Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Journal of Neuroimmunology, 21 (1989) 157–168
Elsevier

JNI 00713

Flavivirus infection up-regulates the expression of class I and class II major histocompatibility antigens on and enhances T cell recognition of astrocytes in vitro

Yang Liu, Nicholas King, Alison Kesson, Robert V. Blanden and Arno Müllbacher

Microbiology Group, Division of Virology and Cellular Pathology, John Curtin School of Medical Research, Australian National University, Canberra, ACT 2601, Australia

(Received 18 April 1988)
(Accepted 11 July 1988)

Key words: Astrocyte; Major histocompatibility complex antigen; Flavivirus, T lymphocyte

Summary

West Nile virus (WNV) infection of astrocytes can up-regulate their expression of both class I and class II major histocompatibility complex (MHC) antigens as determined by flow cytometry with monoclonal antibodies specific for class I and class II MHC antigens. The up-regulation of class I MHC antigen expression could be partly caused by interferon secreted after WNV infection because the synthetic interferon inducer polyinosinic-polycytidylic acid (poly I:C) has similar effects. In contrast the up-regulation of class II MHC antigen expression was not induced by poly I:C.

The increased MHC antigen expression by WNV infection had significant effects on T cell recognition. Thus, WNV and influenza virus A/WSN double-infected astrocytes but not astrocytes infected by A/WSN alone were lysed by influenza virus-immune cytotoxic T cells. Similarly, WNV-infected astrocytes were better stimulators than normal astrocytes for a class II MHC-reactive T cell line, both in terms of T cell proliferation and interleukin release.

Introduction

It is well established that the major histocompatibility complex (MHC) antigens form an essential part of antigenic structures recognised by T lymphocytes (reviewed by Zinkernagel and Doherty, 1979; Schwartz, 1986) and additional evidence suggests that the quantity of the MHC antigens is an important variable determining both the induction and the effect of the immune response (Janeway et al., 1984; Blanden et al., 1987). This principle, however, harbors a paradox concerning the immunological status of the central nervous system (CNS). Cells in the CNS express little, if any, class I MHC antigen and essentially no class II MHC antigen (Williams et al., 1980). On the other hand, existing evidence suggests that immune reactions can take place in the CNS, e.g.

Address for correspondence: Yang Liu, Division of Virology and Cellular Pathology, John Curtin School of Medical Research, Australian National University, GPO Box 334, Canberra, ACT 2601, Australia.

in the case of experimental allergic encephalitis of rats, where transfer of syngeneic T cells specific for myelin basic protein (MBP) results in full expression of the disease (Lipton et al., 1976; Tourtellotte et al., 1978; Kreth et al., 1982; Booss et al., 1983; Wekerle et al., 1985). The paradox has been partly resolved by findings which indicate that at least some cells from the CNS, such as astrocytes, oligodendrocytes, brain endothelial cells and neurons can be induced to express class I and/or class II MHC antigens by lymphokines such as γ-interferon (γ-IFN) and by certain viral infections such as murine hepatitis virus and Theiler's murine encephalomyelitis virus (Hirsch et al., 1983; Massa et al., 1986; Suzumura et al., 1986; Rodriguez et al., 1987).

Additional data suggested that such elevation of the MHC antigens on astrocytes and/or brain endothelial cells can potentiate the antigen-presenting capacity of these cells (Fierz et al., 1985; Male et al., 1987). Thus, the induction of expression of MHC antigens on the cells of the CNS could be an important regulatory mechanism in the initiation, amplification and targeting of the local immune response.

The etiology of a number of neurological disease, most notably, multiple sclerosis, still remains a matter of debate. The preferred hypotheses involve an autoimmune phenomenon in which cellular and/or humoral immune responses are directed against constituents of myelin. However, epidemiological data implicate some kind of acquired factor, most likely an infection, in the etiology of multiple sclerosis (Waksman et al., 1986).

In recent years, evidence has accumulated that viral infections can result in the breakdown of immunological tolerance. Thus both autoreactive B cells (Haspel et al., 1983a, b) and autoreactive T cells (Pfizenmaier et al., 1975; Komatsu et al., 1978) have been observed during viral infection. In this context it would be of interest to study the possible effect of neurotropic viral infection on the local immune response in the CNS.

Flaviviruses are one group of viruses which are responsible for a number of neurological diseases (Monath, 1986). In this study, we address the effect of West Nile virus (WNV) infection on astrocytes in vitro.

Materials and methods

Virus

West Nile virus Sarafend strain (WNV) was obtained from Dr. I.D. Marshall and grown either in Vero cells (WNV-V) or in new-born mouse brain (WNV-B). The stocks were prepared and titrated as described (Reed and Muench, 1938; Taylor and Marshall, 1975). Influenza virus A/WSN was prepared by a standard method (Yap and Ada, 1977).

Mice

Mice were bred under pathogen-free conditions at the Animal Breeding Establishment of the John Curtin School of Medical Research. The strains used were: CBA/H (H-2^k), BALB/c (H-2^d), B10.T(6R) ($K^qI^qD^d$), B10.AQR ($K^qI^kD^d$). Newborn mice were used at 1 or 2 days of age and adult female mice at 6–8 weeks.

The preparation of astrocyte culture

Astrocytes were prepared in essentially the same way as has been described (McCarthy and de Vellis, 1980). Briefly, 1- to 2-day-old CBA/H mice were anesthetized at −20 °C for 15 min. After careful removal of the meninges, the brains were broken down by pipetting and were then subjected to treatment with trypsin/DNase I solution (0.1% trypsin and 0.001% DNase I, w/v) for 20 min, shaken occasionally. The action of trypsin was stopped by adding equal volumes of neural medium (Dulbecco's minimum essential medium (DMEM) supplemented with L-arginine 12 mg/l, L-asparagine 36 mg/l, folic acid 6 mg/l, D-glucose 7 g/l, and non-essential amino acids 1% v/v (Flow Laboratories, Cat. No. 16-810-49). The suspension was centrifuged and the pellet was dispersed. These cells were cultures in 80 cm^2 Nunclon plastic flasks at a density of 10^6/ml. At day 8, the cells were confluent and the medium was changed. One day later, the flasks were shaken for 15–18 h (37 °C, 250 rpm). The medium was then sucked off and the cultures were washed with Puck's saline, trypsinized into single-cell suspensions and cultured again. The secondary cultures were tested by indirect immunofluorescence microscopy with rabbit anti-human glial fibrillary acidic protein (GFAP) serum (Dako, Code A561)

and shown to be more than 95% GFAP positive. The secondary cultures were used in this study.

