisak, and D.E. Pleasure, "Long term culture of oligodendrocytes isolated from rat corpus calosum by Percoll density gradient: lysis by polyclonal anti-galactocerebroside serum", J. Neuropath Exp. Neurol (1983) 42:16-21.
7. A. Suzumura, E. Lavi, S.R. Weiss, D.H. Silberberg, "Coronavirus infection induces H-2 antigen expression on oligodendrocytes and astrocytes", Science, (1986) 232:991-993.
8. D.S. Antanitus, B.H. Choi, L.W. Laphan, "Immunofluorescence staining of astrocytes in vitro using antiserum to glial acidic protein", Brain Res. (1975) 89:363-67.
9. M.C. Raff, R. Mirsky, K.L. Fields, R.P. Lisak, S.H. Dorfman, D.H. Silberberg, N.A. Gregson, S. Leibowitz, M.C. Kennedy, "Galactocerebroside is a specific cell surface antigenic marker for oligodendrocytes in culture", Nature, London (1978) 274:813-816.
10. M. Steinmetz, J.G. Frelinger, D. Fisher, T. Honkapiller, D. Pereira, S.M. Weissman, H. Uehara, S. Nathenson, L. Hood, "Three cDNA clones encoding mouse transplantation antigens. Homology to immunoglobulin genes", Cell (1981) 24:125-134.
11. R.P. Lisak, "Multiple sclerosis: evidence for immunopathogenesis", Neurology, (1980) 30:99.
12. L.A. Lampson, W.F. Hickey, "Monoclonal antibody analysis of MHC expression in human brain biopsies: tissue ranging from "histologically normal" to that showing different levels of glial tumor involvement", J. Immunol. (1986) 136:4054-4062.