

JNI 00992

Differential suppression of interferon- γ -induced Ia antigen expression on cultured rat astroglia and microglia by second messengers

Atsushi Sasaki¹, Steven W. Levison² and Jenny P.-Y. Ting^{1,2,3}

¹ Lineberger Cancer Center, ³ Department of Microbiology-Immunology and ² Curriculum in Neurobiology, University of North Carolina, Chapel Hill, North Carolina 27599, U.S.A.

(Received 4 December 1989)

(Revised, received 1 May 1990)

(Accepted 2 May 1990)

Key words: Ia antigen; Interferon- γ ; Astroglia; Microglia; Cyclic AMP; Protein kinase C

Summary

The roles of intracellular second messengers in interferon- γ (IFN- γ)-induced Ia antigen (Ag) expression by astroglia and microglia were examined. Ia Ag on both glia types was induced by IFN- γ . Reagents known to increase intracellular cAMP or activate intracellular protein kinase C (PKC) reduced IFN- γ -induced Ia Ag expression by astroglia. In contrast, increasing intracellular cAMP had no suppressive effect on Ia Ag expression by microglia. These results indicate (1) cAMP and PKC negatively regulate IFN- γ -induced Ia expression on astroglia, and (2) Ia expression is regulated differentially in astroglia vs. microglia. These findings may explain the frequent observation of Ia⁺ microglia (or macrophages) but not astroglia in various neurodegenerative diseases.

Introduction

Class II major histocompatibility complex (MHC) antigens (Ag) (also known as Immune response associated Ia Ag) are cell surface molecules that play major roles in regulating immune responses (Kaufman et al., 1984). Although the

brain has traditionally been considered an immunologically privileged site, immune responses have been shown to occur in the central nervous system (CNS). An effective immune response to an infectious agent or an altered immune response to auto-antigens in the CNS presumably requires the expression of Ia Ag on brain cells (Watanabe et al., 1983; Sobel et al., 1984; Olsson et al., 1987). In accord with this, *in vitro* studies indicate that Ia Ag can be induced on distinct populations of brain cells such as microglia, astroglia, and endothelial cells (Wong et al., 1985; Frei et al., 1987; Male et al., 1987; Suzumura et al., 1987). Interferon- γ (IFN- γ) is the most potent inducer of Ia Ag on these cells (McCarron et al., 1986; Massa et

Address for correspondence: Jenny P.-Y. Ting, Ph.D., Lineberger Cancer Research Center, CB No. 7295, Room 209, School of Medicine, University of North Carolina, Chapel Hill, NC 27599-7295, U.S.A.

This work was supported by Grant 1785 from The Multiple Sclerosis Society.

al., 1987), although other substances including viruses are known to affect Ia Ag expression on rat astroglia (Massa et al., 1987). In vivo, Ia Ag⁺ microglia have been observed in a large number of neurodegenerative diseases (Hayashi et al., 1988; McGeer et al., 1988); in contrast, the description of Ia Ag⁺ astroglia in vivo has been rarer. In situ, immunohistochemical studies have shown that astroglia in active chronic lesions of multiple sclerosis (MS) are positive for Ia Ag (Traugott et al., 1985; Traugott and Lebon, 1988). However, other MS samples did not have this characteristic (Fontana et al., 1987; for review, see Hayashi et al., 1988). These observations suggest that astroglia may regulate Ia Ag differently from microglia.

IFN- γ is a species-specific protein that exerts potent biological activities by binding to its specific cell surface receptor (Aguet and Mogensen, 1983; Zoon and Arnheiter, 1984). Although the precise mechanism of IFN- γ 's action is poorly understood, several reports have suggested that the second messengers generated after IFN- γ binds to its surface receptor are responsible for its effects (Hamilton et al., 1985; Celada and Schreiber, 1986; Gariglio et al., 1988). A collection of previous studies suggest that the intracellular signals utilized by IFN- γ in the induction of Ia Ag might vary from cell type to cell type (Ina et al., 1987; Koide et al., 1988). Hence, in some cell types protein kinase C (PKC) activation appears to play a role in the induction of Ia Ag expression, while in other cells PKC is not involved. So far, the intracellular signalling molecules involved in IFN- γ -induced Ia Ag expression on brain cells are unknown. This is pertinent considering (1) the importance of Ia Ag in normal and aberrant immune responses in the CNS, and (2) the wealth of CNS mediators that can alter secondary messenger levels and potentially alter the status of Ia Ag expression or nonexpression by glial cells.

Despite the presence of activated T cells and their associated lymphokines in the CNS (Naparstek et al., 1983) only rare Ia Ag-bearing cells have been observed in brains without obvious disease manifestations (Ting et al., 1981; Hauser et al., 1983). In neurodegenerative disease states, reports of Ia Ag⁺ microglia or macrophages are significantly more frequent and consistent than Ia Ag⁺ astroglia. This paucity of Ia Ag on astroglia

in situ led to the hypothesis that astroglial Ia Ag expression may be down-regulated by endogenous CNS mediators (Frohman et al., 1988a, b). Many studies have demonstrated that glia are exposed to and respond to neurotransmitter-like substances released from neurons (Koda and Bloom, 1977; Ariano et al., 1982). Recently, norepinephrine (NE) and vasoactive intestinal polypeptide (VIP) have been shown to inhibit IFN- γ -induced Ia Ag expression on cultured rat astroglia (Frohman et al., 1988a, b). It has been proposed that the cAMP-dependent pathway is involved in the down-regulation of Ia Ag expression generated by both NE and VIP (Frohman et al., 1988a, b). The role of secondary messengers on Ia Ag expression by microglia has not been investigated, most probably due to difficulties in obtaining sufficient numbers of these cells for experimentation.

To understand the intracellular signals that produce IFN- γ -induced Ia Ag expression on glial cells, we have purified cultures of astroglia and ameboid microglia from neonatal rat cerebrum and analyzed the effects of various second messengers on IFN- γ -induced Ia Ag expression. The results indicate that IFN- γ -induced Ia Ag expression on astroglia can be suppressed by a number of agents that alter intracellular secondary messengers and that microglia are not sensitive to this suppression.

