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Expression of major histocompatibility complex (MHC) class I genes in astrocytes correlates with the presence of nuclear factors that bind to constitutive and inducible enhancers

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Summary

The molecular basis of constitutive and inducible major histocompatibility complex (MHC) class I gene expression was studied in murine astrocytes in primary culture. Astrocytes constitutively expressed MHC class I molecules and treatment of these cells with interferon-γ (IFN-γ) further induced expression. The conserved region containing the upstream MHC class I regulatory element (MHC-CRE) and juxtaposed interferon consensus sequence (ICS) enhanced constitutive MHC class I promoter activity. As seen with cell surface expression of MHC molecules, treatment of astrocytes with IFN-γ increased MHC class I promoter activity. Inducible expression required the presence of the MHC-CRE/ICS enhancer region. Nuclear factors that bind to the MHC-CRE and ICS were constitutively expressed in cultured astrocytes and IFN-γ treatment further induced binding activity both to the MHC-CRE and ICS and correlated with induction of MHC class I gene expression. This study identifies the MHC-CRE and ICS as the major *cis* elements in controlling MHC class I promoter activity and suggests that the expression of nuclear factor binding activities to these enhancer elements is a basic transactivating mechanism for the expression of MHC class I genes in astrocytes.

Introduction

The expression of MHC class I molecules on the cell surface is essential for presentation of antigens including viral peptides to $CD8^+$ cytotoxic T lymphocytes (CTL) (Zinkernagel and Doherty, 1979). In the mouse, constitutive major histocompatibility complex (MHC) class I expression is observed in most somatic cells, except in the central nervous system (CNS) where MHC class I molecules are constitutively expressed at extremely low levels during development and in adulthood (Schachner and Hammerling, 1974; Williams et al., 1980; Schnitzer and Schachner, 1981). Because MHC class I mRNA levels are also very low in the CNS compared to other tissues (Chamberlain et al., 1991), regulation at the level of transcription has been postulated (Massa et al., 1989; Chamberlain et al., 1991; Hakem et al., 1991). The significance of the lack of MHC class I expression in the brain is not clear. However, recent studies indicate that low expression in the CNS may relate to the ability of neurotropic viruses to escape CTL recognition and to retarded clearance of virally infected CNS cells (Oldstone et al., 1986; Oldstone, 1989; Joly et al., 1991; Levine et al., 1991).

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The regulation of MHC class I transcription has been extensively investigated in non-neural tissue culture cell lines. These studies identified a conserved *cis* element in the upstream region of classical MHC class I genes, designated the MHC class I regulatory element (MHC-CRE), that acts as a moderate constitutive enhancer (see Fig. 1) (Kimura et al., 1986; Miyazaki et al., 1986; Baldwin and Sharp, 1987; Ehrlich et al., 1988; Silverman et al., 1988; Burke and Ozato, 1989; Burke et al., 1989). The MHC-CRE may also act as an inducible enhancer in mediating the up-regulation of MHC class I molecules by TNF-α (Israel et al., 1989a; Kieran et al., 1990). The MHC-CRE contains two functional subregions (see Fig. 1; regions I and II), that bind to distinct nuclear *trans*-acting factors (Israel et al., 1987; Baldwin and Sharp, 1988; Burke et al., 1989). cDNA clones encoding MHC-CRE binding proteins have recently been described (Shirayoshi et al., 1987; Singh et al., 1988; Hamada et al., 1989; Fan and Maniatis, 1990; Ghosh et al., 1990; Nakamura et al., 1990).

The induction of MHC class I genes by interferons (IFN) α, β and γ is controlled by another *cis* element called the ICS, which is juxtaposed to the MHC-CRE (see Fig. 1) (Friedman and Stark, 1985; Israel et al., 1986; Sugita et al., 1987; Korber et al., 1988; Shirayoshi et al., 1988). Nuclear factors that bind to the ICS are induced following IFN treatment and may be responsible for IFN-mediated induction of MHC class I gene expression (Shirayoshi et al., 1988; Blanar et al., 1989).

