Specific Suppression of Major Histocompatibility Complex Class I and Class II Genes in Astrocytes by Brain-enriched Gangliosides

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Summary

The effect of brain-enriched gangliosides on constitutive and cytokine-inducible expression of major histocompatibility complex (MHC) class I and II genes in cultured astrocytes was studied. Before treatment with gangliosides, astrocytes expressed constitutive MHC class I but not class II molecules, however, the expression of both MHC class I and II cell surface molecules on astrocytes was induced to high levels by interferon γ (IFN-γ). Constitutive and IFN-γ–inducible expression of MHC class I and II molecules was suppressed by treatment of astrocytes with exogenous bovine brain gangliosides in a dose-dependent manner. Constitutive and induced MHC class I and II mRNA levels were also suppressed by gangliosides, indicating control through transcriptional mechanisms. This was consistent with the ability of gangliosides to suppress the binding activity of transcription factors, especially NF-κB–like binding activity, important for the expression of both MHC class I and II genes. These studies may be important for understanding mechanisms of central nervous system (CNS)–specific regulation of major histocompatibility molecules in neuroectodermal cells and the role of gangliosides in regulating MHC-restricted antiviral and autoimmune responses within the CNS.

Cells of the central nervous system (CNS)[1] express extremely low levels of MHC class I and II molecules compared with cells of other tissues (1–3). The lack of MHC class I and II molecules may relate to the suppression of MHC-restricted T cell–mediated immune responses in the CNS as indicated by previous reports demonstrating (*a*) the lack of T cell–mediated clearance of tumors or neurotropic viruses from the CNS compared with other tissues (4, 5); and (*b*) genetic resistance to CNS autoimmune disease in rodent strains that express relatively low amounts of MHC class II molecules on CNS cells compared with those of susceptible strains (6–9).

To study the basis for the lack of MHC class I and II expression in the CNS, astrocytes have been extensively analyzed in vitro. Previous studies (2, 6, 10–13) have shown that astrocytes express little or no MHC class I or II molecules in vivo or when freshly isolated from the brain. However, upon cultivation, these cells constitutively express low levels of MHC class I molecules, and in some instances, class II molecules (10, 11, 14–16). Cultured astrocytes can be further induced by IFN-γ to express high levels of both MHC class I and II molecules (14–17). However, whether astrocytes can be induced by cytokines to express significant amounts of MHC class I and II molecules in vivo during normal immune responses to infectious agents is unclear. Induction of MHC class I and II molecules on astrocytes is generally lacking relative to induction on cells of the monocyte lineage that either reside within the CNS (microglial cells) or migrate into the CNS during an immune response in this tissue (5, 12, 13, 18). Therefore, MHC class I and II genes appear to be negatively regulated in cells of neuroectodermal origin, including astrocytes, relative to other tissue cell types, and may be important for suppressing immunopathogenic reactions within the CNS (5). This is consistent with observations of localized expression of MHC class I and II molecules on astrocytes in some cases of acute immunopathogenic inflammatory T cell responses such as occurs during active lesion formation in multiple sclerosis, experimental allergic encephalomyelitis, and virus-induced encephalomyelitis (19–24).

The present investigation analyzes the possible role of CNS-enriched polysialogangliosides (25, 26) in the suppression of both MHC class I and II molecules on astrocytes. Complex brain-derived gangliosides are highly immunosuppressive in vivo, which is thought to relate to direct inhibitory effects on T cells (27, 28). However, indirect effects of gangliosides

[1]*Abbreviations used in this paper:* a, asialo; BP, binding protein; CaM, Ca^{2+}/calmodulin; CNS, central nervous system; CRE, class I regulatory element; ICAM-1, intracellular adhesion molecule 1; ICS, interferon consensus sequence; MRI, mean fluorescence intensity; NANA, *N*-acetylneuraminic acid; NCAM, neural cell adhesion molecule; PKC, protein kinase C.

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on APCs (29, 30) may also account for immunosuppression. It is shown here that brain-enriched gangliosides profoundly and specifically suppress constitutive and IFN-γ–inducible expression of both MHC class I and II molecules on astrocytes. The MHC suppressive activity of gangliosides may explain the lack of both MHC class I and II molecules on CNS cells in general, and may be the basis for the immunoprivileged status of the CNS.

Materials and Methods

Primary Astrocyte Cultures. BlO.A (H-2^a) newborn mice were obtained from Harlan Sprague Dawley, Inc. (Indianapolis, IN). Astrocytes were prepared from 1–2-d-old mouse neonatal cerebral hemispheres as previously described (10). Astrocytes were treated with IFN-γ and gangliosides at 8 d of primary culture and analyzed at varying times after treatment as indicated in the text. Immunofluorescent staining of cultures with antibody to glial fibrillary acidic protein (GFAP) showed that the cultures consisted of >95% GFAP$^+$ astrocytes.