Infection of astrocytes by West Nile virus

After astrocytes were grown to confluency (approximately 10^5 cell/well in Linbro 24-well plastic tissue culture plates), the medium was sucked off and West Nile viruses (100 plaque-forming units (pfu)/cell of WNV-B and 20 pfu/cell WNV-V, respectively) were added in 100 μl and incubated at 37°C for 1 h. At different times after inoculation, cells were harvested and frozen at −20°C. Free virus was obtained by freeze-thawing plus ultrasonication as described (Taylor and Marshall, 1975).

Interferon induction by poly I:C in astrocyte cultures

The protocol is essentially the same as described by De Clercz (1981). Briefly, astrocytes were grown in 24-well tissue culture trays until confluency; the cultures were then exposed to poly I:C (Sigma, 25 μg/ml) for 8 h and washed with DMEM medium 3 times. Fresh neural medium was added and the cultures were further incubated for 40 h. The supernatants were harvested and IFN activity determined.

Interferon assay

IFN was assayed by inhibition of viral RNA synthesis in L929 cells as described by Morris et al. (1987). The titer of IFN was defined as reciprocal of the final dilution that gave 50% inhibition of Semliki Forest virus RNA synthesis.

Neuralization of IFN activity by IFN-specific antibody

100 μl of 1:20 dilution of supernatants from 48 h WNV-B-infected astrocytes were incubated with an equal volume of medium or 100 units of rabbit antiserum to mouse IFN ($\alpha + \beta$) (Lee Biomedical Research, Cat. No. 21031) at 37°C for 1 h. The IFN activity of these supernatants was titered as described above.

Establishment and maintenance of I^k-reactive T lymphocyte cell lines

Spleen cells from B10.T(6R) mice ($K^qI^qD^d$) were stimulated with those from B10.AQR ($K^qI^kD^d$) as described (King et al., 1986) for three passages. At the fourth passage, an enriched medium for T cell lines (DMEM supplemented with 2×10^{-4} M 2-mercaptoethanol, 10% fetal calf serum (FCS) and 5% concanavalin A (ConA)-activated spleen cell supernatant (CS) prepared as described by Sinickas et al. (1985a)) was added together with allogeneic stimulators.

Generation of cytotoxic T cell

Generation of secondary cytotoxic T cells against influenza virus A/WSN and primary allo-reactive cytotoxic T cells has been described in detail (Yap and Ada, 1977; King et al., 1986).

Cytotoxic T cell assay

The method for the L929 target system has been described (Yap and Ada, 1977). WNV-V-infected or uninfected astrocytes were cultured in flat-bottomed tissue culture plates at 37°C for 48 h. These cultures were then reinfected with influenza virus A/WSN (3 EID_{50}/cell) and labeled with ^{51}Cr by adding 25 μl medium containing 6×10^4 EID_{50}/well influenza virus A/WSN and 1:10 dilution of $Na_2{}^{51}Cr_2O_4$ and incubated at 37°C for 1 h. The rest of the assay was carried out in the same way as that for L929 targets.

T cell proliferation assay

The proliferation of the H-$2I^k$-reactive T cell line was determined by [^{3}H]methyl-thymidine (Amersham) incorporation as has been described (Sinickas et al., 1985b). Both spleen cells and astrocytes were used as stimulators. After growth to confluency in 96-well tissue culture plates (approximately 2×10^4 cells/well), the astrocytes were irradiated with 6000 R from a ^{60}Co γ-ray source. 10 μg/ml of indomethacin was added to the enriched medium of T cell lines and 150 μl of this medium per well was added together with 2×10^4 cells of an H-$2I^k$-reactive T cell line. 24 h later, 0.5 μCi/well of the [^{3}H]methyl-thymidine was added. After another 24 h, the cultures were harvested onto glass fiber filter strips and the incorporation of label into DNA was determined by scintillation counting.

Assay of interleukin release from an H-$2I^k$-reactive cell line

WNV-infected or mock-infected astrocytes grown to confluency in 96-well plates (2×10^4/

well) were irradiated with 2000 R and were used as stimulators of 2×10^4 T cells in 0.2 ml DMEM medium per well supplemented with 10^{-4} M 2-mercaptoethanol, 10 μg/ml indomethacin and 10% FCS. 24 h later the supernatants were collected and their interleukin activity was tested, as described by Lafferty et al. (1980). It is now known that this assay can measure interleukin-2 (IL-2) and/or interleukin-4 (IL-4) activity (Severinson et al., 1987).

Antibodies

Monoclonal antibodies (McAbs) specific for D^k, $I\text{-}A^k$ and $I\text{-}A^d$ antigens were obtained from the supernatants of cultures of hybridoma clones 15-5-5S, 11-5.2.1.9, and MK-D6 (Oi et al., 1978; Ozato et al., 1980; Kappler et al., 1981) (ATCC Nos. HB-24, T1B-94 and HB-3), respectively, obtained from the American Type Culture Collection. The supernatants were concentrated 10-fold on an American P10 membrane. In addition, an McAb specific for K^k (clone 11-4.1) was purchased from Becton-Dickinson. Rabbit anti-mouse immunoglobulin (RAMIg) was raised in a New Zealand white rabbit, separated from whole serum using a protein A-Sepharose column (Pharmacia) and conjugated to fluorescein-isothiocyanate (FITC) by standard methods (Goding, 1976).

Cell labeling

Astrocytes were infected with WNV or treated with recombinant γ-IFN (500 U/ml) or poly I:C for 48 h. Single-cell suspensions of astrocytes were obtained from the culture vessel by gentle trypsinization. The cells were counted and viability, as measured by trypan blue dye exclusion, was invariably > 95%. All subsequent steps of the labeling procedure were carried out at 4° C. Samples of 3×10^5 astrocytes were incubated in 100 μl of anti-MHC antibody for 60 min. After the first antibody incubation the cells were centrifuged through a bed of 500 μl FCS, the supernatant removed, and the astrocytes resuspended in 100 μl of RAMIg-FITC for a further 60 min at a dilution giving saturation labeling (determined by prior titration). Following this incubation, the cells were centrifuged through an FCS bed and resuspended in 250 μl DMEM for flow cytometry.