Materials and methods

Primary astroglia and microglia cultures

Lewis or Sprague-Dawley rats (2 days postnatal at the latest) were used for the present study. Mixed glial cell cultures were established from new-born rat cerebrum as described previously (McCarthy and deVellis, 1978). Cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with glucose (0.9%), L-glutamine (2 mM), sodium bicarbonate, penicillin (50 IU/ml), streptomycin (50 μ g/ml) plus fetal calf serum (FCS) (10% v/v, Gibco), and incubated at 37°C in an atmosphere of 5% CO₂.

The mixed cell cultures became confluent on approximately day 14, then ameboid microglia

and type 1 astroglia were purified by a differential adhesion technique (Sasaki et al., 1989). For microglial cultures, confluent cultures were gently shaken and floating cells were removed. Isolated amoeboid microglia were seeded at a density of 5×10^5 cells/well in 12-well plates (Costar, Cambridge, MA, U.S.A.) and cultured in supple-

mented DMEM containing 10% FCS. Amoeboid microglia were identified as rat CR3-positive, glial fibrillary acidic protein (GFAP)-negative cells using indirect immunofluorescence techniques (Giulian and Baker, 1986; Robinson et al., 1986; Sasaki et al., 1989) (Fig. 1). The purity of this population was > 90%. Type 1 astroglia, which

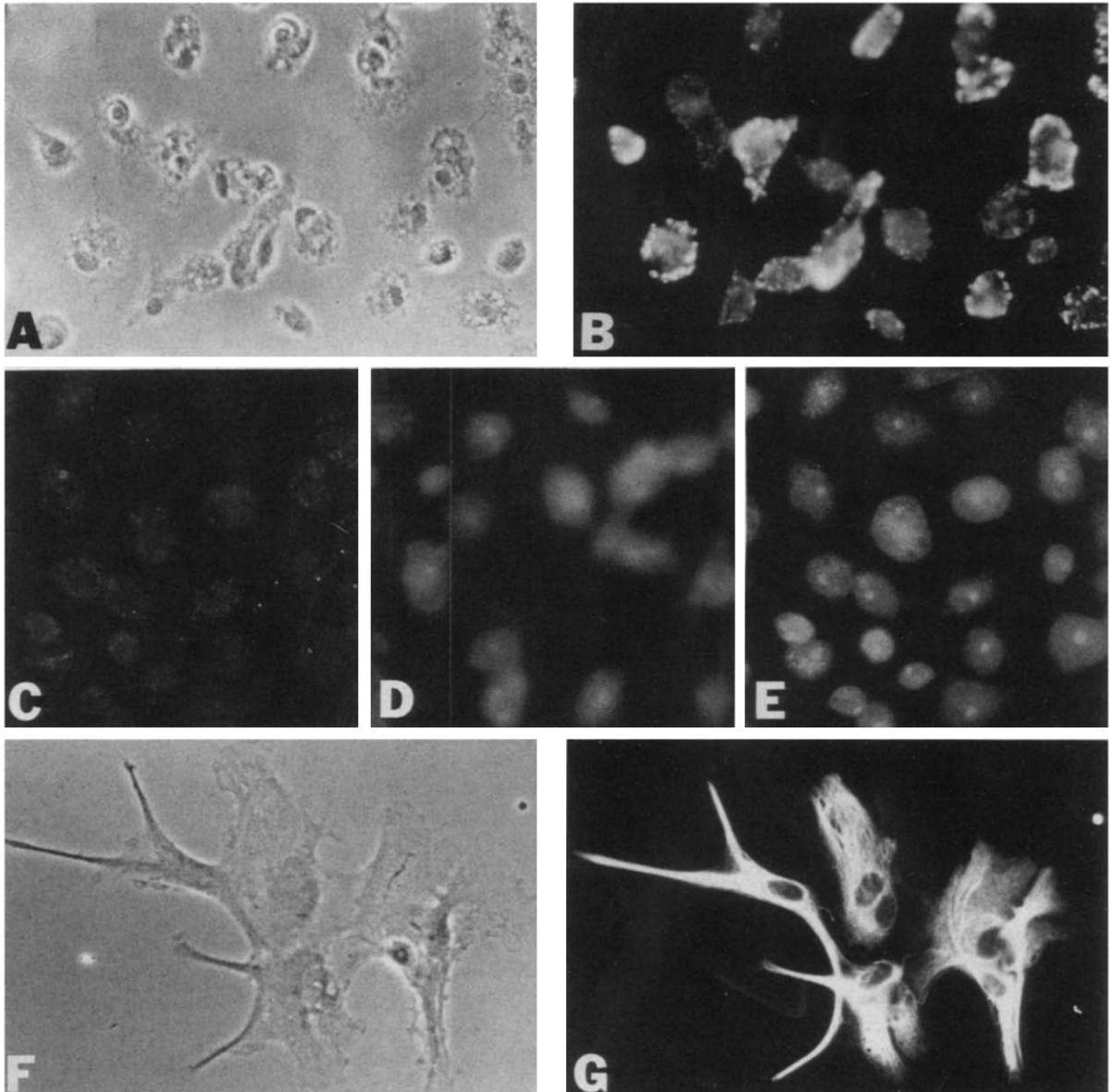


Fig. 1. Amoeboid microglia and type 1 astroglia. Amoeboid microglia-enriched cultures were double stained with monoclonal antibody against CR3 complement receptor (MRC OX-42) (B) and GFAP-specific antiserum (D). Control samples were double stained with normal mouse serum (C) and normal rabbit serum (E). The adherent cell cultures, enriched for type 1 astroglia, were stained with GFAP-specific antiserum (G). (A) and (F) are phase-contrast micrographs of (B) and (G), respectively. $\times 340$.

remained adherent to the culture dish after overnight shaking, were harvested with trypsin. The astroglia were plated at a density of 2×10^4 cells/cm² in 25-cm² flasks (Corning, Corning, NY, U.S.A.) and cultured under the conditions described above. Purity of the cells, as determined by GFAP immunohistochemistry, was > 90% (Fig. 1). Both cell types were used within 5 days of purification.

