Interferon-independent Increases in Class I Major Histocompatibility Complex Antigen Expression Follow Flavivirus Infection

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SUMMARY

Infection of tertiary-passaged mouse embryo fibroblasts by four flaviviruses, West Nile (WNV), Kunjin, Murray Valley encephalitis and Japanese B encephalitis, resulted in a six- to 10-fold increase in the expression of individual H-2K and H-2D class I major histocompatibility complex (MHC) antigens 16 to 48 h after infection. The mechanism(s) by which flaviviruses increased antigen expression has not been fully elucidated, but appears to be mediated partly independently of interferon-β (IFN-β) secretion, as anti-IFN-αβ antibodies partially inhibited the WNV-induced increase but totally prevented increases caused by the addition of (i) pure IFN-β, (ii) IFN-β-containing supernatants from WNV-infected mouse embryo fibroblasts (MEF), or (iii) polyinosinic–polycytidylic acid. Actinomycin D treatment of MEF, which inhibited mRNA synthesis by >90% as determined by [3H]uridine uptake, totally inhibited the increased MHC expression caused by WNV infection. Thus, the increase in class I MHC antigen expression following infection is dependent upon cellular RNA synthesis.

INTRODUCTION

Virus-immune cytotoxic T (Tc) cells are important in the cellular immune response of an organism to virus infections (Blanden, 1974), mediating lysis of infected cells in vitro and presumably in vivo before assembly of progeny virus, thereby preventing continued viral replication. Class I major histocompatibility complex (MHC) surface antigens on infected cells act as restriction elements in the recognition of virus-infected cells by virus-immune Tc cells (Zinkernagel & Doherty, 1979). Furthermore, quantitative variations in class I MHC antigen expression correlate with the efficiency of lysis of virus-infected target cells by MHC-restricted virus-immune Tc cells (O'Neill & Blanden, 1979; King et al., 1985).

We have previously detailed the parameters relating to the generation of flavivirus-immune Tc cells, in particular those for West Nile virus (WNV) (Kesson et al., 1987, 1988). The standard 51Cr release assay described commonly makes use of mouse embryo fibroblasts (MEF) as a target. In this report we show that WNV infection of MEF induces a significant increase in the surface expression of individual class I MHC (H-2K and H-2D) antigens, which is independent of interferon-αβ (IFN-αβ). This unusual phenomenon may have important implications for the combat of flavivirus diseases by the cellular immune system as well as for the strategy of survival of these viruses.

METHODS

Mice. Mice were bred under pathogen-free conditions at the Animal Breeding Establishment of the John Curtin School of Medical Research. Female mice of the strains CBA/H (H-2k), BALB/c (H-2b), C3H. H-2b (H-2o2), DBA/2 (H-2r), B10. A (H-2a), (A/J × DBA/1) F1 (H-2s), (C3H. H-2s × DBA/1) F1 (H-2s/), (B10.BYR × SJL) F1 (H-2byl/s), (BALB/c × C57BL/6) F1 (H-2d) were used between the ages of 6 and 16 weeks.

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Virus stocks. Vero cells were grown in Dulbecco's modified Eagle's medium (Gibco), supplemented with 5% foetal calf serum (FCS) and antibiotics (200 µg/ml streptomycin, 200 units/ml penicillin G and 125 µg/ml neomycin sulphate) (DMEM) in a humidified atmosphere of 5% CO₂ in air at 37 °C [standard culture conditions (SCC)] in 175 cm² tissue culture flasks (Nunc). The medium was removed at confluence, and the cells were infected with 10⁶ p.f.u. of WNV, Kunjin virus, Murray Valley encephalitis virus (MVE) or Japanese B encephalitis (JBE) virus in 1 ml DMEM (i.e. an m.o.i. of 5) for 1 h at 37 °C. The virus was then thoroughly washed off the cells and DMEM was added. After 48 h incubation at 37 °C the flasks were frozen to −70 °C, thawed to release intracellular virus, and the contents centrifuged at 1500 g for 20 min at 4 °C. Further virus was recovered by sonication of the remaining pellet. Pooled supernatants were stored as stock virus in aliquots at −70 °C.