Flow cytometry

Fluorescence was measured using a FACS IV (Beckton-Dickinson) equipped with an argon ion laser set at the standard excitation wavelength of 488 nm for FITC. Emitted fluorescence between 515 and 540 nm was measured. 3×10^4 cells were analyzed from each labeled sample.

Cell size

In no experiment did any of the astrocyte sample populations show changes detectable by low-angle (cell size) or right-angle (membrane configuration) scatter analysis on the FACS. Thus we presume that differences in the MHC fluorescence distributions between astrocyte groups reflect a change in the cell surface MHC antigen concentration.

Results

Characterization of WNV infection in CBA/H astrocyte cultures

Astrocytes grown to confluency in 24-well tissue culture plates were infected with either 100 pfu/cell of WNV-B or 20 pfu/cell of WNV-V, and viral titers at different times after infection were determined. Titers dropped about 100-fold within 12 h (Table 1). However, a clear increase in viral titers was observed by 36 h. The titers reached a plateau at 48 h after infection and remained at a similar level for at least 1 week. Further experiments revealed that viral antigens became detectable after 24 h by immunofluorescence microscopy and by 40 h 50–60% of the astrocytes were expressing detectable viral antigen. The proportion of viral antigen-positive cells remained at a similar level for at least 2 weeks. These data show that WNV can productively infect some cells in astrocyte populations.

Interferon production in CBA/H astrocyte cultures after WNV infection or poly I:C treatment

Astrocyte cultures, either infected with WNV or treated with poly I:C, were tested for their ability to produce IFN. High titers of IFN were detected in the supernatant of astrocytes infected by WNV-B or WNV-V, or treated by poly I:C, all for 48 h (Table 2). The IFN titers were 4-fold

TABLE 1

KINETICS OF MULTIPLICATION OF WEST NILE VIRUS IN CBA/H ASTROCYTE CULTURES

Hours post-infection	Virus titers (log_{10} $TCID_{50}$/ml)	
	WNV-B [a]	WNV-V [b]
0	6.7	6.0
1	5.3	4.0
6	5.0	4.5
12	4.0	4.0
36	6.0	5.7
48	7.0	6.5
60	6.7	6.5
72	6.7	6.3
84	6.7	6.5
96	6.5	6.0
144	6.5	6.5

[a] West Nile virus prepared from mouse brain.
[b] West Nile virus prepared from Verò cell line.

lower in the supernatant of poly I:C-treated astrocytes than that of WNV-infected astrocytes. Furthermore, the IFN produced in WNV-B-infected astrocytes can be neutralized by antibody specific for IFN ($\alpha+\beta$), hence the IFN produced is α- and/or β-IFN (Fig. 1).

The effect of WNV infection on the expression of cell surface class I MHC antigens on astrocytes

The expression of class I MHC antigens on CBA/H astrocytes was investigated by flow cytometry. The single-cell suspensions were stained with McAbs specific either for H-2D^k or H-2K^k. After the astrocytes were infected by WNV-V for 48 h, the expression of H-2K^k as determined by peak fluorescence increased by more than 2-fold compared to mock-infected astrocytes (Fig. 2*a*). The binding of the McAb to the H-2K^k was specific as an isotype control McAb specific for H-2I-A^d (HB-3) did not bind significantly to either normal or WNV-infected astrocytes. Similar enhancement was observed in H-2D^k expression (Fig. 2*b*). As both WNV-B and WNV-V are potent IFN inducers (Table 2), the question arose as to whether the elevation in MHC antigen expression was due to the IFN produced. To investigate this question a synthetic IFN inducer, poly I:C was used. As shown in Fig. 2*c*, poly I:C treatment can significantly enhance the expression of class I MHC antigens, which admits the possibility that the enhanced expression after WNV infection is at least partly the result of virus-induced IFN.

TABLE 2

PRODUCTION OF INTERFERON BY ASTROCYTES AFTER WNV INFECTION OR POLY I:C TREATMENT

Samples [a]	log_2 IFN titers [b]
Normal astrocytes	2
WNV-B-infected astrocytes [c]	10
WNV-V-infected astrocytes [d]	10
Poly I:C-treated astrocytes	8

[a] Supernatants harvested 48 h after treatment.
[b] Reciprocal of the highest dilutions that cause 50% reduction in Semliki Forest virus RNA synthesis.
[c] West Nile virus prepared from mouse brain.
[d] West Nile virus prepared from Vero cell line.

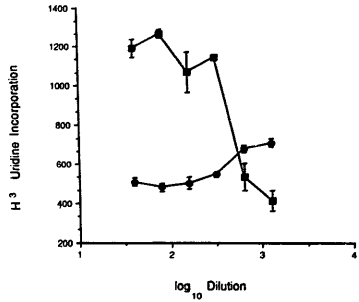

Fig. 1. Replication of Semliki Forest virus (SFV) in L929 cells in the presence of WNV-infected astrocyte supernatant (●) and WNV-infected astrocyte supernatant treated with antibody specific to ($\alpha+\beta$)-IFN at 37°C for 1 h (■). [^{3}H]Uridine incorporation in SFV-infected L929 cells: 1414±108 ($n=6$), [^{3}H]uridine incorporation of normal L929 cells: 426±19.8 ($n=6$).