Culture additives

A stock solution of rat recombinant IFN- γ (Amgen, Thousand Oaks, CA, U.S.A.) was made in phosphate-buffered saline (PBS) at 2×10^4 U/ml. All other chemicals were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. Phorbol 12-myristate 13-acetate (PMA) and Ca²⁺ ionophore A23187 were dissolved in dimethyl sulfoxide (DMSO). 4 α -Phorbol and forskolin were prepared in 95% ethanol. 1-(5-Isoquinolinyln-sulfonyl)-2-methylpiperazine (H7) and *N*-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide (W7) were dissolved in PBS. Aliquots were stored at -20°C or -70°C to avoid repeated thawing and freezing as much as possible. *N*⁶,*O*²-dibutyryl-adenosine 3',5'-cyclic monophosphate (DBcAMP), *N*⁶,*O*²-dibutyryl-guanosine 3',5'-cyclic monophosphate (DBcGMP), and (-)-isoproterenol (IPT) were diluted in the culture media immediately before use.

Antibodies

A monoclonal mouse IgG1 antibody with reactivity against a monomorphic determinant of rat Ia Ag (MRC OX-6) and a monoclonal IgG2a antibody against rat CR3 (MRC OX-42) were obtained from Serotec, Oxford, U.K. Rabbit anti-cow GFAP was purchased from Dakopatts, Denmark. Fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG, FITC-conjugated goat anti-rabbit IgG and tetramethylrhodamine isothiocyanate (TRITC)-conjugated goat anti-mouse IgG were purchased from Cappel (West Chester, PA, U.S.A.) and used as secondary reagents.

Flow cytometry

Astrocytes were prepared as described above followed by an incubation with trypsin (0.25%),

and by two washes with media. Microglia were prepared as described and the cells harvested by gentle scraping with the rubber plunger of a 5 or 10 ml plastic syringe. For analysis, 10^5 - 10^6 cells from each treatment were incubated for 45 - 60 min with MRC OX-6 mAb diluted at 1:100. Bound antibodies were detected by a further 30 min incubation in goat anti-mouse IgG conjugated to FITC (1:20). All incubations were done at 4°C, and all dilutions and washes were in cold Hanks' balanced salt solution (HBSS) with 0.1% bovine serum albumin (BSA) and 0.1% sodium azide. Immediately after staining, labelled cell populations were analyzed on an Ortho CytoFluorograf system 50H (Ortho Diagnostics Systems, Raritan, NJ, U.S.A.) or Coulter EPICS V System (Coulter Electronics, Hialeah, FL, U.S.A.). As described before, astroglia and microglia, respectively, expressed no or little (~ 2%) Ia Ag in the absence of IFN- γ treatment (Sasaki et al., 1989). This background level of Ia Ag for untreated cultures was subtracted from all experimental values to obtain the percentage of cells that became Ia Ag⁺ after treatment.

Results

PKC reduced IFN- γ -induced Ia Ag expression by astroglia

Astroglia and microglia (discussed below) were prepared by a differential adhesion method as discussed in Materials and Methods (Sasaki et al., 1989). Astroglia were identified by positive staining with GFAP, and microglia by staining with CR3 but not GFAP. Ia Ag expression on astroglia was analyzed following a single treatment with a number of reagents (Table 1). The results are summarized in Table 1. The reagents which caused no changes in Ia expression will not be further discussed. Reagents that inhibited IFN- γ -induced Ia expression will be discussed in detail later (in Tables 2-4). As described previously by us and others, treatment with 50 U/ml of IFN- γ alone for 2 days induced Ia Ag expression on approximately 50% of the astroglia. As early as 24 h after exposure to IFN- γ , Ia Ag was detectable by flow cytometry and approximately 35% of the cells were Ia Ag⁺ (data not shown). Astroglia treated

with all other substances at various concentrations for various incubation times (3 h to 5 days) showed no Ia Ag induction. Combined treatment with PMA (10 ng/ml) plus A23187 (0.2 μ M) for 2 days did not induce Ia Ag. Astroglia treated with DBcAMP, forskolin, IPT, or H7 often changed their cell morphology from flat cells to stellate cells (data not shown). However, their viability was not affected as determined by trypan blue exclusion.

To determine if cAMP or PKC are second messengers involved in the IFN- γ response, astroglial cultures were cotreated with IFN- γ and reagents known to affect secondary messengers for 2 days (Table 1). Simultaneous treatment of astroglia with IFN- γ (50 U/ml) and PMA (1–1000 nM) produced a dose-dependent inhibition of Ia Ag expression ranging from 7% at 1 nM of PMA to 56% at 1000 nM (Fig. 2). 4 α -Phorbol (1–1000 nM), an inactive phorbol that has no effect on

TABLE 1
MODULATION OF Ia Ag EXPRESSION ON ASTROGLIA

Pharmacological agent ^a	Ia Ag induction	Inhibition of IFN- γ -induced Ia Ag expression ^c
IFN- γ	+ ^b (51%)	–
DBcAMP	–	+
Forskolin	–	+
IPT	–	+
PMA	–	+
A23187	–	–
PMA + A23187	–	N.D.
4 α -Phorbol	–	–
H7	–	–
W7	–	–
DBcGMP	–	–

^a Primary astroglial cultures were treated with IFN- γ (50 U/ml), DBcAMP (0.1–1 mM), forskolin (10–100 μ M), PMA (1–1000 nM), A23187 (0.2–1 μ M), PMA (10 ng/ml) + A23187 (0.2 μ M), 4 α -phorbol (1–1000 nM), H7 (10–30 μ M), W7 (10–30 μ M) or DBcGMP (1–1000 μ M) for various time periods (3 h to 5 days) and analyzed by flow cytometry for Ia Ag induction.