Astrocytes are parenchymal cells of the CNS that produce cytokines and express MHC molecules during immune responses (Schnitzer and Schachner, 1981; Wong et al., 1984; Massa et al., 1987b, 1989; Lavi et al., 1988; Mauerhoff et al., 1988; Malipiero et al., 1990; Yup Chung et al., 1991). The ability of astrocytes to perform MHC-restricted antigen presentation is of particular interest because persistent virus infections and neoplastic transformation commonly involve these cells. Numerous studies have shown that astrocytes constitutively express MHC class I molecules in vitro (Schnitzer and Schachner, 1981; Wong et al., 1985; Fontana et al., 1986; Mauerhoff et al., 1988; Lavi et al., 1988; Suzumura et al., 1988; Massa et al., 1987b, 1989) and expression can be further induced by interferons or TNF-α (Wong et al., 1985; Fontana et al., 1986; Massa et al., 1987b, 1989; Mauerhoff et al., 1988; Lavi et al., 1988). Because of the potential importance of regulation of MHC class I molecules in the CNS, an analysis of the *cis* DNA enhancer elements and the corresponding *trans*-acting factors that bind to these elements in astrocytes in primary cultures was undertaken. The results demonstrate constitutive and inducible enhancer elements that function in these cells and the existence of specific nuclear factors that bind to these elements.

Materials and Methods

Tissue cultures

BlO.A newborn mice were obtained from Harlan Sprague-Dawley (Indianapolis, IN). Astrocytes were prepared from 1–2-day-old mouse neonatal cerebral hemispheres as previously described (Massa et al., 1989). Cerebral hemispheres, dissected from the rest of the brain, were thoroughly freed of meninges, minced with fine scissors, then triturated in Dulbecco's modified Eagles medium (DMEM) with a Pasteur pipet to dissociate the cells. The cells were centrifuged, resuspended in DMEM containing 4.5 g/l glucose, 10 mM HEPES and 15% fetal bovine serum (FBS) (5 ml/hemisphere), and plated onto Nunc 100 mm diameter dishes (10 ml/dish) coated with bovine type I collagen (Collaborative Research, Bedford, MA). The cells were cultivated at 37°C with maximum humidity and 10% CO_2. Immunofluorescent staining of 1-week cultures with antibody to glial fibrillary acidic protein (GFAP) showed that the cultures consisted of over 95% $GFAP^+$ astrocytes.

Immunofluorescence staining for FACS analyses

Cultures grown for 0, 2, 4, 6, and 8 days in vitro were trypsinized into single-cell suspensions and stained for MHC class I molecules. In some experiments, 6-day cultures were treated for 0.5, 4, 24, and 48 h with 100 U/ml recombinant murine IFN-γ (Genentech Inc., South San Francisco, CA) and similarly processed for FACS analysis. For staining, astrocytes were washed by centrifugation and resuspended in HBSS with 1% FBS (HBSS/FBS) containing a rat monoclonal antibody to mouse MHC class I molecules designated K44 (Ozato et al., 1985). The cells were incubated for 30 min on ice, washed with HBSS/FBS by centrifugation, resuspended in 1:50 goat anti-rat IgG conjugated to fluorescein isothiocyanate (FITC) which had been absorbed with normal mouse serum (Zymed, South San Francisco, CA). After 30 min, the cells were washed again and analysed by flow cytofluorimetry using a FACS (Becton Dickinson Immunocytometry Systems, Mountain View, CA).

Northern blot hybridization

Total RNA was extracted from astrocyte cultures using a guanidine isothiocyanate technique (Chomczynski and Sacci, 1987). 15 μg of RNA from each specimen was electrophoresed in a 0.9% agarose gel and then transferred to a nylon filter. The RNA was hybridized with a ^{32}P-labelled cDNA probe, pH-2^d-3, encoding the conserved third external transmembrane and cytoplasmic region of H-$2L^d$ (Lalanne et al., 1982). An autoradiogram of the filter was analysed by densitometry.