Gangliosides. Mixed bovine brain gangliosides (>98% pure by TLC) contained 21% G_{M1}, 40% G_{D1a}, 16% G_{D1b}, and 19% G_{T1b} (Calbiochem-Novabiochem Corp., San Diego, CA). Purified G_{M1}, asialo(a)-G_{M1}, ceramide, and *N*-acetylneuraminic acid (sialic acid; NANA) were purchased from Sigma Chemical Co. (St. Louis, MO). G_{D1a} and G_{Q1b} were obtained from Calbiochem-Novabiochem Corp. G_{T1b} was purchased from Matreya, Inc. (Pleasant Gap, PA). Sterile stock solutions were stored at 4°C and diluted with serum-free medium (DMEM) directly before adding to astrocyte cultures at concentrations ranging from 1 to 100 μg/ml for mixed gangliosides and at 50 μM for individual gangliosides.

Immunofluorescence Staining for FACS® Analyses. 8-d primary astrocytes were incubated in medium, with or without recombinant murine IFN-γ (Genentech Inc., South San Francisco, CA) and processed for flow cytofluorimetry using a FACS® (Becton Dickinson Immunocytometry Systems, Mountain View, CA) as previously described (31). Some control and IFN-γ–treated cultures also received mixed bovine brain or individual gangliosides as indicated in the text. Mouse or rat mAbs specific for either MHC class I molecules (M1/42.3.9.8) (32), MHC class II I-A^k molecules (OX-6) (Bioproducts for Science, Inc., Indianapolis, IN) (33), murine Thy-1.2 molecules (Accurate Chem. & Sci. Corp., Westbury, NY), murine intercellular adhesion molecule 1 (ICAM-1) (Seikagaku America, Inc., Rockville, MD), or murine neural cell adhesion molecules (NCAM) (Chemicon International, Inc., Temecula, CA) (34) were used. The cells were analyzed by FACS® to determine the mean fluorescence intensities (MFI) and standard errors of samples of 10,000 cells.

Northern Blot Hybridization. Total RNA was extracted from primary astrocyte cultures as previously described (10, 31) using a guanidine isothiocyanate technique (35). 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 ^{32}P-labeled cDNA probes to MHC class I H-2L^d (36), β-actin (37), MHC class II I-A$_\alpha{}^k$ chain (38), and MHC class II I-E$_\beta{}^k$ chain (38). β-actin was used as an internal hybridization control since expression was not affected by IFN-γ or gangliosides. Autoradiograms of the filter were analyzed by densitometry.

Nuclear Extracts from Astrocytes. Nuclear extracts from astrocytes were prepared using a miniprep technique (39), as described previously (31).

Oligonucleotides. All oligonucleotides were synthesized as described (40). The following duplex oligonucleotides contain highly conserved enhancer sequences in the upstream promoter region of MHC class I genes, as previously described (31, 41) and were used as probes or competitors for gel mobility shift assays: (*a*) the MHC class I regulatory element region I (MHC-CRE region I; from −173 to −161 relative to the transcriptional start site position of +1); this sequence is closely related to the NF-κB enhancer binding site shown below (42); and (*b*) the interferon consensus sequence (ICS) (from −167 to −139) of the H-2L^d gene. The NF-κB oligonucleotide (5'CTCAACAGAGGGGACTTTCCGAGAGGCCAT 3') (43) used was previously described (40, 41).

Gel Mobility Shift Assay. Binding of nuclear proteins to the MHC-CRE region I/NF-κB–related or ICS enhancer sequences was studied by the gel mobility shift assay (41, 44, 45) as previously described (31, 46). The density of specific competible bands on autoradiograms was quantified by densitometry.

Results

Ganglioside-mediated Suppression of MHC Class I and II Molecules. Astrocytes in culture constitutively expressed low levels of MHC class I molecules (10, 31) (Fig. 1 *A*), but not class II molecules (Fig. 1 *B*) (7). Induction of MHC class I molecules by IFN-γ treatment for 2 d was dose dependent and resulted in levels five to six times higher than constitutive levels (Fig. 1 *A*). Mixed bovine brain gangliosides suppressed both constitutive as well as IFN-γ–inducible expression of MHC class I molecules in a dose-dependent manner, at 2 d after treatment (Fig. 1 *A*). Treatment with 10 and 100 μg/ml gangliosides resulted in a greater than threefold reduction in constitutive expression of MHC class I molecules. Gangliosides at 100 μg/ml totally blocked IFN-γ induction of MHC class I molecules to levels 10-fold lower than those seen with 1 and 10 U/ml IFN-γ alone and lower than constitutive levels (Fig. 1 *A*).

Treatment of astrocytes with increasing doses of IFN-γ induced MHC class II molecules (I-A^k) to levels well above background in a dose-dependent manner (Fig. 1 *B*). As with MHC class I molecules, gangliosides suppressed the IFN-γ induction of MHC class II molecules (Fig. 1 *B*). Cultures treated with 10 U/ml IFN-γ and 100 μg/ml gangliosides expressed 25-fold lower levels of MHC class II molecules than astrocytes treated with 10 U/ml IFN-γ alone (Fig. 1 *B*).