^b The number in parentheses is the mean percentage of Ia Ag-positive cells from six different experiments where astroglia were treated with 50 U/ml of IFN- γ for 2 days.

^c Astroglia were simultaneously treated with IFN- γ (50 U/ml) and various chemicals for 2 days to determine whether these chemicals could inhibit or augment IFN- γ -induced Ia Ag expression.

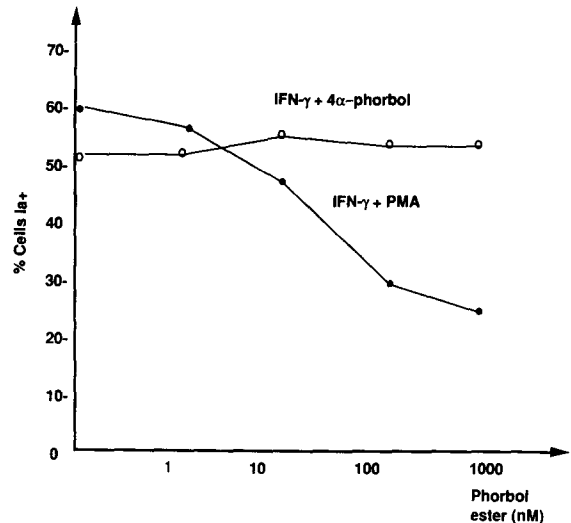


Fig. 2. Effect of PMA and 4 α -phorbol concentration on IFN- γ -induced Ia Ag expression. Primary astroglial cultures were treated with various concentrations of PMA or 4 α -phorbol in addition to 50 U/ml of IFN- γ . After 2 days, cells were stained for Ia Ag and analyzed by flow cytometry to determine the percent of Ia Ag-positive cells. The data for PMA and 4 α -phorbol were obtained from separate experiments.

PKC also had no effect on IFN- γ -induced Ia Ag expression.

The suppressive effect of PMA on IFN- γ -induced Ia Ag expression by astroglia is associated with PKC activation

PMA is known to stimulate PKC in a manner similar to diacylglycerol and elicits many of the physiologic effects of this enzyme. Several controls were used to determine if the suppressive effects of PMA could be attributed to PKC activation. First, preincubation of cells with PMA is known to initiate the degradation of PKC and its sustained disappearance from cells (Young et al., 1987; Ase et al., 1988). Prolonged pretreatment (1 day) with a high dose of PMA (1 μ M) is the standard protocol used to deplete PKC in various cells. Such pretreatment did not suppress the IFN- γ -induced expression of Ia, and actually slightly increased Ia Ag expression on astroglia (Table 2). Conversely, IFN- γ -induced Ia Ag expression on astroglia was suppressed by pretreatment with PMA for a short time (10 min to 1 h). Secondly, the effect of H7, a potent and selective inhibitor of PKC was also tested (Hidaka et al., 1984). Pre-

TABLE 2

EFFECT OF PMA ON IFN- γ -INDUCED Ia Ag EXPRESSION ON ASTROGLIA^a

First treatment	Second treatment	% Inhibition of Ia Ag expression ^b
PMA (160 nM, 10 min)	IFN- γ (50 U/ml, 2 days)	30.0
PMA (50 nM, 15 min)	IFN- γ (50 U/ml, 2 days)	22.7
PMA (160 nM, 1 h)	IFN- γ (50 U/ml, 2 days)	23.1
PMA (1 μ M, 1 day)	IFN- γ (50 U/ml, 2 days)	-11.3 ^c

^a Astroglia were treated with PMA followed by IFN- γ treatment.

^b Values presented are averages of three experiments. The percent inhibition of Ia Ag expression was determined by the following formula: $100 - [(\% \text{ cells Ia Ag}^+ / \% \text{ cells Ia Ag}^+ \text{ of IFN-}\gamma \text{ treatment alone}) \times 100]$.

^c The percent of Ia Ag⁺ cells was higher (~34%) than that of IFN- γ alone in two experiments, but lower (8%) in one experiment.

treatment with H7 (20 μ M) for 1 h prior to the addition of IFN- γ did not inhibit Ia Ag expression on astroglia (Table 3). Neither was an inhibition of IFN- γ -induced Ia Ag expression observed after longer pretreatment with H7 (10 μ M, 3 h). On the contrary, H7 reversed the suppressive effect of PMA on Ia Ag induction. Collectively these results suggest that PKC activation is not necessary for IFN- γ -induced Ia Ag expression on astroglia

TABLE 3

EFFECT OF PROTEIN KINASE INHIBITOR H7 ON IFN- γ -INDUCED Ia Ag EXPRESSION ON ASTROGLIA^a

First treatment	Second treatment	% Inhibition of Ia Ag expression ^b
H7 (20 μ M), 1 h	IFN- γ (50 U/ml), 2 days	-8.6
None	IFN- γ (50 U/ml) + PMA (100 nM), 2 days	21.2
H7 (10 μ M), 3 h	IFN- γ (50 U/ml) + PMA (100 nM), 2 days	-17.0

^a Astroglial cultures were treated with or without H7, and then treated with IFN- γ alone or IFN- γ plus PMA.

^b Values are average of two separate experiments with similar results.

TABLE 4

INHIBITION OF IFN- γ -INDUCED Ia Ag EXPRESSION ON ASTROGLIA BY TREATMENT WITH DBcAMP, FORSKOLIN, IPT, OR PMA^a

Additions	Dose (μ M)	% Inhibition of Ia Ag expression ^b
DBcAMP	100	25.3 \pm 10.7 (n = 4)
	1000	69.3 \pm 7.8 (n = 3)
Forskolin	10	33.8 \pm 11.7 (n = 4)
	100	43.7 \pm 12.1 (n = 3)
IPT	1	34.5 \pm 3.5 (n = 2)
	10	42.0 \pm 12.7 (n = 2)
	100	44.5 \pm 17.7 (n = 2)
PMA	0.1	40.0 \pm 12.5 (n = 6)

^a Astroglia were treated with IFN- γ (50 U/ml) and drugs simultaneously. After 2 days, cultures were then stained for Ia Ag and analyzed by flow cytometry.