Purified virus was prepared using a modification of the method described by Lobigs et al. (1986). Briefly, Vero cell monolayers, grown to confluence under SCC in 850 cm² roller bottles (Corning) were infected with WNV at an m.o.i. of 10. Twelve h later the culture medium was removed and the infected cells were incubated with actinomycin D (Sigma) at 5 µg/ml in 20 ml 30% DMEM for 30 min. This medium was then replaced with Eagle's MEM (Gibco) containing 2% dialysed FCS, 20 mM-HEPES buffer pH 8.0 and [5-3H]uridine (5 µCi/ml) (Amersham). Culture fluids were harvested 36 and 48 h after infection, pooled, and clarified by centrifugation. The virus was concentrated from this clarified culture fluid by addition of 40% polyethylene glycol 6000 to a final concentration of 8% with stirring at 4 °C, and was banded by centrifugation on a 10% to 30% glycerol gradient in a SW28 rotor at 20000 r.p.m. for 3.5 h at 4 °C in a Beckman L5-50 ultracentrifuge. Fractions containing virus were collected on an Isco fraction collector and radioactivity was counted in a Beckman scintillation counter. Fractions with counts above background were divided into samples and stored at −70 °C for future use.

Titres of all virus stocks were determined by serial dilutions and titrations on Vero cell monolayers as described by Taylor & Marshall (1975).

Cell culture. MEF were obtained by trypsinization of BALB/c, CBA/H, C3H.H-2b or B10.A foetuses at 16 days of gestation. The cells, designated primary MEF, seeded at 10⁶.3 cells/80 cm² plastic flask (Nunc) were grown under SCC for 4 days, after which they were removed to room temperature and kept for use as required for up to 3 weeks before subculturing. Four days before they were required, primary MEF were trypsinized and reseeded at 10³ cells/80 cm² flask, grown under SCC and designated secondary MEF. Secondary MEF were used after 4 days as the source of tertiary MEF (Sinickas et al., 1985).

Interferons and anti-interferons. Purified murine IFN-β (batch 83001) was used at 200 units/ml. Affinity-purified polyclonal rabbit anti-mouse IFN-αβ globulin (IFN-αβ antibody) (batch 21031) was used at 200 units/ml. Both reagents (Cytoimmune Reagents, Lee Biomolecular, San Diego, Ca., U.S.A.) were titrated before use on MEF using MHC labelling (see below). The concentration of IFN-β causing maximal increase in class I MHC antigen expression after 48 h was used. The IFN-αβ antibody inhibited this effect of IFN-β on a unit for unit basis.

Treatment of cells with polyinosinic acid : polycytidylic acid (poly l : C). MEF were grown in Petri dishes (Kayline, Australia) for 24 h, divided into three groups for treatment (see Fig. 4) and incubated under SCC for 48 h. To approximate conditions of viral infection, poly l : C was left in the culture supernatant for the duration of the treatment.

Treatment of cells with actinomycin D (AMD) and measurement of [3H]uridine incorporation. MEF grown either in Petri dishes or 96-well flat-bottomed tissue culture plates (Nunc) for 24 h were drained of medium, and 10 p.f.u./cell of WNV in DMEM was added (2 ml to Petri dishes; 30 µl in each well of the 96-well plates). After incubation for 1 h under SCC, the cells were washed, AMD (Sigma) was added at a concentration of 5 µg/ml in DMEM (2 ml total volume in Petri dishes; 30 µl in each well of the 96-well plates) and the cells were incubated for a further 30 min under SCC. This concentration of AMD (previously determined by titration) reduced the incorporation of [3H]uridine by more than 90%. The MEF were then washed twice with fresh DMEM at 37 °C and incubated for 24 h under SCC. [3H]Uridine incorporation by MEF during incubation in the 96-well tissue culture plate was measured after 25 µl of [3H]uridine (Amersham) (25 µCi/ml) had been added for a 6 h interval, 18 h after beginning the incubation. Labelled MEF were harvested onto glass-fibre paper using a multiple sample harvester (Titertek 530; Flow Laboratories) and β emissions were counted in a liquid scintillation counter (Beckman). Twelve samples in each group were assayed and the mean c.p.m. and standard errors of the mean (s.e.m.) were calculated. Significance was determined by Student's t-test and P values of < 0.05 were considered significant.

Antisera. Anti-H-2K/k/Iaα and H-2D/k antisera were raised in (A/J × DBA/1) F₁ and (C3H. OH × DBA/1) F₁ mice, respectively, injected intraperitoneally at weekly intervals with 10⁵–10⁶ DBA/2 spleen cells for 4 weeks and bled 1 week after the final injection. Anti-K α sera were raised in (B10. BYR × SJL) F₁ mice injected with B10. A cells, and anti-D/k sera in (BALB/c × C57BL/6) F₁ mice injected with C3H.OH cells using the above protocol. Preimmune sera from relevant F₁ mice were used as normal serum controls.