Susceptibility of CBA/H astrocytes to influenza virus-immune cytotoxic T cells (Tc)

As class I MHC antigens are the major restriction elements for recognition by Tc, the effect of WNV-enhanced class I MHC antigen expression on influenza virus-immune Tc-mediated lysis of astrocytes was investigated. CBA/H (H-2^k) astrocytes doubly infected with WNV-V and influenza virus were lysed by CBA/H influenza

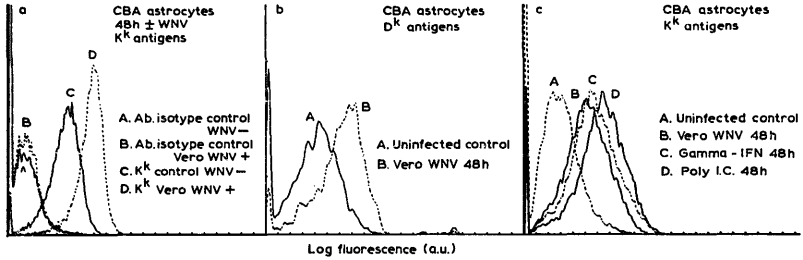

Fig. 2. Effects of WNV infection on the expression of cell surface class I MHC antigen in CBA/H astrocytes ($H\text{-}2^k$) as determined by flow cytometry. *a*: Fluorescence intensity on normal (A, C) and WNV-V-infected (B, D) astrocytes with McAb 11-4.1 ($H\text{-}2K^k$ specific, C, D) and HB-3 ($H\text{-}2I\text{-}A^d$ specific A, B, isotype control). *b*: Fluorescence intensity on normal (A) and WNV-V-infected (B) astrocytes, determined by labeling with McAb HB-24 ($H\text{-}2D^k$ specific). *c*: Fluorescence intensity after labeling with McAb 11-4.1 ($H\text{-}2K^k$ specific) on normal astrocytes (A), infected by WNV-V (B), treated with γ-IFN (500 U/ml) (C), or poly I:C (25 μg/ml) (D). In each case astrocytes were treated for 48 h.

virus-immune Tc (Table 3). In contrast, astrocytes infected by influenza virus or WNV alone were not lysed by influenza virus-immune Tc more than normal astrocytes. The activity of the Tc was confirmed as they lysed influenza virus-infected L929 ($H\text{-}2^k$) cells, but not mock-infected L929 cells. This result revealed that WNV infection enhances MHC-restricted virus-specific Tc recognition on astrocytes.

Lysis of WNV-infected astrocytes by BALB/c anti-CBA/H alloreactive T cells was significantly higher than that of normal astrocytes while comparable to the lysis of γ-IFN-treated astrocytes (Fig. 3), hence WNV infection enhances alloreactive Tc recognition on astrocytes.

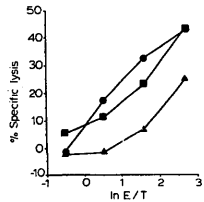

Fig. 3. Susceptibility of normal astrocytes (▲), WNV-V-infected astrocytes (■), and γ-IFN (500 U/ml)-treated astrocytes (●) to BALB/c anti-CBA/H alloreactive Tc. The results presented are means of triplicates. Standard errors are not shown but were all below 4%.

TABLE 3

THE EFFECT OF WNV INFECTION ON THE SUSCEPTIBILITY OF CBA/H ($H\text{-}2^k$) ASTROCYTES TO LYSIS BY INFLUENZA VIRUS-SPECIFIC Tc CELLS

Percentage of ^{51}Cr release from virus-infected or uninfected targets over a 6 h period. Killer-to-target ratio was 15:1 derived from 3-point linear regression analysis. All points were means of triplicates with SE of the mean all below 4.2%.

	L929 targets		Astrocyte targets			
	A/WSN infected	Uninfected	A/WSN infected	Uninfected	A/WSN and WNV infected	WNV infected
Specific lysis (%)	53.7	3.74	18.6	14.0	34.3	17.9
r	0.987	0.379	0.960	0.939	0.999	0.990

Effect of WNV-B infection on the expression of cell surface class II MHC antigens in astrocytes

The expression of cell surface class II MHC antigens on CBA/H astrocytes was also determined by flow cytometry. Astrocytes were labeled with H-2I-A^k-specific McAb TIB94; control cells were treated with HB-3, an McAb specific for H-2I-A^d. Normal CBA/H (H-2^k) astrocytes express little or no detectable class II MHC antigens, as their binding to TIB94 (H-2I-A^k-specific) is not significantly higher than to HB-3 (H-2I-A^d-specific) (Fig. 4*a*). However, after the astrocytes were infected by WNV-B for 48 h, a distinct increase in expression of H-2I-A^k antigen was detected Fig. 4*b*), although the amount of H-2I-A^k expressed on WNV-infected astrocytes was not as

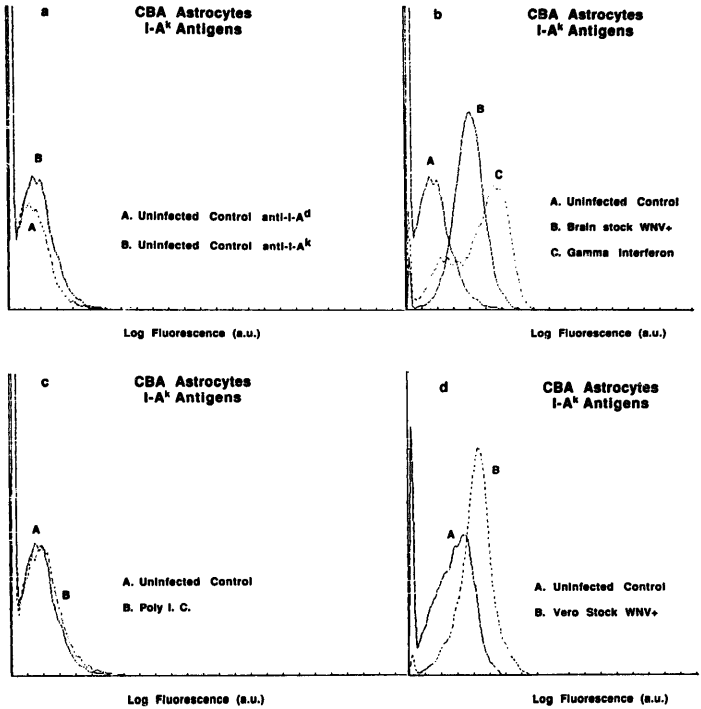

Fig. 4. Effects of WNV infection on the expression of cell surface class II MHC antigens on CBA/H astrocytes as determined by flow cytometry. *a*: Fluorescence intensity of normal CBA/H astrocytes after labeling by McAb specific for H-2I-A^k (TIB94) (B), and H-2I-A^d (HB-3) (A). *b*: Fluorescence intensity of CBA/H astrocytes after labeling of H-2I-A^k with TIB94 on normal astrocytes (A), WNV-B-infected astrocytes (B), and astrocytes treated with γ-IFN (C). *c*: As for *b*, but normal astrocytes (A) and astrocytes treated with poly I:C (B). *d*: As above but astrocytes infected by WNV-V. Astrocytes were subject to pre-treatment with WNV or IFN or poly I:C for 48 h before labeling.

