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Induction of MHC class I antigens on glial cells is dependent on persistent mouse hepatitis virus infection

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Summary

H-2 class I antigens, but not class II antigens, were detected on the surface of glial cells persistently infected with mouse hepatitis virus strain A59 (MHV-A59) as late as 90 days post-infection. Uninfected glial cells remained negative for H-2 class I and class II surface antigens. We have previously shown that conditioned media from infected glial cell cultures (supernatants) contain a factor unrelated to infectious virus and capable of inducing H-2 class I antigens on uninfected glial cells. The synthesis of this factor appears to be dependent on production of infectious virus since the H-2 inducing activity could not be detected 3 days following the addition of neutralizing antibodies to the cultures. This suggests that H-2 inducing activity contains an unstable component, the synthesis of which is dependent on continual virus production. Persistent MHV infection and H-2 class I antigen expression may play a role in MHV-induced demyelination.

Mouse hepatitis virus, strain A59 (MHV-A59), a neurotropic coronavirus, causes limbic encephalitis followed by subacute and chronic demyelination (Lavi et al., 1984, 1988; Fishman et al., 1985). The chronic disease is characterized by persistence of viral nucleic acid in the white matter (Lavi et al., 1984). The mechanism of demyelination is unknown. However, in the case of JHM, a closely related strain of MHV, there is evidence both for cytolytic destruction of oligodendrocytes during MHV-induced disease (Lampert et al., 1973) and for a mechanism involving an autoimmune reaction against myelin basic protein (Watanabe et al., 1983).

MHV infection of glial cells, either cells infected in vitro or cells cultured from the brains of pre-infected mice, results in persistent production of moderate levels of virus with little cytopathic

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effect for as long as the cells survive (Lavi et al., 1987). The infected glial cells, both astrocytes and oligodendrocytes, show detectable levels of H-2 class I, but not class II, surface antigens when assayed either by immunofluorescence or by radioimmunoassay (Suzumura et al., 1986, 1988) 3 days post-infection. In contrast, uninfected glial cells do not contain detectable levels of these antigens. Previously we have shown that H-2 induction was due to a soluble factor(s) synthesized in the infected glial cultures, probably by astrocytes (Suzumura et al., 1986, 1988). The activity of the factor was not dependent on infectious virus since infected glial cell supernatants, treated with ultraviolet (UV) light, which inactivates infectious virus, could induce H-2 class I surface antigens when added to uninfected glial cells. This factor was also shown not to be interferon, another factor known to induce H-2, class I antigens on glial cells (Wong et al., 1984; Suzumura et al., 1988), or virus particles (Suzumura et al., 1988). In this communication we demonstrate that the continued production of the H-2 inducing factor by cultured glial cells depends on persistent MHV infection.

We have assayed for H-2 class I expression in glial cells at much later times after infection than previously examined. For the experiment described in Fig. 1, 5-day-old C57BL/6 mice were infected intracerebrally with 400 $TCID_{50}$ of MHV-A59; one day later mixed glial cultures were derived from these mice by previously described techniques (McCarthy et al., 1980; Suzumura et al., 1984, 1988). These cultures typically contain > 90% astrocytes as determined by immunostaining with antiserum directed against glial fibrillary acidic protein (GFAP), an astrocyte-specific protein; the remainder of the cells are a mixture of oligodendrocytes, fibroblasts and microglia (Suzumura et al., 1984, 1985; Lavi et al., 1987). These cells remained in culture and continued to produce infectious virus for up to 90 days (Fig. 1). A radioimmunoassay using a monoclonal antibody directed against H-$2D^bK^b$ (supernatant of cell line 28-8-16S, Litton Bionetics) and a secondary ^{125}I-labeled rabbit anti-mouse immunoglobulin (Amersham) were used to measure H-2 class I expression in these cultures 7, 28 and 90 days post-infection (Suzumura and Silberberg,