In addition, monoclonal antibodies (MAbs) against K/k/D/k (clone 34-1-25; American Type Culture Collection no. HB-79; Ozato et al., 1982), Kα (clone 11-4.1; Becton-Dickinson), I-A/k (clone 11-5.2.1.9; American Type Culture Collection no. TIB-94; Oi et al., 1978) and I-Aα (clone MK-M6; American Type Culture Collection no.
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HB-3; Kappler et al., 1981) were used to label class I MHC antigens. Supernatants from hybridoma cultures were concentrated tenfold and used at dilutions giving saturation labelling of the relevant antigens. Sera and supernatants were stored at -20 °C until required, thawed, and stored thereafter at 4 °C. The same batch of each antiserum or supernatant was used throughout. Reagents were used for no more than 6 weeks after thawing.

Rabbit anti-mouse immunoglobulin–fluorescein isothiocyanate (RAMIg–FITC) was prepared by standard methods from antiserum raised in a New Zealand white rabbit against mouse Ig (Goding, 1976). RAMIg–FITC was never frozen, but was stored at 4 °C in light-proof vials.

Cell labelling for fluorescence-activated cell sorting (FACS) analysis. Secondary MEF were trypsinized and seeded in 7 ml DMEM at 10^5 cells/ml into tissue culture plastic Petri dishes for labelling and FACS analysis. MEF were then infected/treated with WNV/IFN-β/polyI:C and/or anti-IFN-αβ antibody and incubated as described above. After this, MEF were trypsinized off the plastic, washed once in DMEM to inactivate the trypsin, once in Hanks’ balanced salt solution (HBSS), and suspended for 30 min at 4 °C in fresh periodate–lysine–paraformaldehyde, a gentle fixative which maintains the serological detectability of surface H-2 (King & Parr, 1982). After fixing, MEF were washed first in HBSS and then in 50% normal rabbit serum in HBSS, to reduce non-specific binding of antibody during labelling. All steps of this and subsequent procedures were carried out at 4 °C.

Aliquots of 10^5-7 BALB/c MEF were incubated for 60 min in 100 µl anti-K<sup>d</sup>/I<sup>a</sup> or anti-D<sup>4</sup> diluted to give maximum specific binding. Aliquots were then washed three times in DMEM and incubated for 60 min in 150 µl RAMIg–FITC diluted to give maximum specific binding. After three washes, each aliquot was suspended in 0.5 ml DMEM for flow cytometry. Controls were BALB/c MEF incubated with anti-K<sup>k</sup>, and anti-D<sup>4</sup> or normal mouse serum (NMS) and RAMIg–FITC, or with RAMIg–FITC alone. BALB/c MEF labelled with anti-K<sup>d</sup>/I<sup>a</sup> or anti-D<sup>4</sup> plus RAMIg–FITC were always significantly more fluorescent than controls. MEF are I-Ad-negative, thus only K<sup>k</sup> antigens were detected by the anti-K<sup>k</sup>/I<sup>a</sup> serum (King et al., 1985).

WNV-infected and mock infected MEF from CBA/H (H-2<sup>k</sup>), C3H.H-2<sup>2</sup> (H-2<sup>2</sup>) and B10.A (H-2<sup>b</sup>) strains were also labelled for class I MHC antigens in a protocol identical to the above, using polyclonal antibodies as follows: anti-K<sup>k</sup> and anti-D<sup>4</sup> for CBA/H, anti-K<sup>d</sup>/I<sup>a</sup> and anti-D<sup>4</sup> for C3H.H-2<sup>2</sup>, and anti-K<sup>k</sup> and anti-D<sup>4</sup> for B10.A. Control MEF samples from the respective strains were labelled with an irrelevant polyclonal antibody and NMS from the above panel.

Labelling of WNV-infected and mock infected MEF from the above strains was also carried out using MAbs against K<sup>k</sup>/D<sup>d</sup>, K<sup>k</sup>, I-A<sup>d</sup> and I-A<sup>b</sup> (see Antisera). Class I MHC antigen labelling with MAbs gave results identical to those obtained with polyclonal antisera.

