Coronavirus Infection Induces H-2 Antigen Expression on Oligodendrocytes and Astrocytes

AKIO SUZUMURA, EHUD LAVI, SUSAN R. WEISS, DONALD H. SILBERBERG

Infection of the central nervous system by mouse hepatitis virus strain A59, a murine neurotropic coronavirus, induces class I major histocompatibility complex antigens on mouse oligodendrocytes and astrocytes, cells that do not normally express these antigens on their surfaces. This induction, which occurs through soluble factors elaborated by infected glial cells, potentially allows immunocytes to interact with the glial cells and may play a critical role in the pathogenesis of virus-induced, immune-mediated demyelination in the central nervous system.

NEURAL CELLS DO NOT NORMALLY express either class I or class II major histocompatibility complex (MHC) antigens (*1*). However, class I antigens on most neural cells (*2, 3*) and class II antigens on some astrocytes (*2, 4*) have recently been induced by factors secreted from activated T cells, or by γ-interferon (INF-γ). MHC antigens are key elements in the pathogenesis of viral infection, restricting immunologic recognition of viral antigens (*5*) or functioning as viral receptors (*6*). Although an autoimmune mechanism has been implicated for the pathogenesis of some viral infections in the central nervous system (CNS) (*7*), little is known about the relation between viral infection and host immune responses to host antigens in the CNS. We have found that CNS infection by mouse hepatitis virus strain A59 (MHV-A59), a murine neurotropic coronavirus, induces H-2 antigen expression on oligodendrocytes and astrocytes in vivo and in vitro.

The induction of MHC antigen expression on neural cells by immune factors (*2–4*) led us to investigate whether viral infection could induce MHC antigen expression on neural cells before their contact with activated T cells or their secreted products. As a model, we selected the infection of C57BL/6 mice with MHV-A59, which produces chronic CNS demyelination accompanied by lymphocytic cellular infiltration (*8*); the mechanism of MHV-A59–induced demyelination is still unknown.

We examined the effect of direct viral infection on the induction of MHC expression in oligodendrocytes and astrocytes in vitro. Oligodendrocytes were isolated from primary dissociated mixed brain cell cultures of newborn C57BL/6 mice at 10 days of age as described (*9*). Astrocyte-enriched cultures were prepared from the same primary cultures after oligodendrocytes and other loosely attached cells in the upper layer had been removed by mechanical agitation (*9, 10*). More than 80% of the cells in oligodendrocyte-enriched cultures reacted with antibody to galactocerebroside (anti-GalC), and more than 90% in astrocyte-enriched cultures reacted with antibody to glial fibrillary acidic protein (anti-GFAP) 3 days after isolation, as assessed by indirect immunofluorescence. Contamination of cultures by macrophage-microglia was less than 1%, as determined by phagocytosis of latex particles. Fewer than 1% of the cells present at the time of infection of oligodendrocyte-enriched cultures were GFAP-positive astrocytes (*9*). After 1 day of isolation, cells were infected with MHV-A59. Briefly, MHV-A59 (*11*), prepared as a cell lysate of 17 clone-1 (Cl-1) mouse fibroblasts, was applied to either oligodendrocyte or astrocyte cultures at a multiplicity of infection (MOI) of approximately one plaque-forming unit (PFU) per cell. We have confirmed, by means of indirect immunofluorescence and titration of infectious virus, that oligodendrocytes and astrocytes were productively infected with MHV-A59 in vitro (*12*). This MHV-A59 infection did not induce detectable MHC antigen (either H-2 or Ia) expression on oligodendrocytes as measured by indirect immunofluorescence with a single monoclonal antibody to H-2D^b and H-2K^b or antibody to I-A^b (*3, 4*), when examined 1, 3, 5, 7, 14, and 21 days after inoculation. Astrocytes, however, expressed H-2 antigen, but not Ia antigen, after 3 days. Infection at different multiplicities of infection of MHV-A59 (0.1 or 5.0) gave similar results.