Transfection experiments

Chloramphenicol-acetyl-transferase (CAT) constructs. pL^d-CAT constructs listed in Fig. 1 were previously described and characterized (Miyazaki et al., 1986; Sugita et al., 1987). The upstream regions of the H-$2L^d$ gene in these constructs are connected to the CAT reporter gene (Gorman et al., 1982).

Transfection of astrocytes. Primary cultures of astrocytes were transfected 5 days after plating. The cultures were washed twice with Dulbecco's phosphate-buffered saline (D-PBS) and then transfected using the DEAE-dextran method (McCutchan and Pagano, 1968). Briefly, cultures at 50% confluency were co-transfected with two plasmids consisting of one of various pL^d constructs listed in Fig. 1 at 5 μg/dish and pCH-110, a SV-40-β-galactosidase gene construct (Pharmacia Fine Chemicals, Piscataway, NJ) at 5 μg/dish. 15 min after adding the transfection medium, the cultures were washed and fresh medium containing antibiotics was added. 1 day after transfection the cultures were fed with fresh medium with or without 100 U/ml recombinant murine IFN-γ. 2 days later (8 days after plating), the cells were processed for CAT assay.

CAT assay. 3 days after transfection, cytoplasmic extracts were prepared and assessed for CAT activity (Gorman et al., 1982). CAT activity was determined by the degree of acetylation of [^{14}C]chloramphenicol after 4 h reaction and quantified after separation of acetylated from unacetylated by TLC development. Radioactivity of acetylated [^{14}C]chloramphenicol was quantified using an Ambis Radioanalytic Imaging System (Ambis Systems, San Diego, CA) and reported as net counts above background or radioactivity was cut from the TLC sheets and analysed in a scintillation counter. β-Galactosidase activities were assessed to determine the relative transfection efficiencies of each culture. CAT activity of each sample was normalized to β-galactosidase activity to control for differences in transfection efficiencies between samples.

Gel mobility shift experiments

Nuclear extracts from tissue culture cells. Nuclear extracts from astrocytes were prepared using a mini-prep technique, as described previously (Lee et al., 1988). 6-day astrocyte cultures were fed fresh medium with or without 100 U/ml of recombinant murine IFN-γ for up to 2 days as specified in the text. Astrocytes (10^6-10^7 cells) were washed with PBS and then harvested using a Teflon policeman. The cells were pelleted, resuspended in lysis buffer and triturated using a syringe fitted with a 25-gauge hypodermic needle to break open the cells. The nuclei and cellular debris were centrifuged and the pellet was resuspended in nuclear extraction buffer. The nuclei were mixed with a stir bar on ice for 30 min and then pelleted. PMSF and DTT were added to all buffers. The supernatant was extensively dialysed against buffer D (Lee et al., 1988), aliquotted and stored under liquid nitrogen until used. Protein determinations were performed using the Biorad protein assay kit (Biorad Laboratories, Richmond, CA).

Oligonucleotides. All oligonucleotides were synthesized as described (Burke et al., 1989). The following duplex oligonucleotides were used as probes or competitors: the MHC-CRE spanning nucleotide positions -203 to -161; region I (from -173 to -161); region II (from -203 to -185); and the ICS (from -167 to -139) of the H-$2L^d$ gene (see Fig. 1). The NF-κB (5′-CTCAACAGAGGGGACTTTCCGAGAGGC-CAT-3′) (Picard and Schaffner, 1984) and AP-1 oligonucleotides used were previously described (Shirayoshi et al., 1987; Burke et al., 1989).