FACS analysis. Fluorescence was measured using a FACS IV (Becton–Dickinson) with an argon ion laser set at 488 nm for FITC. Emitted fluorescence between 515 and 540 nm was measured. From each labelled sample, 10<sup>5</sup> cells were analysed. We found no differences in any of the MEF populations detectable by low angle (cell size) or right angle (membrane configuration) scatter analysis on the FACS (see Melamed et al., 1979). Thus differences in the MHC fluorescence distributions between any of the MEF groups cannot be explained by cell size differences between or within sample groups.

RESULTS

Flavivirus infection induces increased class I MHC antigen expression in MEF

BALB/c MEF (H-2<sup>d</sup>) were infected with 40 p.f.u./cell of purified WNV for 16 h and 40 h and the level of cell surface class I MHC antigen expression of both infected and uninfected control MEF was measured by fluorescent labelling and FACS analysis. Fluorescence profiles of the WNV-infected cells showed a significant increase in class I MHC antigens compared with uninfected control MEF (Fig. 1), and the level of fluorescence was higher 40 h after infection than after 16 h. Class II MHC antigens were not detected either before or after infection with WNV in BALB/c MEF (data not shown). After this first demonstration, all subsequent experiments used Vero cell-grown WNV seeded from brain stocks and harvested after the first passage to reduce possible variations in virulence.

Increases in class I MHC antigen expression were demonstrated after 48 h infection at 10 p.f.u./cell with the four Vero cell-grown flaviviruses tested, WNV, Kunjin, MVE and JBE (Fig. 2). Furthermore, similar increases in class I MHC antigen concentration were demonstrated in CBA/H (K<sup>k</sup> and D<sup>b</sup>), BALB/c (K<sup>k</sup> and D<sup>4</sup>), C3H. H-2<sup>12</sup> (K<sup>d</sup> and D<sup>4</sup>) and B10.A (K<sup>k</sup> and D<sup>4</sup>) MEF infected with these flaviviruses, indicating that mouse strain background or H-2 haplotype had no profound influence on the phenomenon (data not shown).
Fig. 1. Induction of increased expression of H-2K\textsuperscript{d} on BALB/c MEF by WNV infection. Profiles A, B and C show control untreated MEF, MEF infected with WNV for 16 h and MEF infected with WNV for 40 h, respectively. The plots (from FACS analysis) show fluorescence intensity divided into channels (abscissa) against cell number in each channel (ordinate).

Fig. 2. Induction of increased expression of H-2K\textsuperscript{d} on BALB/c MEF by flavivirus infection. The four panels show MEF infected with flaviviruses (a) WNV, (b) Kunjin, (c) MVE and (d) JBE for 48 h (broken line) compared with uninfected control MEF (solid line). The data presented as in Fig. 1.
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Flavivirus-induced increases in class I MHC antigen expression in MEF occurs by mechanism(s) partly independent of IFN-β secretion

IFN-β can induce increased class I MHC antigen expression on cells (Wallach et al., 1982). Theoretically, WNV infection of MEF could result in IFN-β production by the infected MEF which could up-regulate MHC expression of the whole population, including the MEF that remained uninfected. Therefore, anti-IFN-αβ antibody was added to MEF cultures at the time of WNV infection in an attempt to inhibit the action of any endogenously secreted IFN-β and to determine whether the increase in class I MHC after WNV infection is due solely to the action of virus-induced secreted IFN-β or whether WNV infection can up-regulate MHC expression by some other mechanism.

Fig. 3 (a) shows that the increase in class I MHC antigen expression caused by WNV infection (profile A) was partially inhibited by the presence of anti-IFN-αβ antibody (profile B); however, some 66% of the MEF population remained significantly more fluorescent than the control MEF.

To ascertain whether all secreted IFN-β in group B was neutralized by the anti-IFN-αβ antibody and therefore whether the increase in class I MHC antigen expression seen was due to residual IFN or another mechanism(s), a second experiment was performed. We have previously demonstrated that WNV does not affect MHC antigen expression after irradiation with 10^6.7 rads from a 60Co source but IFN-β remains active (data not shown). We therefore irradiated the supernatants of the MEF cultures of profiles A and B with 10^6.7 rads to inactivate any remaining virus. These supernatants were then added to fresh MEF cultures for 48 h and the level of class I MHC antigen expression was measured. IFN-β was also added to a control group of normal, irradiated MEF supernatants to determine whether the nutrients in the medium were still sufficient to support an increase in MHC expression in MEF in response to IFN-β.