much as that on γ-IFN-treated astrocytes. The specificity of the labeling was ascertained in two ways: (a) HB-3 (anti-H-2I-A^d) McAb did not bind WNV-infected CBA/H (H-2^k) astrocytes (Fig. 2*a*); (b) as HB-3 is of the IgG_{2a} subclass, while TIB94 is IgG_{2b}, another IgG_{2b} McAb specific for murine CD4 was tested and shown not to bind either WNV-infected or normal astrocytes (data not shown). It seems unlikely that material other than WNV in WNV-B (e.g. murine IFN) is responsible for the up-regulation of class II MHC antigen, as WNV-V that was passaged 4 times in Vero cells (a permanent cell line originating from monkey kidney that does not secrete murine IFN) also consistently up-regulated class II MHC antigen, though to a lesser extent than WNV-B in this particular experiment (Fig. 4*d*). Furthermore, poly I:C, which induced IFN production and enhanced class I MHC antigen expression in astrocyte cultures (Fig. 2*c*, Table 2) did not induce class II MHC antigen expression (Fig. 4*c*). The mechanism of the induction of class II MHC antigen is at present unknown.

Astrocytes as stimulators for H-2I^k-reactive T cell lines

An alloreactive B10.T(6R) anti-B10.AQR T cell line specific for H-2I^k antigens was used to determine if the WNV-induced expression of class II MHC antigens on astrocytes can be recognised by T cells. The T cell line was induced to proliferate by spleen cells from mice bearing H-2I^k antigens, e.g. B10.AQR and CBA/H mice, but not by autologous B10.T(6R) spleen cells (Table 4, Expt. 1).

WNV-infected CBA/H astrocytes also stimulated the proliferation of the H-2I^k-reactive T cell line (Table 4, Expt. 2). The stimulation was comparable to CBA/H spleen cells; mock-infected astrocytes had no stimulation effect. To test whether the induced stimulation effect of astrocytes was due to induced class II MHC antigens on their surface, the H-2I-A^k-specific McAb TIB94 was added. TIB94 blocked the proliferation of the H-2I^k-specific cell line stimulated by either B10.AQR spleen cells or by WNV-V-infected CBA/H astrocytes (Table 4, Expt. 3).

WNV-infected and uninfected astrocytes were also compared for their ability to stimulate IL-2 and/or IL-4 release from the H-2I^k-specific T cell line described above. WNV-infected CBA/H astrocytes stimulated significant release of IL-2 and/or IL-4, whereas uninfected CBA/H astrocytes were much poorer stimulators (Fig. 5). The quantity of IL-2 and/or IL-4 released was about 25-fold higher with WNV-infected astrocytes than with normal astrocytes. Specificity of the stimula-

TABLE 4

THE EFFECT OF WNV INFECTION ON ABILITY OF CBA/H ASTROCYTES TO STIMULATE THE PROLIFERATION OF AN I^k-REACTIVE T CELL LINE

Expt.	Passages of the responder (2×10^4/well)	Stimulators [a] (5×10^4/well)	TIB94 (1:40)	^{3}H cpm (means ± SE)
1	6	Nil	–	278 ± 39.7
		CBA/H spleen	–	2249 ± 144.6 *
		B10.AQR spleen	–	2170 ± 117.5 *
		B10.T6R spleen	–	377 ± 69.5
2	8	Nil	–	188 ± 69.5
		CBA/H spleen	–	2708 ± 91.0 *
		WNV-CBA/H astro [b]	–	2129 ± 165 *
		CBA/H astrocyte	–	135 ± 6.5
3	11	Nil	–	222 ± 84.6
		B10.AQR spleen	–	1505 ± 200 *
		B10.AQR spleen	+	147 ± 37.3
		WNV-CBA/H astro [b]	–	1058 ± 124.5 *
		WNV-CBA/H astro [b]	+	538 ± 42.2 *

[a] H-2 haplotype: CBA/H, H-2^k, B10.AQR, $K^qI^kD^d$; B10.T(6R), $K^qI^qD^d$.

[b] Astrocytes from CBA/H mice were infected by WNV-V for 24 h before irradiation.

* cpm significantly higher than the medium control ($P < 0.01$).

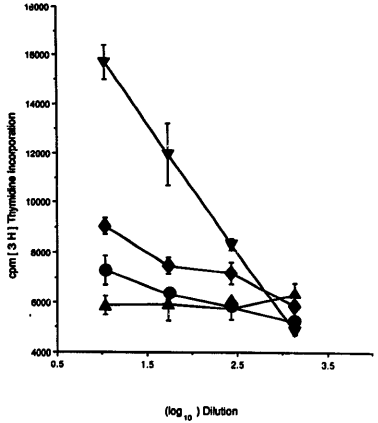

Fig. 5. Interleukin-2 and/or interleukin-4 (IL-2/IL-4) release from an H-2I^k-specific T cell line (passage 11). IL-2/IL-4 activity was determined by ability to maintain proliferation of ConA-stimulated T lymphoblasts ([^{3}H]thymidine incorporation). Stimulators were: WNV-infected CBA/H astrocytes (▼), WNV-infected CBA/H astrocytes in the presence of anti-H-2I-A^k monoclonal antibody TIB94 (●), normal CBA/H astrocytes (◆), and normal CBA/H astrocytes in the presence of TIB94 (▲).

tion for H-2I-A^k was confirmed as the IL-2 and/or IL-4 release was blocked by McAb TIB94.

To address the question as to whether the T cell line we used is representative of an anti-H-2I^k T cell population, a bulk culture of primary anti-I^k T cells was obtained by in vitro stimulation of B10.T(6R) spleen cells with B10.AQR spleen cells. WNV-infected and uninfected CBA/H astrocytes were compared for their ability to stimulate the T cell population.