Gel mobility shift assay. Binding of nuclear proteins to the MHC-CRE or ICS was studied by the gel mobility shift assay (Fried and Crothers, 1981; Garner and Rezvin, 1981; Shirayoshi et al., 1987). DNA probes were prepared by end-labelling MHC-CRE, NF-κB, or ICS oligonucleotides with γ-[^{32}P]ATP using T4 polynucleotide kinase. These probes (0.1–0.5 ng DNA having 5000–10000 cpm) were incubated with 10–20 μg of nuclear extract in the presence of 1–4 μg poly (dI-dC) (Pharmacia, Piscataway, NJ) for 40 min on ice in the presence or absence of various unlabelled competitor MHC-CRE or ICS oligonucleotides at 50-fold molar excess to the probe. The probe was added last, 10 min after mixing the other components. The reaction buffer contained 20 mM Tris buffer (pH 7.6), 50 mM KCl, 1 mM $MgCl_2$, 0.2 mM EDTA, 5% glycerol, 0.5 mM DTT, and 0.1 mM PMSF. The reaction mixtures were electrophoresed through a 4% polyacrylamide gel and the gels were processed for autoradiography on X-ray film.

Results

Transfection studies

Constitutive expression. Primary cultures of astrocytes were transfected with constructs in which the reporter gene CAT was under the control of the MHC class I gene promoter (Fig. 1). The cells were first transfected with either a plasmid containing the basic promoter of the H-$2L^d$ gene lacking upstream MHC-CRE or ICS enhancers (pL^d-CAT-123) or the pL^d-CAT-237 con-

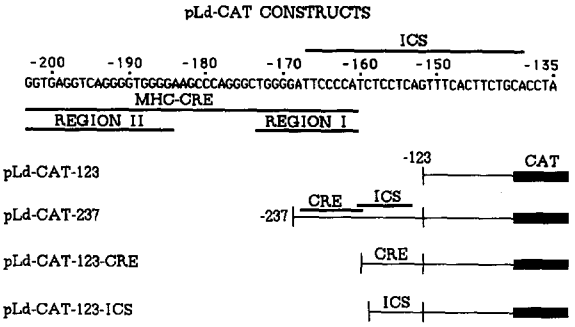

Fig. 1. The upstream nucleotide sequence of the H-2L^d class I gene of the mouse at the MHC-CRE (CRE) and ICS is shown with subregions I and II of the MHC-CRE. Nucleotides are numbered relative to the transcriptional start site (cap site). Below are listed the pL^d-CAT constructs used for transfection of astrocytes. Construct designations are given to the left.

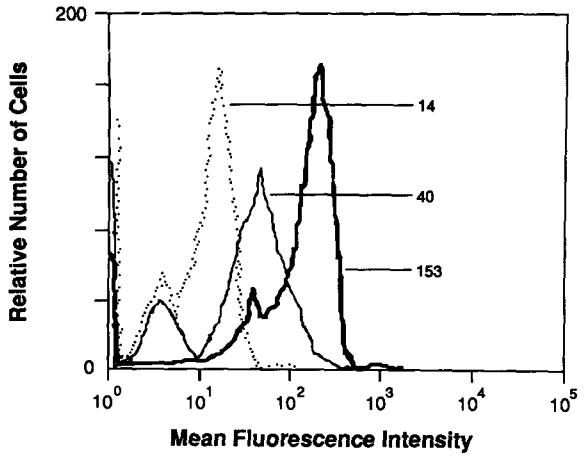

Fig. 2. FACS analysis of cell surface expression of MHC class I molecules on astrocytes cultivated for 8 days. The solid-lined profiles represent astrocytes incubated in a rat monoclonal antibody (K44) that reacts with MHC class I molecules followed by a secondary FITC-labelled goat anti-rat IgG reagent. The thin solid line represents astrocytes not treated with IFN-γ and the thick solid line represents astrocytes treated for 2 days with 100 U/ml IFN-γ. The dotted curve represents cells incubated with an irrelevant primary monoclonal antibody followed by the secondary FITC-labelled goat anti-rat IgG (control). The mean fluorescence intensity of each sample of 10000 cells is given on the histograms.