The results from this experiment (Fig. 3b) show that while the supernatants from MEF infected with WNV for 48 h caused increased MHC expression on BALB/c MEF (profile G), those from WNV-infected MEF treated with anti-IFN-αβ antibody from the start of infection contained insufficient free IFN-β to cause a detectable increase in the surface class I MHC antigens (profile E). Thus, anti-IFN-αβ antibody was able to inhibit completely the MHC-increasing effect of endogenously produced and secreted IFN-β acting through MEF surface.
IFN receptors. This strongly suggests that increased class I MHC expression in WNV-infected MEF treated with anti-IFN-αβ antibody was due to mechanism(s) independent of secreted IFN-β. It is also clear from these experiments that secreted, WNV-induced IFN-β contributed to increased MHC expression in infected MEF.

The effect of polyI:C on the induction of class I MHC antigen expression in MEF

Treatment of BALB/c MEF with polyI:C brought about a significant increase in the level of expression of class I MHC antigens over and above control MEF (Fig. 4); this was completely inhibited by the addition of 200 units of anti-IFN-αβ antibody. We assumed, therefore, that polyI:C-induced IFN-β was completely neutralized by the anti-IFN-αβ antibody, particularly as previous experiments titrating the anti-IFN-αβ antibody on polyI:C-treated MEF showed an inhibition of the class I MHC antigen increase proportional to the concentration of anti-IFN-αβ antibody used (data not shown).

Increased class I MHC antigen expression by WNV-infected MEF is dependent upon host cell mRNA synthesis

WNV infection could increase the expression of class I MHC antigen expression by one of several mechanisms. Theoretically, this could result from increasing cellular mRNA synthesis, increasing translation of mRNA already transcribed or increasing the transport of MHC antigens to the cell surface membrane. To investigate these possibilities further, MEF were treated with AMD to block new mRNA transcription, and the level of class I MHC antigen expression was determined by FACS analysis in parallel with measurement of [3H]uridine incorporation into RNA. The replication of flavivirus RNA is not inhibited by AMD (Leary & Blair, 1983) and it is used in a method of isolating purified virus (Lobigs et al., 1986).

As shown in Fig. 5, treatment with AMD totally inhibited the WNV-induced increase in MHC antigens. [3H]Uridine incorporation was reduced by more than 90% in the AMD-treated groups, indicating that cellular RNA synthesis had been virtually abolished by the AMD treatment (Table 1), while more than 95% of the MEF remained alive, as assessed by trypan blue exclusion and low angle scatter parameters in the FACS (data not shown). Thus, we conclude
Flavivirus increases class I MHC expression

Table 1. Effect of AMD on [3H]uridine incorporation in WNV-infected MEF

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>[3H]Uridine incorporation (c.p.m.)†</th>
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<tbody>
<tr>
<td>Untreated</td>
<td>25107 ± 98</td>
</tr>
<tr>
<td>AMD</td>
<td>1975 ± 12</td>
</tr>
<tr>
<td>WNV</td>
<td>20988 ± 111</td>
</tr>
<tr>
<td>WNV + AMD</td>
<td>1245 ± 9</td>
</tr>
</tbody>
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* BALB/c MEF were either untreated, treated with AMD, infected with WNV or infected with WNV and then treated with AMD as described in Methods.
† Mean ± s.e.m. in 12 replicates.

that the WNV-induced increase in class I MHC antigen expression is dependent upon cellular mRNA synthesis. Whether this reflects a requirement for new mRNA synthesis, or replacement of short-lived mRNA is not known.

DISCUSSION

Tc cell lysis of cells infected with intracellular pathogens is crucial not only for controlling the spread of intracellular organisms and viruses, but also for their eventual eradication from the host (Kees & Blanden, 1976; Yap et al., 1978; Oldstone et al., 1986). In view of the central role played by class I MHC antigens in the recognition of virus-infected cells by virus-immune MHC-restricted Tc cells, the increase in class I MHC gene expression caused by flavivirus infection described here is an important immunological phenomenon.

WNV was used as the prototype model for detailed investigations, although infection for 48 h by all four of the flaviviruses tested so far (WNV, Kunjin, MVE and JBE) produced the phenomenon. Analysis was usually performed on MEF infected for 24 to 48 h; nevertheless, class I MHC increases on BALB/c MEF were detectable by FACS analysis as early as 8 h after WNV infection and increased up to at least 96 h after infection (data not shown). Inhibition of MHC increases in WNV-infected MEF by AMD treatment indicated that increased MHC expression requires transcription of mRNA from host cell DNA, but detailed mechanism(s) by which MHC expression increases remain unclear.