^b Values given are means \pm SD obtained from separate experiments. n = number of experiments.

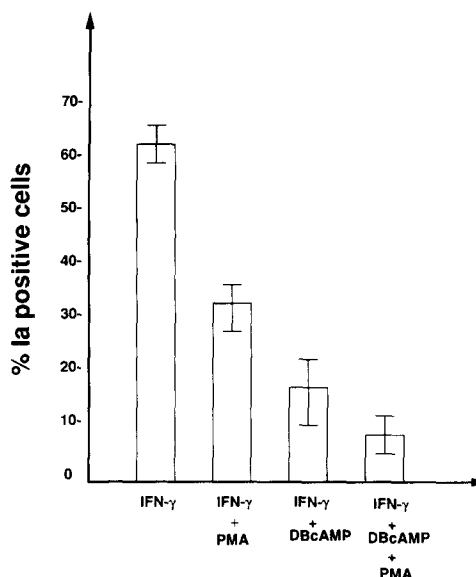


Fig. 3. Synergism of PMA and DBcAMP on Ia Ag inhibition. Astroglial cultures were treated with IFN- γ (50 U/ml) alone, IFN- γ (50 U/ml) plus PMA (100 nM), IFN- γ (50 U/ml) plus DBcAMP (1 mM) or IFN- γ (50 U/ml) plus PMA (100 nM) plus DBcAMP (1 mM) for 2 days. The cells were then stained for Ia Ag and analyzed by flow cytometry to determine the percent of cells that were Ia Ag positive. The histograms represent results from two separate experiments and are expressed as the mean \pm SD.

and that PKC activation actually reduced IFN- γ -induced Ia Ag induction.

Cyclic AMP reduced IFN- γ -induced Ia Ag expression by astroglia

The cotreatment of astroglia with IFN- γ and 100–1000 μ M DBcAMP, 10–100 μ M forskolin or 1–100 μ M IPT produced a dose-dependent inhibition of Ia Ag expression which ranged from 25 to 69%, 34 to 44% or 35 to 45%, respectively (Table 4). All these reagents are known to increase intracellular cAMP in primary astroglia. Therefore, these results strongly suggest that cAMP can suppress IFN- γ -induced Ia Ag expression on astroglia as reported previously (Frohman et al., 1988a). Simultaneous treatment with DBcAMP (1 mM) and PMA (100 nM) almost abolished the IFN- γ -induced Ia Ag expression (Fig. 3). These results imply that PKC activation and increased cAMP can inhibit IFN- γ -induced Ia Ag expression. Various concentrations of other substances such as the calcium ionophore A23187, the calmodulin antagonist W7 (Hidaka et al., 1981), and DBcGMP did not inhibit or augment IFN- γ -induced Ia Ag expression (Table 1).

IFN- γ -induced Ia Ag expression by microglia was not affected by cAMP

Our previous report showed that IFN- γ induced Ia Ag expression on ameboid microglia

more readily than astroglia (Sasaki et al., 1989). To determine if the IFN- γ -induced expression of Ia Ag on ameboid microglia and astroglia were regulated similarly, microglia were treated with analogs of second messengers or inducers of second messengers and analyzed for Ia Ag expression (Table 5). In contrast to astroglia, forskolin (10 μ M), IPT (10 μ M) or DBcAMP (100 μ M) did not suppress IFN- γ -induced Ia Ag expression on microglia. This suggests that increased cAMP has little suppressive effect on IFN- γ -induced Ia Ag expression by ameboid microglia. Treatment with PMA (100 nM) induced many microglia to alter their morphology to giant cells or to die, therefore, we could not analyze the effects of PMA on IFN- γ -induced Ia Ag expression by microglia.

Discussion

The two novel observations in this report are (1) PKC activation suppresses the IFN- γ -induced Ia Ag expression by astroglia, and (2) the elevation of intracellular cAMP selectively suppresses IFN- γ -induced Ia Ag expression by astroglia but not ameboid microglia. The findings with astroglia imply that two major signal-transducing molecules, cAMP-dependent protein kinase (PKA) and PKC, act as negative regulatory signals of the IFN- γ -induced Ia Ag expression on these cells. In fact, simultaneous activation of both PKA and PKC abrogated Ia Ag expression (90% suppression of Ia Ag expression).

Negative effects of PKC on IFN- γ -induced Ia Ag expression by astroglia have not been previously reported. A priori, PKC would appear to be a likely signalling molecule for astroglia based on the following findings: (1) the receptor-linked inositol lipid pathway can be activated in astroglia (Girard et al., 1985; Mochley-Rosen et al., 1987), and (2) astroglia are rich in immunoreactive PKC (Pearce et al., 1986; Ritchie et al., 1987). In fact, one report showed that PMA per se could induce Ia Ag expression on rat astroglia (Frohman et al., 1988a). We extensively investigated the role of PKC in Ia Ag induction using a combination of PMA, an inactive phorbol ester 4 α -phorbol, and an inhibitor of PKC, H7. Our results demonstrate that (1) PKC activation does not appear to be

TABLE 5

COMPARISON OF cAMP'S EFFECT ON IFN- γ -INDUCED Ia Ag EXPRESSION BY ASTROGLIA AND MICROGLIA^a

Treatment	% Inhibition of Ia Ag expression	
	Astroglia	Microglia
IFN- γ (50 U/ml)	0	0
+ DBcAMP (100 μ M)	17.4	-10.6 ^b
+ Forskolin (10 μ M)	28.7	-2.8 ^b
+ IPT (10 μ M)	25.1	-5.5 ^b

^a Both astroglial cultures and ameboid microglia cultures were treated with IFN- γ (50 U/ml alone), IFN- γ plus DBcAMP (100 μ M), IFN- γ plus forskolin (10 μ M) or IFN- γ plus IPT (10 μ M). Values are average of at least two experiments.