We sought to determine whether MHV-A59 infection could induce MHC expression on oligodendrocytes through soluble factors elaborated by infected CNS cells. Primary dissociated mixed brain cell cultures were prepared from newborn C57BL/6 mice and grown in 75-cm^2 culture flasks (Falcon, two brains per flask) for 10 days. Then cultures were infected with 0.5 ml of stock MHV-A59 at 1×10^7 PFU per flask. Supernatant media from infected cultures (Sup) was collected at days 1, 3, and 5 after inoculation and twice a week thereafter. Supernatant media from parallel uninfected cultures and cultures inoculated with unin-

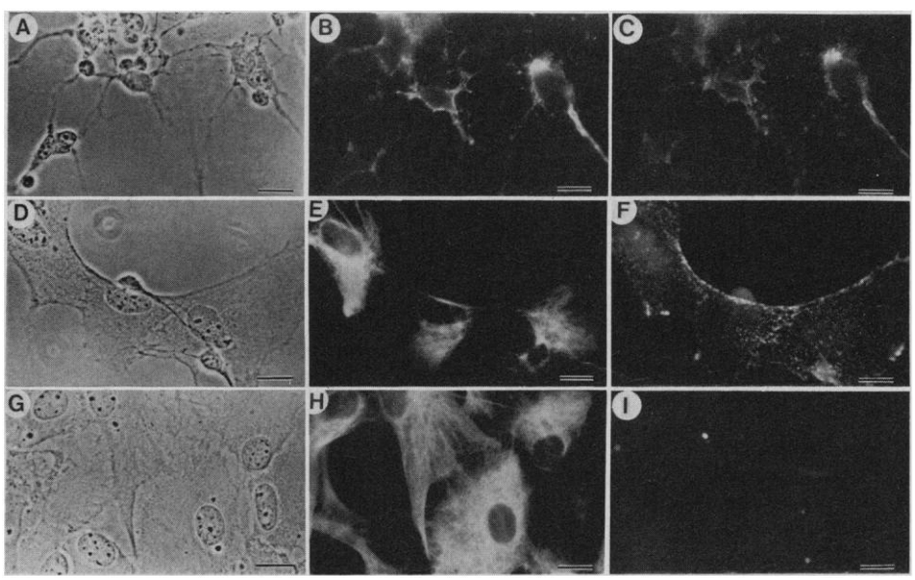

Fig. 1. In vitro induction of H-2 antigen expression on oligodendrocytes and astrocytes by supernatant from mixed brain cell cultures infected with MHV-A59. The expression of MHC antigens was assessed by indirect immunofluorescence of unfixed viable cells. Monoclonal antibodies against mouse H-2 were obtained from Bionetics Laboratory (Charleston, SC) (*3*). Cultures were then double-labeled with antibodies to H-2 and antibodies to GalC or GFAP (*3, 9*). Oligodendrocytes (A–C) and astrocytes (D–F) stimulated with 10% Sup for 2 days. Astrocyte cultures stimulated with supernatant from uninfected mixed brain cell cultures did not express detectable H-2 antigen (G–I). Viewed with phase-contrast (A, D, and G), fluorescein [GalC (B), GFAP (E and H)], and rhodamine [H-2D^b and H-2K^b (C, F, and I)] optics. Bar, 15 μm.

A. Suzumura and D. H. Silberberg, Department of Neurology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.
E. Lavi and S. R. Weiss, Department of Microbiology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