Increases in class I MHC antigen concentration were only partially inhibited by treating the MEF with anti-IFN-αβ antibody at the time of infection. This treatment was shown to neutralize all MHC-increasing activity that was either present in supernatants of infected MEF (probably due to IFN-β) or deliberately added as pure IFN-β, suggesting that increased class I MHC antigen expression on WNV-infected MEF was not caused solely by secretion of virus-induced IFNs. The possibility that some IFN-β was bound to receptors on the MEF before being neutralized by the antibody, or that WNV-induced IFN-β was able to act internally, i.e. without being secreted by the cell, cannot be excluded, although published evidence (Yarden et al., 1984; Leeuwenberg et al., 1987), and the results of the polyI:C experiments reported here, make the latter unlikely. Furthermore, from other work (N. J. C. King et al., unpublished data), it is evident that WNV infection can increase class I MHC antigen expression independently of secretion of virus-induced IFNs. Primary trophoblast giant cell outgrowths from preimplantation blastocysts hatched in vitro may be induced to express class I MHC antigens de novo following infection with WNV for 16 h. These cells neither secrete virus-induced IFNs (Barlow et al., 1984), nor are they susceptible to the increase of MHC antigen concentration induced by extracellular IFNs (Ozato et al., 1985; King et al., 1987).

The heterogeneity in the MHC fluorescence distribution of WNV-infected MEF in the presence of anti-IFN-αβ antibody revealed by profile B in Fig. 3 therefore presumably occurs because MEF are influenced individually and unequally by WNV infection per se to increase MHC antigen expression, in contrast to MEF exposed to IFN-α, -β or -γ, or WNV plus WNV-induced IFN-β, which show an increase in MHC fluorescence as a whole population (i.e. in a log10 normal distribution) identical to the fluorescence distribution of an untreated control MEF population (King et al., 1985; N. J. C. King, unpublished data).
Profile B in Fig. 3 further reveals two subpopulations of MEF. The first, some 66% of the total cell population, exhibited an increase in MHC antigen expression not inhibited by anti-IFN-αβ antibodies, and presumably caused by WNV infection via intracellular mechanism(s). The remainder showed an MHC antigen increase caused by secreted WNV-induced IFNs, and inhibited by anti-IFN-αβ antibodies. This suggested that the second population (34%) was not infected. However, immunofluorescence studies on acetone-fixed WNV-infected MEF using hyperimmune anti-WNV antiserum showed >80% of MEF nuclei to be brightly stained for WNV antigens 48 h after infection. Thus, while 15 to 20% of the population were probably not infected, 10 to 15% were WNV-infected, able to respond to WNV-induced secreted IFNs, but refractory to the intracellular MHC-increasing effects of WNV.

The heterogeneous MHC-increasing response of MEF to WNV infection may relate, among other factors, to time and m.o.i. of individual MEF. We have found, for example, the magnitude of WNV-induced MHC increase over 24 h is directly related to m.o.i. (data not shown). We are also currently investigating the possibility that cell cycle position at the time of infection may alter either the susceptibility or the MHC-increasing response of MEF to WNV infection, or both. Lastly, some fibroblasts, e.g. L929, readily infected by WNV (Kesson et al., 1987) and sensitive to exogenously added IFNs, are refractory to the MHC-increasing effects of WNV (data not shown). It seems possible therefore that polyclonal tertiary MEF populations may contain elements partially or completely refractory to the MHC-increasing effects of WNV.

Previous studies have demonstrated that virus-immune, class I MHC-restricted Tc cells are capable of triggering clearance of established viral infections in vivo (Kees & Bladen, 1976; Yap et al., 1978; Oldstone et al., 1986). The mechanisms by which T cells mediate virus clearance are not fully elucidated, but cytopathic activity is likely to be a contributor (Bladen, 1974). Flaviviruses infect a wide range of both vertebrate and arthropod hosts, and replicate in a wide variety of cultured cells (Brinton, 1986). In vitro, the latent phase of the flavivirus replication cycle in Vero cells lasts approximately 12 h, after which progeny viruses begin to be released, maximal virus titres occurring some 24 h after infection (Trent & Naeve, 1980). The relevance of these times to events in vivo is not fully understood, but our findings suggest that there could be a significant increase in MHC antigen concentration on infected cells before assembly of maximal numbers of progeny virions. It follows, therefore, that a flavivirus-induced increase in class I MHC antigen expression could contribute to more efficient lysis by virus-specific immune Tc cells in vivo and thus to limitation of virus replication.

REFERENCES


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