^b The percent Ia Ag⁺ cells were always a little higher than that of IFN- γ alone: ~16% for DBcAMP-treated cells, 7% for forskolin and 13% for IPT.

involved in the positive regulation of astroglial Ia Ag by IFN- γ because H7 does not affect this process, (2) PMA does not induce Ia Ag even in the presence of A23187, and (3) most interestingly, PKC activation can actually inhibit IFN- γ -induced Ia Ag expression by astroglia. This is in contrast to the observation of Massa et al. (1987b) that both PMA and Ca ionophore A23187 induced Ia Ag expression on rat astroglia. However, in their study Ia Ag induction by these inducers seems to occur at a narrow concentration and specific treatment interval (for example, only at 10 ng/ml of PMA for 5 days). We duplicated their treatment protocol and still did not observe any Ia Ag induction. The reason for this discrepancy is not clear.

The effect of cAMP on Ia Ag induction was investigated by the administration of DBcAMP (cAMP analogue), forskolin (activator of adenylate cyclase) or IPT (β -adrenergic receptor agonist). Our results show that all treatments examined which increased intracellular cAMP levels in astroglia inhibited IFN- γ -induced Ia Ag expression in a dose-dependent manner. This data is compatible with a report by Frohman et al. (1988a).

This analysis did not reveal the nature of second messengers utilized by IFN- γ in inducing Ia Ag expression on cultured astroglia. A study of the human promyelocytic monocytic line U937 suggested that PKC might be a positive regulator of IFN- γ -induced Ia Ag expression because the inhibition of PKC suppressed the IFN- γ effect. This is not the case with astroglia. These divergent findings suggest that different intracellular signals generated by IFN- γ may regulate Ia Ag in astroglia versus U937 (Ina et al., 1987; Koide et al., 1988). A recent study has shown that sodium and intracellular pH are involved in IFN- γ -induced Ia Ag expression in primary macrophages (Prpic et al., 1989). It is of interest to examine the role of sodium in IFN- γ -induced Ia Ag expression on astroglia.

This study supports the hypotheses that activation of PKC and/or PKA down-regulates IFN- γ -induced Ia Ag expression on rat astroglia, and that Ia Ag expression on astroglia is subject to negative control (Frohman et al., 1988b). Compatible with these hypotheses, a number of studies indicate that cultured astroglia express receptors

that regulate phosphatidylinositol metabolism, which activate PKC (Pearce et al., 1985; De George et al., 1986). Astroglia also express the β -adrenergic and α -adrenergic receptors for norepinephrine, which increase cAMP and diacylglycerol levels. Many endogenous brain substances are able to modulate cAMP or PI metabolism via surface receptors and these may suppress the expression of Ia Ag on astroglia. Further studies, including transcriptional and translational analyses, must be carried out to understand the mechanism of negative control of Ia Ag expression on astroglia. A preliminary Northern hybridization analysis indicates that IFN- γ induction and DBcAMP inhibition are both regulated at the transcriptional level (unpublished data).

Recent reports have suggested that microglia may play important roles as the major CNS cell type that expresses Ia Ag and participates in immune processes (Matsumoto et al., 1986; Hayes et al., 1987; McGeer et al., 1988). In accord with this, our previous report showed that IFN- γ induced greater numbers of Ia Ag-expressing microglia than astroglia (Sasaki et al., 1989). To further examine the regulation and expression of Ia Ag on microglia this study showed that in contrast to astroglia, forskolin, IPT or DBcAMP did not affect Ia Ag expression by microglia. The regulation of Ia Ag on microglia also may be different from macrophages since a previous report indicates that cAMP inhibits the induction of macrophage Ia Ag expression (Steeg et al., 1982). This discrepancy may be due to the different assays used in the two studies, or a more interesting explanation is that ameboid microglia constitute a distinct class of tissue macrophages.

The differential sensitivity of microglia and astroglia to cAMP suppression is intriguing. In vitro both microglia and astroglia can be induced to express Ia Ag by IFN- γ and can function as APC (Fontana et al., 1987). However, in vivo immunohistochemical studies of brain tissues indicate that most Ia Ag-expressing cells in disease states are most likely microglia. This is true for rat experimental allergic encephalomyelitis (Hickey et al., 1985; Matsumoto et al., 1986) and a large number of human neurological diseases (McGeer et al., 1988). As a cautionary note, however, microglia-specific markers need to be used in such studies to

distinguish microglia from blood-derived macrophages. Conversely, the observation of Ia Ag⁺ astroglia in brain tissue sections is rare. This study presents a potential basis for the differential expression of Ia Ag by microglia and astroglia. The predominance of Ia Ag⁺ microglia in neurologic disease is in accord with our results revealing their sensitivity to IFN- γ as a positive regulator of Ia Ag, and their insensitivity to increased intracellular cAMP levels as a negative regulator of Ia Ag.

The differential regulation of Ia Ag on astroglia and microglia is in accord with molecular biologic studies performed in the laboratory. Analysis of an octamer DNA element that is critical for HLA-DRA gene expression has shown that this element is only critical for HLA-DRA gene expression in B cells but not in non-B cells, including a glioblastoma multiforme. The function of this element in B cell is achieved through DNA-protein interactions most likely via complex formation with a B cell-specific DNA binding protein (Sherman et al., 1989).

Acknowledgements

We thank Ms. Jane Radford for preparation of the manuscript and Harold Moses for critical comments.