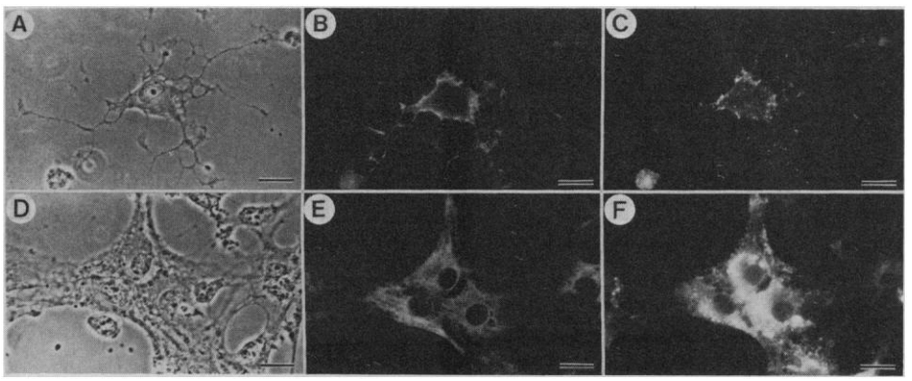

Fig. 2. H-2 antigen expression on glial cells from infected live mice. C57BL/6 mice at 5 to 7 days of age (Charles River) were inoculated intracerebrally with 20 μl of stock virus at 400 PFU per inoculum (*8*). Two days after inoculation, brains were dissected out, minced, and digested with trypsin (Gibco, 2.5 mg/ml) and deoxyribonuclease I (Sigma, 10 μg/ml) for 30 minutes in a shaking water bath at 37°C. The cell suspensions were plated on poly-L-lysine–coated cover slips at a density of 5×10^4 per square centimeter. Oligodendrocytes were isolated from the enzymatically dissociated cell suspension by Percoll density gradient (*19*) and plated in the same manner. At 1 or 3 days in vitro, they were examined for MHC expression by indirect immunofluorescence (*3*). (A to C) GalC-positive oligodendrocytes in isolated oligodendrocyte cultures; (D to F) GFAP-positive astrocytes in dissociated brain cell cultures. (A and D) Phase-contrast optics; (B) GalC and (E) GFAP, fluorescein optics; and (C and F) H-2, rhodamine optics.

fected 17Cl-1 cell lysate were collected in the same manner and served as controls. Oligodendrocyte- and astrocyte-enriched cultures were treated with 1% to 50% (in final concentration) Sup for 2 days starting 1 day after isolation, and examined by indirect immunofluorescence for their MHC (H-2 and Ia) antigen expression at day 3 (*3*). The Sup induced H-2 antigen expression of the corresponding haplotype on oligodendrocytes and astrocytes (Fig. 1), but did not induce detectable Ia antigen expression on these cells. Monoclonal antibodies against noncorresponding H-2 haplotypes, normal mouse serum, or supernatant of SP2/0 (*3*) did not stain these cells. Thus, the H-2 expression on these cells is unrelated to the induction of Fc receptors that may occur in certain viral infections (*13*). Although fluorescence after treatment with antibodies to H-2 on oligodendrocytes was weaker than that on astrocytes, more than 80% of oligodendrocytes and almost all astrocytes expressed detectable H-2 antigen. The minimum requirement for stimulation of H-2 induction was 1% Sup for 2 days or 5% Sup for 1 day. Sup obtained 1, 3, 5, and 17 days after inoculation induced H-2 antigen expression on glial cells with no obvious difference noted from each other.

Since direct viral inoculation in vitro did not induce MHC expression on oligodendrocytes, this induction of H-2 expression by Sup was probably mediated by factors from MHV-A59–infected cells in the primary cultures of mixed brain cells and not by infectious virus. To study this, we applied Sup after inactivation of virus. The ultraviolet (UV) light irradiation of MHV-A59 for either 3 hours or overnight inactivated infectious virus but did not alter the ability of Sup to induce H-2 activity. We also examined UV-inactivated MHV-A59 to see if it could induce primary cultures of mixed brain cells to elaborate the H-2–inducing factors. The supernatant media from cultures inoculated with inactivated MHV-A59 had no stimulatory effect on MHC induction. Thus, the H-2–inducing factors in the Sup were soluble factors from MHV-A59–infected cells in primary mixed brain cell cultures, but not MHV-A59 itself.

To identify the cells responsible for the production of the H-2–inducing factors, we infected different glial cell cultures and assayed them, with UV-irradiated supernatant, for their ability to induce MHC expression on oligodendrocytes and astrocytes. Astrocyte-enriched cultures and mixed glial cell cultures from which oligodendrocytes or fibroblasts were deleted (*14*) produced H-2–inducing factors. However, supernatant from oligodendrocytes, meningeal fibroblasts, or spleen cell cultures of the same strain of mouse, infected with MHV-A59 in the same manner, did not induce MHC antigen expression on glial cells. These observations indicated that astrocytes are more likely to play a predominant role in the production of the factors, although we could not rule out the possible participation of macrophage-microglia in the production of the H-2–inducing factors.