struct containing an additional 114 base pair upstream region including the MHC-CRE and ICS (Fig. 1). The pL^d-CAT-237 construct showed significantly higher constitutive CAT activity than pL^d-CAT-123 (approx. 8-fold) (Table 1). Expression of pL^d-CAT-237 was consistent with the constitutive expression of MHC class I molecules and mRNA in astrocytes in parallel cultures (Fig. 2 and Table 2). The individual contributions of the ICS and MHC-CRE to this activity were analysed using two constructs consisting of either an MHC-CRE or ICS oligonucleotide fused to the basic pL^d-CAT-123 construct (Fig. 1) (pL^d-CAT-123-CRE and pL^d-CAT-123-ICS) (Miyazaki et al., 1986; Sugita et al., 1987). Both constructs gave significantly higher CAT activity than pL^d-CAT-123 in astrocytes (Table 1) and the sum of the CAT activities of these two constructs approximated the CAT activity of pL^d-CAT-237. These results suggest that the MHC-CRE and ICS contribute to constitutive enhancement of MHC class I gene promoter activity in astrocytes.

TABLE 1

Constitutive and inducible enhancer function of the MHC-CRE and ICS in astrocytes

Construct	Mean CAT Activity (S.D.)		Mean fold induction (SD)	P
	−IFN-γ	+IFN-γ		
pL^d-CAT-123	1.0 (0.2)	1.1 (0.3)	1.1 (0.2)	0.5
pL^d-CAT-237	8.0 (0.4)	30.6 (5.7)	3.9 (0.8)	0.005 [a]
pL^d-CAT-123-CRE	3.1 (1.2)	3.5 (1.1)	1.2 (0.3)	0.5
pL^d-CAT-123-ICS	6.2 (0.4)	7.4 (1.4)	1.2 (0.2)	0.5

Analysis of *cis* regulation of constitutive and IFN-γ-mediated inducible promoter activity of MHC class I genes in astrocytes. Astrocytes were transfected with various CAT constructs described in Fig. 1. Mean CAT activities with standard deviation (SD) were determined from three individual experiments. Mean CAT activities are reported as relative to pL^d-CAT-123. Fold induction is equal to CAT activity with IFN-γ divided by CAT activity without IFN-γ. Cultures were treated with 100 U/ml IFN-γ for 2 days.

[a] Fold induction is statistically greater than pL^d-CAT-123 (Student's t-test).

TABLE 2

Kinetics of constitutive and inducible expression of MHC class I genes and in astrocytes

A. Constitutive expression

	Days in vitro				
	0	2	4	6	8
mRNA	1.0	38.9	65.1	80.0	79.9
protein	1.3	5.9	9.8	13.4	16.8

B. IFN-inducible expression

	Hours post-treatment				
	0	0.5	4	24	48
mRNA	72.9	84.5	132.6	156.1	154.0
protein	13.2	14.1	14.0	27.5	59.5
NF-κB	1.0	3.3	3.4	3.4	2.8

Expression of MHC class I genes in astrocytes was analysed with respect to (1) mRNA levels on Northern blots using a ^{32}P-labelled MHC class I probe, quantified as autoradiographic densities relative to mRNA levels at 0 days in culture and (2) cell surface expression of MHC class I molecules (protein), quantified by FACS as mean fluorescence intensity relative to background as in Fig. 2. IFN-inducible expression represents 6-day cultures treated with 100 U/ml IFN-γ for increasing amounts of time as indicated. Increase of NF-κB binding activity at various timepoints following IFN-γ treatment was quantified from autoradiographic densities of gel shift assays relative to 0 h treatment. A ^{32}P-labelled NF-κB oligonucleotide probe and nuclear extracts from astrocytes were used as outlined in Fig. 3b.