WNV-infected CBA/H astrocytes stimulated significantly IL-2 and/or IL-4 release from the primary H-2I^k-reactive T cell population, while the normal astrocytes induced essentially no IL-2 and IL-4 release (Fig. 6).

Discussion

Since it was established that MHC antigens formed an important part of the antigenic structure recognised by T lymphocytes (Zinkernagel and Doherty, 1979; Schwartz, 1986), there has been great interest in the regulation of MHC antigen expression on potential antigen-presenting cells. It is well documented that different virus infections may either inhibit or enhance the expression of MHC antigens. Notably, infection by adenovirus (Pääbo et al., 1986), herpes simplex virus type 2 (Jennings et al., 1985), ectromelia virus (Gardner et al., 1975) and measles virus (Rager-Zisman et al., 1981) can down-regulate class I MHC antigen expression of their host cells and may make the latter less susceptible to cytotoxic T cells. On the other hand, murine hepatitis virus (Massa et al., 1986; Suzumura et al., 1986), Epstein-Barr virus (McCune et al., 1975), Moloney murine leukemia virus (Flyer et al., 1985) and Theiler's murine encephalomyelitis virus (Rodriguez et al., 1987) can up-regulate class I and/or class II MHC antigen expression. Our results described here revealed that flavivirus infection can

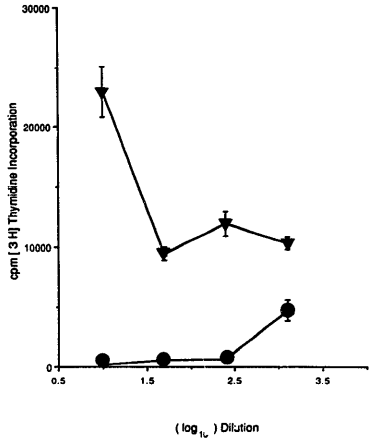

Fig. 6. Interleukin-2 and/or interleukin 4 release from B10.T(6R) (H-2K^{q}I^{q}D^d) anti-B10.AQR (H-2K^{q}I^{k}D^d) T cell populations generated in primary culture. Stimulators were: WNV-infected CBA/H astrocytes (▼), and normal CBA/H astrocytes (●).

up-regulate the expression of cell surface class I and class II MHC antigens.

It has been documented that interferon can up-regulate class I and II MHC antigen. All three classes of IFN known (α, β, γ) have been reported to enhance class I MHC antigen expression, while only γ-IFN can up-regulate the expression of class II MHC antigens (Wong et al., 1985). In our experiment, any treatment that can induce IFN production, e.g. WNV-B and WNV-V infection or poly I : C treatment, can up-regulate class I MHC antigens; it is possible that the up-regulation of class I MHC antigen expression by WNV infection is at least partly the result of IFN produced after virus infection. In contrast, poly I : C treatment of astrocytes, which induces good IFN production and increases class I MHC antigen expression equal to or more than WNV infection, fails to induce class II MHC antigen expression. This finding together with the data that IFN produced in WNV-B-infected astrocyte culture can be neutralized by anti-($\alpha + \beta$)-IFN antibody strongly suggests that the induction of class II MHC antigen by WNV does not depend on IFN produced. Addition of antibodies that neutralize γ-IFN to WNV-infected astrocytes culture would help to answer the question if free γ-IFN was involved in class II MHC antigen induction. However, the question as to what extent virus replication per se and induced interferon actually contribute to enhanced class I MHC expression is more problematic. Some obvious approaches used by others (Massa et al., 1986), i.e. use of ultraviolet-inactivated virus and neutralizing McAb to the virus cannot provide definite information, because it is not known to what extent UV inactivation will inhibit viral protein and nucleic acid synthesis and the mechanism of viral neutralization is at present not well understood. Furthermore, addition of IFN antibody may not help to exclude the role of IFN due to the autocrine activity without the need of secretion (Sanceau et al., 1987).

It seems unlikely that the binding of H-2I-A^k-specific McAb to WNV-infected but not normal astrocytes of CBA/H($H\text{-}2^k$) was due to the molecular mimicry by a viral product of an epitope on the I^k molecule, because an McAb specific for H-2I-A^d can bind to WNV-infected astrocytes from BALB/c($H\text{-}2^d$) mice but cannot bind to WNV-infected astrocytes from CBA/H($H\text{-}2^k$) mice; WNV can replicate productively in astrocytes from both strains of mice (data not shown).

A number of studies have shown that treatment which up-regulates the expression of cell surface class I MHC antigens increases the susceptibility of cells to lysis by appropriately sensitised Tc cells, probably due to recognition by low-affinity clones that could not exhibit cytotoxic activity without the elevation of MHC antigen expression (Shimonkevitz et al., 1985). Infection by viruses that down-regulate cell surface MHC antigen expression, on the other hand, results in reduced susceptibility of the infected cells to lysis by virus-specific cytotoxic T cells. Thus it has been shown that herpes simplex virus type 2 infection, which down-regulates cell surface MHC expression, reduces the susceptibility of an SV40- and HSV2-double-infected targets to lysis by SV40-specific Tc cells, as compared to target cells infected with SV40 alone (Jennings et al., 1985). Adenovirus type 5 infection inhibits the cell surface expression of class I MHC antigen via the E3 protein. This phenomenon results in less efficient recognition by adenovirus-specific Tc cells of target cells infected by this wild-type virus compared to target cells infected by an E3 deletion mutant which has no effect on host MHC antigen expression (Müllbacher and Bellett, personal communication) and could be responsible for the tumorigenicity of the wild-type virus (Eager et al., 1985). A Moloney murine leukemia virus that increases cell surface class I MHC antigen expression was also reported to increase the susceptibility of host cells to lysis by alloreactive Tc cells (Flyer et al., 1985). However, no attempt was made to address the effect of up-regulated MHC antigen expression on the recognition of MHC-restricted, antigen-specific T cells. In this paper, we compared WNV-infected astrocytes with uninfected controls for their ability to act as targets of influenza virus-specific Tc cells and as stimulators for an I^k-reactive T cell line. The results showed that WNV infection has significant effects on T cell recognition of astrocytes. Thus, after infection by influenza virus, normal astrocytes are poor targets of influenza virus-specific Tc, while doubly infected (with WNV and influenza) astrocytes can

be well recognized by influenza-specific Tc. WNV-infected astrocytes from CBA/H mice can also stimulate I^k-reactive T cells significantly better than the normal astrocytes, in terms of both T cell proliferation and interleukin release. The latter function was also enhanced in populations of primary anti-H-$2I^k$ T cells. These findings are particularly interesting because astrocytes are a large population of cells in the CNS, presumably important both for the physiology and immunology of the CNS (Fontana et al., 1985). Elevated T cell recognition of astrocytes could have an important effect on the organism. These findings also raise interesting questions regarding self tolerance. If T cell tolerance to self antigens is determined quantitatively (Blanden et al., 1987) the up-regulation of MHC antigens in the CNS could possibly break T cell self tolerance in the CNS. This may explain the autoimmune/viral etiology of some neurological diseases.