It is possible that MHV-A59–infected brain cells produce IFN's that may stimulate induction of H-2 expression, although IFN production by MHV-infected brain cells is reportedly low (*15*). The addition of antibodies to mouse interferon [anti–IFN-α/β (*16*)] in various concentrations (10 to 10^6 neutralization units per milliliter) into cultures with 10% Sup did not abolish the ability of Sup to induce H-2. Thus, the H-2–inducing factors in Sup were probably unrelated to IFN-α/β. Since our brain cell cultures do not usually contain lymphocytes, and since Sup from spleen lymphocytes inoculated with MHV-A59 did not induce MHC antigens on glial cells, it is also unlikely that the factors are related to IFN-γ. However, the precise characterization of the factors awaits further elucidation.

To confirm whether the H-2 induction by MHV-A59 infection occurs in vivo, we inoculated mice intracerebrally with MHV-A59 (*8*) and examined brain cell cultures for MHC expression. MHV-A59 induced expression of H-2 antigen on GalC-positive oligodendrocytes and GFAP-positive astrocytes (Fig. 2). We did not detect H-2 antigen expression on oligodendrocytes and astrocytes prepared from uninfected mice or from the mice inoculated with uninfected cell lysate of 17 Cl-1. These H-2–positive cells were still observed at 28 days in vitro without any additional stimulation, but oligodendrocytes and astrocytes did not express detectable amounts of Ia antigen throughout the culture period.

Our results, combined with the results of previous studies on H-2 induction by the factors from activated T cells (*2, 3*), indicate at least two ways for neural cells to express H-2 antigen in certain pathological conditions—immune-mediated and virus-mediated mechanisms. Schrier *et al.* (*17*) have shown that expression of MHC class I antigen in rat kidney cells is switched off when the cells are transformed by a highly oncogenic adenovirus. More recently, however, Rosenthal *et al.* (*18*) have shown that infection of mouse embryo cells with either oncogenic or nononcogenic adenovirus results in transcriptional activation of the H-2K gene. Despite the large increase in cytoplasmic H-2K messenger RNA, they could find only a marginal increase in surface H-2K antigen by radioimmunoassay. However, as we have shown, expression of detectable surface H-2 antigen on oligodendrocytes and astrocytes is induced during neurotropic MHV-A59 infection. This H-2 induction may play a critical role in the interaction between virus-infected cells and immunocytes to initiate virus-induced, immune-mediated demyelination in the CNS, a possible animal model of multiple sclerosis.

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20. Supported by NIH grants NS11037 and NS21954, and grant RG1421 from the National Multiple Sclerosis Society. We thank D. M. Murasko for the use of antibody to IFN; L. A. Lampson, R. P. Lisak, and D. E. Pleasure for critically reading the manuscript; and L. Buell for typing the manuscript.

30 September 1985; accepted 24 January 1986

Hyperphoretic Dispersal of a *Pyxidiophora* Anamorph

MEREDITH BLACKWELL, J. ROBERT BRIDGES, JOHN C. MOSER, THELMA J. PERRY

It has been suggested that *Thaxteriola* species and other minute, nonmycelial fungi associated with arthropods have phylogenetic relationships with the Laboulbeniales. However, direct development of the thallus of *Thaxteriola* from an ascospore of *Pyxidiophora* has now been discovered. *Thaxteriola* is specialized for dispersal by mites carried on pine bark beetles; other fungi dispersed by arthropods in this symbiotic assemblage rely primarily on arthropod specializations.

A PREVIOUSLY UNKNOWN METHOD of fungal anamorph (asexual state) development with extreme specialization for arthropod dispersal has been discovered. The new information provides the only evidence of the phylogenetic affinity of any of the minute, nonmycelial, entomogenous fungi first reported early in this century (*1*).