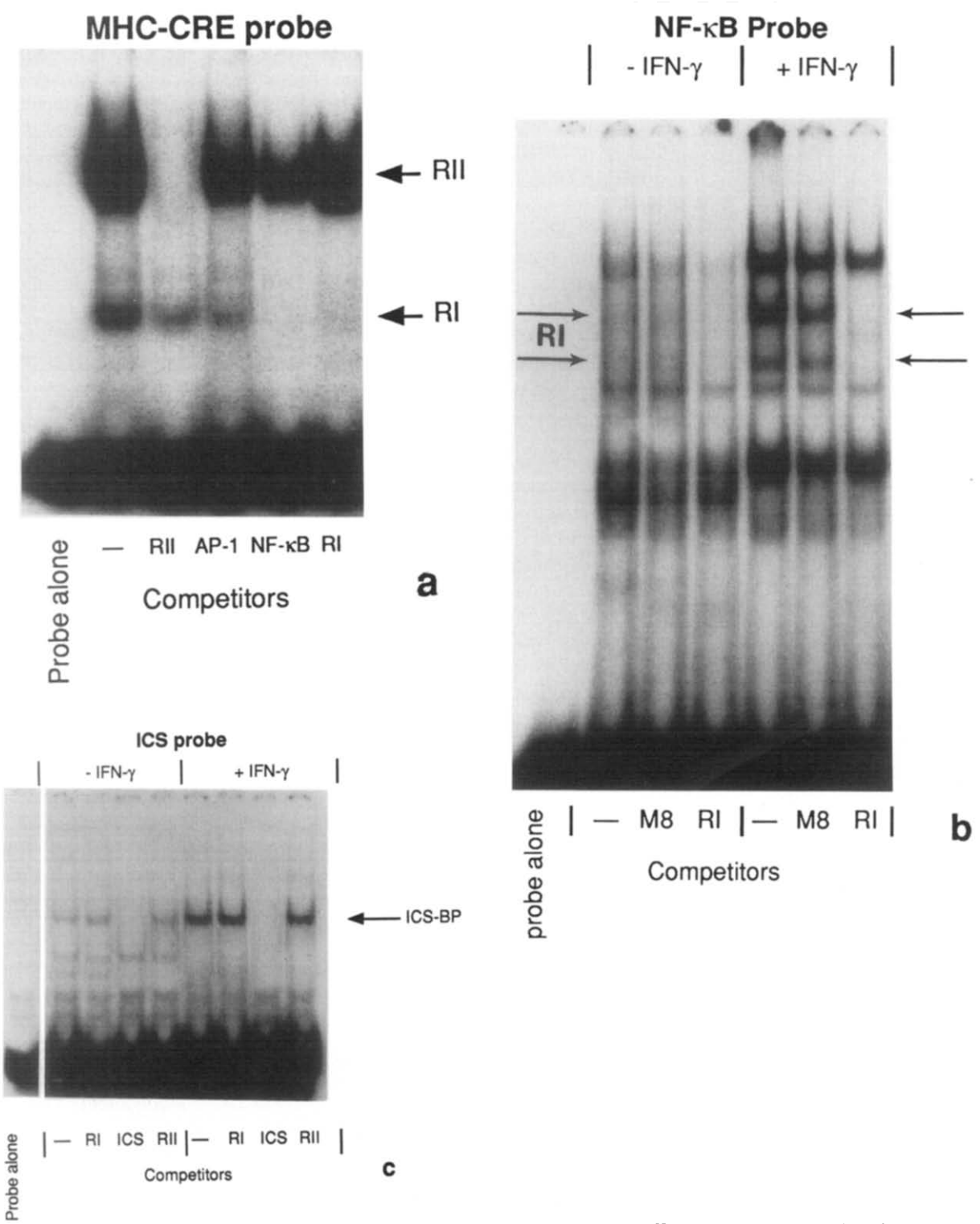

Fig. 3. **a**. Gel mobility shift assays of nuclear proteins extracted from cultured astrocytes using ^{32}P-labelled MHC-CRE (CRE) probe containing both region I and region II binding sites. Region I (RI), region II (RII), AP-1, and NF-κB oligonucleotide competitors (50 × molar excess to the probe) are indicated at the bottom of the gel. 20 μg of extract and 4 μg of poly (dI : dC) was added to each reaction and loaded in each lane. **b**. Gel mobility shift assay using a ^{32}P-labelled NF-κB probe and nuclear extracts from astrocytes treated or untreated with IFN-γ. Unlabelled region I oligonucleotide (50 × molar excess) was used as competitor as indicated at the bottom of the lanes. A region I mutant oligonucleotide (M8) (G at −172 and −171 converted to C; see Fig. 1) which does not compete with region I/NF-κB (Shirayoshi et al., 1987) was also included as a competitor. 10 μg of extract and 1 μg of poly (dI : dC) was added to each reaction and loaded in each lane. **c**. ICS binding activities analysed by gel mobility shift assay. Nuclear extracts were prepared from astrocytes incubated in medium with or without IFN-γ. Unlabelled region I (RI), region II (RII), and ICS competitors (50 × molar excess to the probe) were used as indicated at the bottom of the lanes. 20 μg of extract and 4 μg of poly (dI : dC) was added to each reaction. In **a** and **b**, arrows indicate specific retarded bands in the gel.

IFN-γ-mediated inducible expression. The effect of IFN-γ treatment on MHC class I promoter activity in astrocytes was also studied. Astrocytes transfected with pL^d-CAT-237 showed on average an approx. four-fold induction in CAT activity after IFN-γ treatment (Table 1), which was consistent with the induction of MHC class I expression at the cell surface and mRNA (Fig. 2 and Table 2). No significant induction of expression was seen with pL^d-CAT-123. As well, no substantial increases in CAT activity were observed with both pL^d-CAT-123-CRE and pL^d-CAT-123-ICS constructs in response to IFN-γ, suggesting that both the MHC-CRE and ICS were required for IFN-γ induction of the MHC class I genes as previously shown in cell lines (Israel et al., 1986; Sugita et al., 1987).

Gel mobility shift studies