Acknowledgements

We thank Boehringer Ingelheim for the kind gift of recombinant γ-IFN and Barb Geary for typing the manuscript. Part of the work was carried out when one of us (Y.L.) was a recipient of a World Health Organisation Fellowship.

References

Blanden, R.V., Hodgkin, P.D., Hill, A., Sinickas, V.G. and Müllbacher, A. (1987) Quantitative considerations of T cell activation and self-tolerance. Immunol. Rev. 98, 75–93.

Booss, J., Esiri, M.M., Tourtellotte, W.M. and Mason, D.Y. (1983) Immunohistological analysis of T lymphocyte subsets in the central nervous system in chronic progressive multiple sclerosis. J. Neurol. Sci. 62, 219–232.

De Clercq, E. (1981) Interferon induction by polynucleotides, modified polynucleotides, and polycarboxylates. Methods Enzymol. 78, 227–236.

Eager, K.B., Williams, J., Breiding, D., Pan, S., Knowles, B., Appella, E. and Ricciardi, R. (1985) Expression of histocompatibility antigens H-K, -D, and -L is reduced in adenovirus-12-transformed mouse cells and is restored by interferon. Proc. Natl. Acad. Sci. U.S.A. 82, 5525–5529.

Fierz, W., Endler, B., Reske, K., Wekerle, H. and Fontana, A. (1985) Astrocytes as antigen-presenting cells. I. Induction of Ia antigen expression on astrocytes T cells via immune interferon and its effect on antigen presentation. J. Immunol. 134, 3785–3793.

Flyer, D.C., Burakoff, S.J. and Faller, D.V. (1985) Retrovirus induced changes in major histocompatibility complex antigen expression influence susceptibility to lysis by cytotoxic T lymphocytes. J. Immunol. 135, 2287–2292.

Fontana, A. and Fierz, W. (1985) The endothelium-astrocyte immune control system. Springer Semin. Immunopathol. 8, 57–70.

Gardner, I.D., Bowern, N.A. and Blanden, R.V. (1975) Cell mediated cytotoxicity against ectromelia virus-infected target cells. Eur. J. Immunol. 5, 122–127.

Goding, J.W. (1976) Conjugation of antibodies with fluorochromes: modifications to the standard methods. J. Immunol. Methods 13, 215–226.

Haspel, M.V., Onodera, T., Prabhakar, B.S., Horita, M., Suzuki, H. and Notkins, A.L. (1983a) Viral induced autoimmunity: monoclonal antibodies that react with endocrine tissues. Science 220, 304–306.

Haspel, M.V., Onodera, T., Prabhakar, B.S., McClintock, P.R., Essani, K., Ray, U.R., Yagihashi, S. and Notkins, A.L. (1983b) Multiple organ-reactive monoclonal autoantibodies. Nature 304, 73–76.

Hirsch, M.-R., Wietzerbin, J., Pierres, M. and Goridis, C. (1983) Expression of Ia antigens by cultured astrocytes treated with γ-interferon. Neurosci. Lett. 41, 199–204.

Janeway, Jr., C.A., Bottomly, K., Babich, J., Conrad, P., Conzen, S., Jones, B.K., Katz, M., McVay, L., Murphy, D.B. and Tite, J. (1984) Quantitative variation in Ia antigen expression plays a central role in immune regulation. Immunol. Today 5, 99–105.

Jennings, S.R., Rice, P.L., Klosewski, E.D., Anderson, R.W., Thompson, D.L. and Tevethia, S.S. (1985) Effect of herpes simplex virus type 1 and 2 on surface expression of class I major histocompatibility complex antigens on infected cells. J. Virol. 56, 757–766.

Kappler, J.W., Skidmore, B., White, J. and Marrack, P. (1981) Antigen-inducible, H-2-restricted interleukin-2-producing T cell hybridomas lack of independent antigen and H-2 recognition. J. Exp. Med. 153, 1198–1214.

King, N.J.C., Müllbacher, A. and Blanden, R.V. (1986) Relationship between surface H-2 concentration, size of different target cells and lysis by cytotoxic T cells. Cell. Immunol. 98, 525–532.

Komatsu, Y., Nawa, Y., Bellamy, A.R. and Marbrook, J. (1978) Clones of cytotoxic lymphocytes can recognize uninfected cells in a primary response against influenza virus. Nature 274, 802–804.

Kreth, H.W., Dunker, R., Rodt, H. and Meyermann, R. (1982) Immunohistochemical identification of T lymphocytes in the central nervous system of patients with multiple sclerosis and subacute sclerosing panencephalitis. J. Neuroimmunol. 3, 177–183.

Lafferty, K.J., Prowse, S.J., Al-adra, A., Warren, H., Vasalli, J. and Reich, E. (1980) An improved assay for interleukin-2 (lymphocyte growth factor) produced by mitogen activated lymphocytes. Aust. J. Exp. Biol. Med. Sci. 58, 533–544.

Lipton, H.L. and Dal Canto, M.C. (1976) Theiler's virus-induced demyelination: prevention by immunosuppression. Science 192, 62–64.

McCarthy, K.D. and de Vellis, J. (1980) Preparation of separate astroglial and oligodendroglial cell cultures from rat cerebral tissue. J. Cell Biol. 85, 890–902.