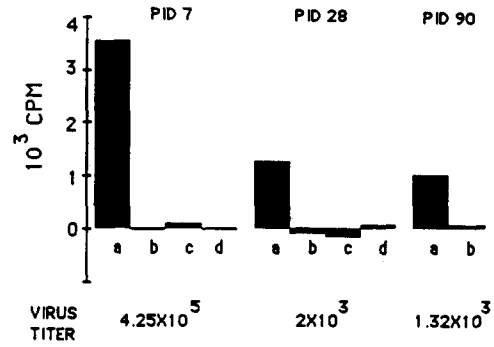

Fig. 1. Quantitation of H-2 antibody binding to the surface of glial cells by radioimmunoassay. Mixed glial cell cultures were derived from infected and mock-infected mice. After 7, 28 and 90 days post-infection (PID) (or after 6, 27 and 89 days in culture), cells were assayed for H-2 surface antigen by radioimmunoassay using monoclonal antibodies as primary antibodies and ^{125}I-labeled anti-mouse immunoglobulin as secondary antibody. (a) Infected cells, anti-H-$2D^bK^b$; (b) infected cells, anti-Ia^b; (c) infected cells, anti-H-$2D^kK^k$; (d) mock-infected cells, anti-H-$2D^bK^b$. Antibody dilutions were 1:2 for anti-H-$2D^bK^b$, undiluted for anti-H-$2D^kK^k$ and 1:3 for anti-I-a^b. Each column represents mean ^{125}I cpm value of triplicate coverslips after subtraction of parallel mean value of triplicate control coverslips incubated with supernatant from non-producing myeloma SP2/0 cells supplemented with 100 μg/ml of IgG. Supernatants from infected cells at each time point were assayed for infectious virus titer by a $TCID_{50}$ assay in L-2 fibroblasts (Lavi et al., 1987). For values shown in columns (a), $P < 0.001$ as compared to controls.

1985). For up to 90 days after infection (89 days post-culturing), significant levels of H-2^b surface antigens could be detected in the cultures. Expression of H-2, class II surface antigens (as measured by use of a monoclonal antibody directed at Ia^b (supernatant of cell line 26-18-8S, Litton Bionetics) remained negative throughout the experiment (Fig. 1, b lanes) as did the expression of H-2, class I (Fig. 1) and class II (data not shown) on uninfected cells. Controls for haplotype specificity were provided by a monoclonal antibody directed against H-$2K^kD^k$ (supernatant of cell line 16-1-2N, Litton Bionetics). (Positive control for the Ia^b antibody was provided by reaction with microglia derived from mouse brain. Positive control for the H-$2D^kK^k$ antibody was provided by reaction with glial cells derived from AKR mice (Suzumura et al., 1985).) The same results were obtained for cultures that were derived from uninfected mice and subsequently infected in vitro (data not

shown). Thus persistent H-2 expression is found on glial cells during persistent MHV-A59 infection, for up to at least 90 days post-infection.

Expression of H-2 class I in these cultures was detectable on approximately 80% of the cells while only about 20% were viral-antigen positive (as detected by immunofluorescence) as previously described for cultures examined at early times after infection (Suzumura et al., 1986; Lavi et al., 1987). The majority of H-2 class I-positive cells were astrocytes (as measured by staining with anti-GFAP) as previously described for cultures examined at early times after infection (Suzumura et al., 1985) although some were microglia and oligodendrocytes (not shown). The fact that the majority of cells were positive for H-2 surface antigens, while only 20% expressed viral antigen has been attributed to a soluble factor released by infected cells (Suzumura et al., 1985).

To determine whether the production of the glial cell factor that induces H-2 expression in these cultures is dependent on continual viral replication, cultures were treated with neutralizing anti-viral antibody (Table 1). Mixed glial cultures were infected with MHV-A59 (1 $TCID_{50}$/cell) and incubated at 37°C for 3 days. On days 3 and 6 post-infection, the medium was replaced with fresh medium containing 10% normal rabbit serum or 10% neutralizing anti-MHV-A59 rabbit serum. On day 9 post-infection, the antiserum containing medium was replaced with fresh medium lacking antibodies. The level of infectious virus remained below the level of detection for as long as the antibodies were present in the medium. As shown in Table 1, during the period of decreased viral production, the glial cell supernatants lacked the ability to induce H-2 antigens when added to uninfected glial cultures as assessed by indirect immunofluorescence. However, by 21 days post-infection, 12 days after the antibody containing media was replaced with fresh media, infectious virus was again produced and culture supernatants regained the ability to induce H-2 when added to uninfected glial cells (Table 1).