The transfection studies provided evidence that the MHC-CRE and ICS are involved in controlling constitutive and inducible MHC class I gene promoter activity in astrocytes. The binding of nuclear factors was investigated using an MHC-CRE oligonucleotide probe which includes both region I and region II. Both region I and II binding activities were constitutively expressed in astrocytes (Fig. 3a). Region I binding activity was competible by excess unlabelled region I as well as a related NF-κB oligonucleotide (Fig. 3a), suggesting that region I binding activity in astrocytes is NF-κB or a related factor. The region II binding activity in astrocytes was competed by excess region II but not by an AP-1 oligonucleotide (Fig. 3a), as seen previously in other cells (Burke et al., 1989).

IFN-γ increased levels of region I/NF-κB binding activities in astrocytes (Fig. 3b) with kinetics parallel with that of IFN induction of MHC class I molecules and mRNA (Table 2). Two prominent bands induced by IFN-γ treatment were specifically competible by MHC-CRE region I oligonucleotide (Fig. 3b, arrows). In contrast, MHC-CRE region II binding activity was not induced by IFN-γ (not shown). These observations show that IFN-γ treatment induces an NF-κB or related factor(s) in astrocytes.

Nuclear extracts from astrocytes were also examined using an ICS oligonucleotide probe. Astrocytes constitutively expressed ICS binding activity (Fig. 3c) and, as with MHC-CRE region I/NF-κB binding activities, specific ICS binding activity was induced by IFN-γ (Fig. 3c). In sum, IFN-γ induced both region I and ICS binding activities and this correlated with the induction of MHC class I gene promoter activity in astrocytes.

Discussion

To evaluate MHC class I transcriptional regulation in the CNS, MHC class I gene promoter activity and nuclear DNA binding factors were examined in cultured astrocytes. The *cis* elements that increase MHC class I gene promoter activity in astrocytes were mapped to the MHC-CRE/ICS region, which is highly conserved in all classical murine MHC class I genes (Burke and Ozato, 1989). The MHC-CRE has been demonstrated previously to elicit constitutive enhancer activity (Miyazaki et al., 1986; Kimura et al., 1986; Baldwin and Sharp, 1987; Silverman et al., 1988; Burke et al., 1989; Israel et al., 1989a) whereas the ICS has been shown to mediate inducible transcription of MHC class I genes in cells treated with IFNs (Israel et al., 1986; Sugita et al., 1987; Korber et al., 1988; Blanar et al., 1989). Taken together, the present findings suggest that the MHC-CRE and the ICS function as constitutive enhancers and these elements mediate IFN-γ-enhancement of MHC class I transcription in astrocytes.