References

- Aguet, M. and Mogensen, K.E. (1983) Interferon receptors. In: I. Gresser (Ed.), *Interferon 5*, Academic Press, New York, pp. 1-22.
- Ariano, M.A., Briggs, C.A. and McAfee, D.A. (1982) Cellular localization of cyclic nucleotide changes in rat superior cervical ganglion. *Cell. Mol. Neurobiol.* 2, 143-155.
- Ase, K., Berry, N., Kikkawa, U., Kishimoto, A. and Nishizuka, Y. (1988) Differential down-regulation of protein kinase C subspecies in KM3 cells. *FEBS Lett.* 244, 775-779.
- Celada, A. and Schreiber, R.D. (1986) Role of a protein kinase C and intracellular calcium mobilization in the induction of macrophage tumoricidal activity of interferon- γ . *J. Immunol.* 137, 2373-2379.
- De George, J.J., Morell, P., McCarthy, K.D. and Lapetina, E.G. (1986) Adrenergic and cholinergic stimulation of arachidonate and phosphatide metabolism in cultured astroglia cells. *Neurochem. Res.* 11, 1061-1071.
- Fontana, A., Frei, K., Bodmer, S. and Hofer, E. (1987) Immune-mediated encephalitis: on the role of antigen-presenting cells in brain tissue. *Immunol. Rev.* 100, 185-201.
- Frohman, E.M., Frohman, T.C., Vayuvegula, B., Gupta, S. and van den Noort, S. (1988a) Vasoactive intestinal polypeptide inhibits the expression of the MHC class II antigens on astrocytes. *J. Neurol. Sci.* 88, 339-346.
- Frohman, E.M., Vayuvegula, B., Gupta, S. and van den Noort, S. (1988b) Norepinephrine inhibits γ -interferon-induced major histocompatibility class II (Ia) antigen expression on cultured astrocytes via β_2 -adrenergic signal transduction mechanism. *Proc. Natl. Acad. Sci. U.S.A.* 85, 1292-1296.
- Gariglio, M., Franco, A., Cavallo, G. and Landolfo, S. (1988) Evidence for a GTP-binding protein involved in interferon- γ transduction signal. *J. Interferon Res.* 8, 463-472.
- Girard, P.R., Mazzei, G.J., Wood, J.G. and Kuo, J.F. (1985) Polyclonal antibodies to phospholipid/Ca²⁺-dependent protein kinase and immunocytochemical localization of the enzyme in rat brain. *Proc. Natl. Acad. Sci. U.S.A.* 82, 3030-3034.
- Giulian, D. and Baker, T.J. (1986) Characterization of ameboid microglia isolated from developing mammalian brain. *J. Neurosci.* 6, 2163-2178.
- Hamilton, T.A., Becton, D.L., Somers, S.D., Gray, P.W. and Adams, D.O. (1985) Interferon- γ modulates protein kinase C activity in murine peritoneal macrophages. *J. Biol. Chem.* 260, 1378-1382.
- Hauser, S.L., Bhan, A.K., Gilles, F.H., Hoban, C.J., Reinherz, E.L., Schlossman, S.F. and Weiner, H.L. (1983) Immunohistochemical staining of human brain with monoclonal antibodies that identify lymphocytes, monocytes and the Ia antigen. *J. Neuroimmunol.* 5, 197-205.
- Hayashi, T., Morimoto, C., Burks, J.S., Kerr, C. and Hauser, S.L. (1988) Dual-label immunocytochemistry of the active multiple sclerosis lesion: major histocompatibility complex and activation antigens. *Ann. Neurol.* 24, 523-531.
- Hayes, G.M., Woodroffe, M.N. and Cuzner, M.L. (1987) Microglia are the major cell type expressing MHC class II in human white matter. *J. Neurol. Sci.* 80, 25-37.
- Hickey, W.F., Osborn, J.P. and Kirby, W.N. (1985) Expression of Ia molecules by astrocytes during acute experimental allergic encephalomyelitis in the Lewis rat. *Cell. Immunol.* 91, 528-535.
- Hidaka, H., Inagaki, M., Kawamoto, S. and Sasaki, Y. (1984) Isoquinolinesulfonamides, novel and potent inhibitors of cyclic nucleotide dependent protein kinase and protein kinase C. *Biochemistry* 23, 5036-5041.
- Hidaka, H., Sasaki, Y., Tanaka, T., Endo, T., Ohno, S., Fujii, Y. and Nagata, T. (1981) *N*-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide, a calmodulin antagonist, inhibits cell proliferation. *Proc. Natl. Acad. Sci. U.S.A.* 78, 4354-4357.
- Ina, Y., Koide, Y., Nezu, N. and Yoshida, T.O. (1987) Regulation of HLA class II antigen expression: intracellular signalling molecules responsible for the regulation by IFN- γ and cross-linking of Fc receptors in HL-60 cells. *J. Immunol.* 139, 1711-1717.
- Kaufman, J.F., Anfray, C., Korman, A.J., Shackelford, D.A. and Strominger, J. (1984) The class II molecules of the