The perithecial ascomycete *Pyxidiophora* had been known from dung and fungal substrates (*2, 3*). We report here an undescribed species that is associated with the southern pine beetle symbiotic assemblage. Lundqvist (*2*) first noticed the similarity between ascospores of *Pyxidiophora* and the presumed hyphomycetes, *Thaxteriola* species and *Acariniola* species, from mites in bark beetle habitats in Poland and Louisiana (*1, 2*). However, conidia were not produced in the specimens, and they were regarded merely as ascospores (*2*).

The anamorph is characterized by a nonmycelial thallus consisting of two cells and a darkened holdfast in linear arrangement (*1*). Although *Thaxteriola* was first reported in 1914 (*1*), its development has not been observed until now. We found that the ascospores of *Pyxidiophora* differentiate into a *Thaxteriola* state while still within the ascus and ascocarp (Fig. 1A). Morphological differentiation of the ascospore begins with early development of a septum to divide the spore into two unequal cells, followed by gradual loss of the gelatinous membrane surrounding the ascospore, formation of a darkened holdfast (Figs. 1, A and B) at the end of the distal cell as it is positioned within the ascocarp, and attenuation of the proximal cell tip into a spine (Fig. 1C); an additional septum may be formed in the distal cell (*4*). The ascal products are released to the perithecial ostiole, holdfast-first, in a mucilaginous mass. Mites have been observed crawling over the perithecial necks, where they apparently acquire often multiple infestations of *Thaxteriola* thalli that adhere by the holdfast (Fig. 1C); endoconidium formation occurs later in the terminal cell (Fig. 1C). Presumably, endoconidia germinate by a germ tube to produce the teleomorphic (sexual) thallus (Fig. 2).

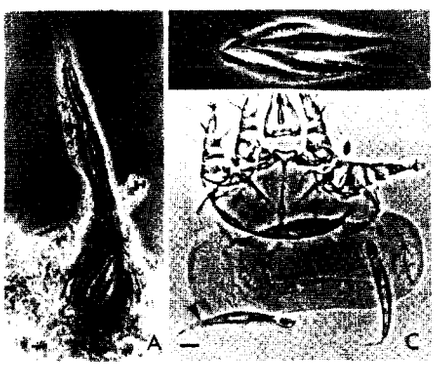

Fig. 1. *Pyxidiophora* and its *Thaxteriola* anamorph. (A) Perithecium containing anamorph that has developed from ascospores. Thalli are oriented so that the holdfast emerges first (arrows). (B) Contents of an ascus showing holdfast that has already developed (large arrow) and remnant of spore membrane (small arrow). (C) Two thalli of *Thaxteriola* attached to *Tarsonemus krantzi*. Endospores are present at arrow. Scale bars, 10 μm

Thus the mystery of the phylogenetic position of *Thaxteriola* has been elucidated in a most unexpected way. A *Pyxidiophora* teleomorph could not have been predicted for *Thaxteriola* because this manner of anamorph production by direct development of the ascospore has not been observed before. Previously described ascomycete anamorphs are single-celled (yeasts) or mycelial. In mycelial forms the anamorph is derived from an ascospore or conidium that germinates by a germ tube. Ascospore germ tube formation has been suppressed, and the *Thaxteriola* anamorph is differentiated from the ascospore itself. Now that the possibility of nonmycelial anamorph production in this manner is recognized, additional species in six genera of minute entomogenous fungi should be reexamined. *Acariniola*, *Amphoromorpha*, *Amphoropsis*, *Endosporella*, *Entomocosma*, and *Myriapodophila* (*1*) have characteristics in common with *Thaxteriola*. All have a small number of cells arranged linearly with a darkened holdfast at one end, lack haustoria, lack a mycelium, form nonwalled or thin-walled endospores within one to several terminal cells, and are associated with arthropods, primarily insects. It has been suggested that these organisms, including *Thaxteriola*, might be related to the Laboulbeniales as reduced (*1*) or even asexual forms (*5*). However, Thaxter (*1*) and Benjamin (*6*) did not believe that there was evidence for a

M. Blackwell, Department of Botany, Louisiana State University, Baton Rouge, LA 70803.
J. R. Bridges, J. C. Moser, T. J. Perry, Southern Forest Experiment Station, U.S. Department of Agriculture, Forest Service, Pineville, LA 71360.