In previous experiments (Suzumura et al., 1986), we determined that H-2 factor can be detected in the supernatant of infected cells within one day after initial infection. The experiment in Table 1 suggests that it takes longer for detection of the

TABLE 1

THE EFFECT OF NEUTRALIZATION ON PRODUCTION OF H-2 CLASS I INDUCING FACTOR BY GLIAL CELLS PERSISTENTLY INFECTED WITH MHV-A59

	PID [d]	Virus titer [e]	H-2 induction [f]
Mock-infected [a]	3	$<10^2$	−
	6	$<10^2$	−
	9	$<10^2$	−
	14	$<10^2$	−
	21	$<10^2$	−
MHV + normal rabbit serum [b]	3	1.77×10^6	+
	6	4.25×10^4	+
	9	1.77×10^6	+
	14	4.25×10^4	+
MHV + anti-MHV serum [c]	3	1.77×10^6	+
Culture 1	6	$<10^2$	−
	9	$<10^2$	−
	14	$<10^2$	−
	21	1.55×10^3	+
Culture 2	3	1.55×10^6	+
	6	$<10^2$	−
	9	$<10^2$	−
	14	$<10^2$	+/−
	21	1.55×10^3	+

[a] Mixed glial cells were mock-infected with uninfected 17Cl-1 cell lysate.

[b] Mixed glial cells were infected with MHV-A59 (MOI = 1 $TCID_{50}$/cell). The medium was replaced with medium containing 10% normal rabbit serum at PID 3 and again at PID 6. At PID 9, medium containing antiserum was replaced with normal medium.

[c] Mixed glial cells were infected with MHV-A69 (MOI = 1 $TCID_{50}$/cell) and incubated with media containing 10% anti-MHV-A59 serum (neutralization titer of 1:10240) at day 3 and again at day 6. At day 9 medium containing antiserum was replaced with normal medium. This part of the experiment was carried out in duplicate.

[d] PID = post-infection days.

[e] Virus titer contained in supernatants, expressed as $TCID_{50}$/ml, was measured in L-2 mouse fibroblasts (Lavi et al., 1987).

[f] Portions of supernatants were treated with ultraviolet light to inactivate virus and tested for induction of H-2 class I antigens 2 days later by indirect immunofluorescence on uninfected mixed glial cells using a monoclonal antibody against $H\text{-}2D^bK^b$ (dilution of 1:2) (Suzumura et al., 1986). Each experimental point represents the average of duplicate coverslips. As negative controls, coverslips were incubated with SP2/0 cell supernatant supplemented with 100 μg/ml IgG. As positive controls for H-2 class I antigen induction coverslips were incubated with 100 μg/ml γ-interferon (Suzumura and Silberberg, 1985; Suzumura et al., 1986, 1988).

H-2 inducing factor after removal of antibody. This could be because it may take time to completely deplete the system of antibody and for viral release to return to previous levels. In the data in Table 1 it is apparent that H-2 inducing factor is detectable either before or at the same time that the titer of viral released is 1.55×10^3. Titers in excess of this may be observed by one day after initial infection. It may be the titer of virus released that is important, not the time period between infection and assay.

We have shown previously (Suzumura et al., 1986) and in Table 1, that UV-irradiated infected cell supernatant can induce H-2 class I antigens on uninfected glial cells. Two lines of evidence suggest that this induction is not mediated by virus. First, virus particles can be removed from infected-cell supernatants by centrifugation (Suzumura et al., 1988) without affecting the induction of H-2 antigens. Second, treatment of supernatants from infected cells with neutralizing anti-viral antiserum does not alter the ability of those supernatants to induce H-2 antigens on uninfected cells (Suzumura et al., 1988). These observations suggest that the factor in infected-cell supernatants responsible for the induction does not require infectious virus. However, the addition of neutralizing anti-viral antiserum to infected glial cells does prevent the production of a supernatant capable of inducing H-2 antigens on infected cells, suggesting that the synthesis of an active H-2 inducing factor is dependent on the concomitant production of infectious virus. It is possible that the soluble H-2 inducing factor is induced in astrocytes by active viral replication but that the factor is not very stable and therefore continual production of the factor depends on continual viral replication.

These results suggest that persistent coronavirus infection of glial cells results in the continued production of a soluble factor that causes enhanced expression of H-2 class I antigens on glial cells. This expression could result in virus-induced demyelination by allowing cytotoxic T lymphocytes to recognize either a viral or cellular antigen in an H-2-restricted manner and promote the destruction of glial cells. This hypothesis is presently being investigated.

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