- human and murine major histocompatibility complex. *Cell* 36, 1–13.
- Koda, L.Y. and Bloom, F.E. (1977) A light and electron microscope study of noradrenergic terminals in the rat dentate gyrus. *Brain Res.* 120, 327–335.
- Koide, Y., Ina, Y., Nezu, N. and Yoshida, T.O. (1988) Calcium influx and the Ca^{2+} -calmodulin complex are involved in interferon- γ -induced expression of HLA class II molecules on HL-60 cells. *Proc. Natl. Acad. Sci. U.S.A.* 85, 3120–3124.
- 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. and ter Meulen, V. (1987) Analysis of Ia induction on Lewis rat astrocytes in vitro by virus particles and bacterial adjuvants. *J. Neuroimmunol.* 13, 259–271.
- Massa, P.T., ter Meulen, V. and Fontana, A. (1987) Hyperinducibility of Ia antigen on astrocytes correlates with strain-specific susceptibility to experimental autoimmune encephalomyelitis. *Proc. Natl. Acad. Sci. U.S.A.* 84, 4219–4223.
- Matsumoto, Y., Hara, N., Tanaka, R. and Fujiwara, M. (1986) Immunohistochemical analysis of the rat central nervous system during experimental allergic encephalomyelitis, with special reference to Ia-positive cells with dendritic morphology. *J. Immunol.* 136, 3668–3676.
- McCarron, R.M., Spatz, M., Kempski, O., Hogan, R.N., Meuhl, L. and McFarlin, D.E. (1986) Interaction between myelin basic protein-sensitized T lymphocytes and murine cerebral vascular endothelial cells. *J. Immunol.* 137, 3428–3435.
- McCarthy, K.D. and de Vellis, J. (1978) Alpha-adrenergic receptor modulation of β -adrenergic, adenosine and prostaglandin E_1 increased adenosine 3',5'-cyclic monophosphate levels in primary culture of glia. *J. Cyclic Nucleotide Res.* 4, 15–26.
- McGeer, P.L., Itagaki, S. and McGeer, E.G. (1988) Expression of the histocompatibility glycoprotein HLA-DR in neurological disease. *Acta Neuropathol.* 76, 550–557.
- Mochley-Rosen, D., Basbaum, A.I. and Koshland, Jr., D.E. (1987) Distinct cellular and regional localization of immunoreactive protein kinase C in rat brain. *Proc. Natl. Acad. Sci. U.S.A.* 84, 4660–4664.
- Naparstek, Y., Ben-Nun, A., Holoshitz, J., Reshef, T., Frenkel, A., Resenberg, M. and Cohen, I.R. (1983) T lymphocytes lines producing or vaccinating against autoimmune encephalomyelitis (EAE). Functional activation induces peanut agglutinin receptors and accumulation in brain and thymus of line cells. *Eur. J. Immunol.* 13, 418–423.
- Olsson, T., Maehlen, J., Love, A., Kjareskog, L., Norrby, E. and Kristensson, K. (1987) Induction of class I and class II transplantation antigens in rat brain during fatal and non-fatal measles virus infection. *J. Neuroimmunol.* 16, 215–224.
- Pearce, B., Cambray-Deakin, M., Morrow, C., Grimble, J. and Murphy, S. (1985) Activation of muscarinic and of α_1 -adrenergic receptors on astrocytes results in the accumulation of inositol phosphates. *J. Neurochem.* 45, 1534–1540.
- Pearce, B., Morrow, C. and Murphy, S. (1986) Receptor-mediated phospholipid hydrolysis in astrocytes. *Eur. J. Pharmacol.* 121, 231–243.
- Prpic, V., Yu, S.-F., Figueiredo, F., Hollenbach, P.W., Gawdi, G., Herman, B., Uhing, R.J. and Adams, D.O. (1989) Role of Na^+/H^+ exchange by interferon- γ in enhanced expression of JE and I-A β genes. *Science* 244, 469–471.
- Ritchie, T., Cole, R., Hun-Sou, K., de Vellis, J. and Noble, E.P. (1987) Inositol phospholipid hydrolysis in cultured astrocytes and oligodendrocytes. *Life Sci.* 41, 32–39.
- Robinson, A.P., White, T.M. and Mason, D.W. (1986) Macrophage heterogeneity in the rat as delineated by two monoclonal antibodies MRC OX-41 and MRC OX-42, the latter recognizing complement receptor type 3. *Immunology* 57, 239–247.
- Ting, J.P.-Y., Shigekawa, B.L., Linthicum, S., Weiner, L.P. and Frelinger, J.A. (1981) Expression and synthesis of murine immune response-associated (Ia) antigens by brain cells. *Proc. Natl. Acad. Sci. U.S.A.* 78, 3170–3174.
- Sasaki, A., Levison, S.W. and Ting, J.P.-Y. (1989) Comparison and quantitation of Ia antigen expression on cultured macroglia and ameboid microglia from Lewis rat cerebral cortex: analyses and implications. *J. Neuroimmunol.* 25, 63–74.
- Sherman, P.A., Basta, P.V., Heguy, A., Wloch, M.K., Roeder, R.G. and Ting, J.P.-Y. (1989) The octamer motif is a B-lymphocyte-specific regulatory element of the HLA-DR α promoter. *Proc. Natl. Acad. Sci. U.S.A.* 86, 6739–6743.
- Sobel, R.A., Blanchette, B.W., Bhan, A.K. and Colvin, R.B. (1984) The immunopathology of experimental allergic encephalomyelitis. *J. Immunol.* 132, 2402–2407.
- Steeg, P.S., Johnson, H.M. and Oppenheim, J.J. (1982) Regulation of murine macrophage Ia antigen expression by an immune interferon-like lymphokine: inhibitory effect of endotoxin. *J. Immunol.* 129, 2402–2406.
- Suzumura, A., Meztitis, S.G.E., Gonatas, N.K. and Silberberg, D.H. (1987) MHC antigen expression on bulk isolated macrophage-microglia from newborn mouse brain: induction of Ia antigen expression by γ -interferon. *J. Neuroimmunol.* 15, 263–278.
- Traugott, U. and Lebon, P. (1988) Interferon- γ and Ia antigen are present on astrocytes in active chronic multiple sclerosis lesions. *J. Neurol. Sci.* 84, 257–264.
- Traugott, U., Scheinberg, L.C. and Raine, C.S. (1985) On the presence of Ia-positive endothelial cells and astrocytes in multiple sclerosis lesions and its relevance to antigen presentation. *J. Neuroimmunol.* 8, 1–14.
- Watanabe, R., Wege, H. and ter Meulen, V. (1983) Adoptive transfer of EAE-like lesions from rats with corona virus-induced demyelinating encephalomyelitis. *Nature* 305, 150–153.
- Wong, G.H.W., Bartlett, P.F., Clarke-Lewis, I., McKimm-Breshkin, J.L. and Schrader, J.W. (1985) Interferon-gamma induces the expression of H-2 and Ia Ag antigens on brain cells. *J. Neuroimmunol.* 7, 255–278.
- Young, S., Parker, P.J., Ullrich, A. and Stabel, S. (1987) Down-regulation of protein kinase C is due to an increased rate of degradation. *Biochem. J.* 244, 775–779.
- Zoon, K.C. and Arnheiter, H. (1984) Studies of the interferon receptors. *Pharmacol. Ther.* 24, 259–278.