The functional importance of factor binding to the MHC-CRE region I for MHC class I promoter activity has been documented in cell lines (Israel et al., 1987; Baldwin and Sharp, 1987, 1988; Burke et al., 1989). Similarly, constitutive and IFN-enhanced region I binding activity is likely to contribute to MHC class I promoter activity seen in astrocytes. The region I binding activity in astrocytes appears to be NF-κB or a related protein, since the binding activity was competed by the Ig NF-κB oligonucleotide (Baldwin and Sharp, 1988; Israel et al., 1989b). It will be of interest to determine whether this binding factor is related to the recently cloned NF-κB gene (Ghosh et al., 1990; Kieran et al., 1990) or to PRDII-BFI (Singh et al., 1988; Fan and Maniatis, 1990; Nakamura et al., 1990) both of which have been shown to bind both the MHC-CRE region I and the IgG NF-κB site, and whether binding activity is transcriptionally or post-transcriptionally induced (Sen and Baltimore, 1986).

The lack of inducible activity of pL^d-CAT-123-ICS following IFN-γ treatment suggests a requirement for the juxtaposed MHC-CRE for ICS function in responding to interferons as observed previously in other cells (Israel et al., 1986; Sugita et al., 1987). That the increase in MHC class I promoter activity produced by IFN-γ correlated with the enhancement of both ICS and MHC-CRE region I binding activities is consistent with this possibility. This is also consistent with the observation that induction of MHC class I genes by tumor necrosis factor-α is mediated by induction of NF-κB binding activity to the MHC-CRE region I element (Israel et al., 1989a). However, a definitive answer on the independent and cooperative effects of the MHC-CRE and ICS in constitutive and inducible expression of MHC class I genes in astrocytes will require systematic mutation of these enhancers in the pL^d-CAT-237 construct.

ICS binding activity is both constitutively expressed and enhanced by IFN-γ in cultured astrocytes. Since

the MHC class I gene promoter activity in astrocytes may be, in part, mediated by the ICS, both constitutive and enhanced ICS binding activities may have a functional role. However, as with region I activity, the mechanisms governing the expression of ICS binding activity are not known. It has been reported that ICS binding activities, induced in cell lines by IFN or by viruses, have at least two distinct components, one newly synthesized after stimulation, and the other induced by a post-translational change of a preexisting protein (Shirayoshi et al., 1988; Blanar et al., 1989; Driggers et al., 1990), perhaps involving nuclear translocation (Levy et al., 1989).

A number of studies have demonstrated differential regulation of MHC class I molecules among cell types within the CNS, including astrocytes, oligodendrocytes, and neurons (Schnitzer and Schachner, 1981; Wong et al., 1985; Fontana et al., 1986; Mauerhoff et al., 1988). This regulation may relate to relative susceptibility or resistance to lysis by cytolytic T cells during an anti-viral immune response within the CNS (Oldstone et al., 1986; Joly et al., 1991; Levine et al., 1991). Additional complexity is related to strain-specific regulation of MHC molecules in CNS cells (Massa et al., 1989) which may contribute to genetic control of cellular immune responses within the CNS (Massa et al., 1987a,c). Therefore, the regulation of MHC class I molecules in CNS cells may be uniquely important for the regulation of cellular immune responses in this tissue. The present study forms a basis upon which CNS-specific regulation of MHC class I molecules may be further investigated. It would be of importance to determine the identity of enhancer binding proteins and how these proteins are regulated in astrocytes.

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