McCune, J.M., Humphreys, R.E., Yocum, R.R. and Strominger, J.L. (1975) Enhanced representation of HL-A antigens on human lymphocytes after mitogenesis induced by phytohemagglutinin of Epstein-Barr virus. Proc. Natl. Acad. Sci. U.S.A. 72, 3206–3209.

Male, D.K., Pryce, G. and Hughes, C.C.W. (1987) Antigen presentation in brain: MHC induction on brain endothelium and astrocytes compared. Immunology 60, 453–459.

Massa, P.T., Dorries, R. and ter Meulen, V. (1986) Viral particles induce Ia antigen expression on astrocytes. Nature 320, 543–546.

Monath, T.P. (1986) Pathobiology of flaviviruses. In: S. Schlesinger and M.J. Schlesinger (Eds.), The Togaviridae and Flaviviridae, Plenum Press, New York – London, pp. 375–440.

Morris, A., Tomkins, P.T., Maudsley, D.J. and Blackman, M. (1987) Infection of cultured murine brain cells by Semliki Forest virus: effect of interferon-$\alpha\beta$ on viral replication, viral antigen display, major histocompatibility complex antigen display and lysis by cytotoxic T lymphocytes. J. Gen. Virol. 68, 99–106.

Oi, V.T., Jones, P.O., Goding, J.W., Herzenberg, L.A. and Herzenberg, L.A. (1978) Properties of monoclonal antibodies to mouse Ig allotypes, H-2 and Ia antigens. Curr. Top. Microbiol. Immunol. 81, 115–129.

Ozato, K., Mayer, N. and Sachs, D.H. (1980) Hybridoma cell lines secreting monoclonal antibodies to mouse H-2 and Ia antigens. J. Immunol. 124, 533–540.

Pääbo, S., Nilsson, T. and Peterson, P.A. (1986) Adenoviruses of subgenera B, C, D, and E modulate cell-surface expression of major histocompatibility complex class I antigens. Proc. Natl. Acad. Sci. U.S.A. 83, 9665–9669.

Pfizenmaier, K., Trostman, H., Rollinghoff, M. and Wagner, H. (1975) Temporary presence of self-reactive cytotoxic T lymphocytes during murine lymphocytic choriomeningitis. Nature 258, 1238–1240.

Rager-Zisman, B., Ju, G., Rajan, R.V. and Bloom, B.R. (1981) Decreased expression of H-2 antigens following acute measles virus infection. Cell. Immunol. 59, 319–329.

Reed, L.J. and Muench, H. (1938) A simple method of estimating fifty percent end points. Am. J. Hyg. 27, 493–497.

Rodriguez, M., Pierce, M. and Howie, E.A. (1987) Immune response gene products (Ia antigens) on glial and endothelial cells in virus induced demyelination. J. Immunol. 138, 3438–3442.

Sanceau, J., Sondermayer, P., Beranger, F., Falcoff, R. and Vagero, C. (1987) Intracellular γ-interferon triggers an antiviral state in transformed L cells. Proc. Natl. Acad. Sci. U.S.A. 84, 2906–2910.

Schwartz, R.H. (1986) Immune response (Ir) gene of the murine major histocompatibility complex. Adv. Immunol. 38, 31–201.

Severinson, E., Naito, T., Tokumoto, H., Fukushima, D., Hirano, A., Hama, K. and Honjo, T. (1987) Interleukin 4 (IgG_1 induction factor): a multifunctional lymphokin acting also on T cells. Eur. J. Immunol. 17, 67–72.

Shimonkevitz, R., Leuscher, B., Cerottini, J.-C. and MacDonald, H.R. (1985) Clonal analysis of cytolytic T lymphocyte-mediated lysis of target cells with inducible antigen expression: correlation between antigen density and requirement for Ly-2/3 function. J. Immunol. 135, 892–899.

Sinickas, V.G., Ashman, R.B. and Blanden, R.V. (1985a) The cytotoxic response to murine cytomegalovirus. I. Parameters in vivo. J. Gen. Virol. 66, 747–755.

Sinickas, V.G., Ashman, R.V. and Blanden, R.V. (1985b) The cytotoxic response to murine cytomegalovirus. II. In vitro requirement for generation of cytotoxic T cells. J. Gen. Virol. 66, 757–765.

Suzumura, A., Lavi, E., Weiss, S.R. and Silbereg, D.H. (1986) Coronavirus infection induces H-2 antigen expression on oligodendrocytes and astrocytes. Science 232, 991–993.

Taylor, W.P. and Marshall, I.D. (1975) Adaption studies with Ross River virus: laboratory mice and cell cultures. J. Gen. Virol. 28, 59–72.

Tourtellotte, W.W. and Ma, I.B. (1978) Multiple sclerosis: the blood–brain barrier and the measurement of de novo central nervous system of Ig synthesis. Neurology 28, 76–83.

Waksman, B. and Reingold, S.C. (1986) Viral etiology of multiple sclerosis: where does the truth lie? Trends Neurosci. 9, 388–391.

Wekerle, H. and Fierz, W. (1985) T lymphocyte auto-immunity in experimental autoimmune encephalomyelitis. Concepts Immunopathol. 2, 102–127.

Williams, K.A., Hart, D.N.J., Fabre, J.W. and Morris, P. (1980) Distribution and quantitation of HLA-ABC and DR (Ia) antigens on human kidney and other tissues. Transplantation 29, 274–279.

Wong, G.H.W., Bartlett, P.F., Clark-Lewis, I., Battye, F. and Schrader, J.W. (1984) Inducible expression of H-2 and Ia antigens on brain cells. Nature 310, 688–691.

Wong, G.H.W., Bartlett, P.F., Clark-Lewis, I., McKimm-Breschkin, J.L. and Schrader, J.W. (1985) Interferon-γ induces the expression of H-2 and Ia antigen on brain cells. J. Neuroimmunol. 7, 255–278.

Yap, K.L. and Ada, G.L. (1977) Cytotoxic T cells specific for influenza virus infected target cells. Immunology 32, 151–159.

Zinkernagel, R.M. and Doherty, P.C. (1979) MHC-restricted cytotoxic T cells: studies on the role of polymorphic major transplantation antigens determining T cell restriction-specificity, function and responsiveness. Adv. Immunol. 27, 51–177.