The effect of gangliosides on MHC class I and II molecules appeared specific for IFN-γ-inducible genes because (*a*) the level of constitutive cell surface expression of both NCAM (34) and Thy-1 (11) molecules was not affected by either IFN-γ or gangliosides; and (*b*) both constitutive and IFN-γ–inducible ICAM-1 molecules were affected similarly to MHC molecules (Table 1).

Gangliosides Specifically Suppress Levels of MHC Class I and II mRNA. IFN-γ and ganglioside treatments that affect the expression of MHC class I and II molecules at the cell surface also affect, in parallel, the expression of MHC class I and II mRNA in astrocytes. MHC class I mRNA was constitutively expressed in astrocytes (Table 2) (10, 31) and treatment with 50 μg/ml gangliosides for 2 d decreased constitutive MHC class I mRNA to undetectable levels (Table 2). IFN-γ (10 U/ml) induced MHC class I mRNA by approximately threefold over constitutive levels, and addition of gan-

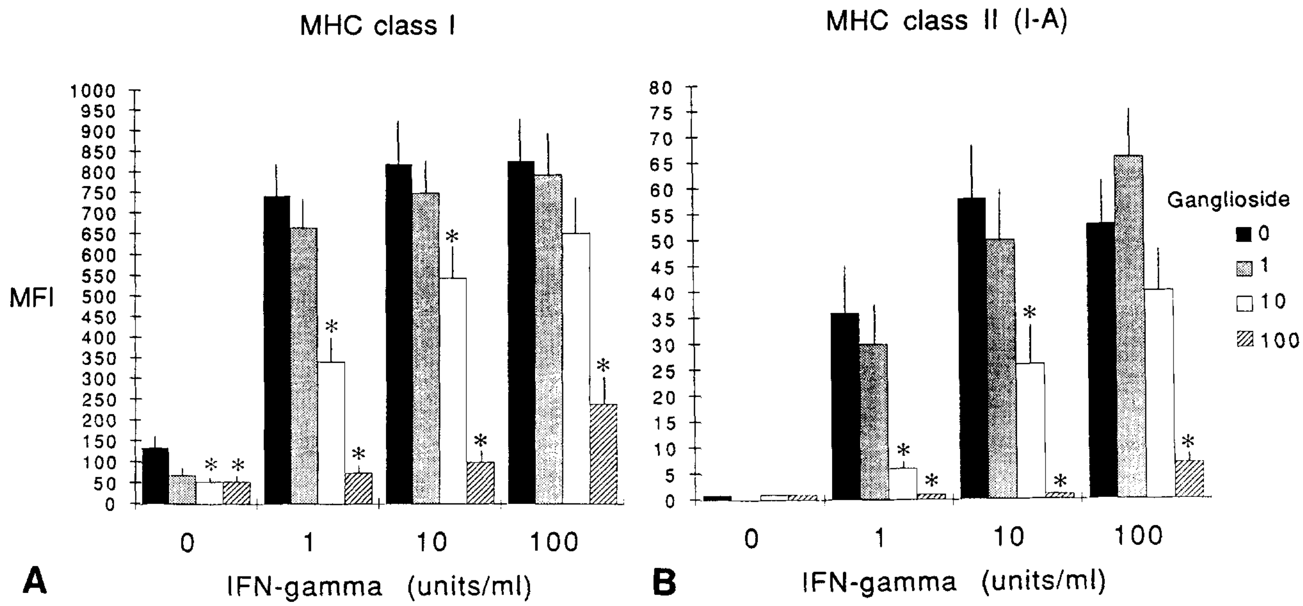

Figure 1. Suppression of constitutive and IFN-γ–inducible cell surface expression of MHC class I and II molecules by gangliosides. Primary mouse astrocytes were treated with varying doses of IFN-γ in the presence or absence of different concentrations of bovine brain gangliosides (μg/ml) as indicated in the figure. After 2 d of treatment, the cells were stained for MHC class I and II molecules and analyzed by FACS®. mAbs to MHC class I molecules (M1/42.3.9.8) and MHC class II (I-A^k) (Ox-6) were used. (*MFI*) Mean fluorescence intensity. 10,000 cells were analyzed per specimen. The SE of the means is indicated by vertical lines at the top of the bars. Absence of a vertical line above a bar indicates a SE of <1. The means at 10 and 100 μg/ml ganglioside were significantly lower (*) than the means for cultures without gangliosides (Student's *t* test; p <0.05–0.001).

gliosides to parallel IFN-γ–treated cultures suppressed this increase in MHC class I mRNA to basal constitutive levels (Table 2).

MHC class II mRNA (I-A_α^k and I-E_β^k) was undetectable in cultures not treated with IFN-γ (Table 2), which was consistent with the absence of constitutive cell surface expression. IFN-γ (10 U/ml) induced I-A_α and I-E_β mRNA to detectable levels (Table 2). Gangliosides suppressed IFN-γ induction of mRNA by over 12-fold for I-A_α and to undetectable levels for I-E_β (Table 2).

Suppression of MHC Molecules by Gangliosides Is Dependent on Sialylation. The bovine brain ganglioside preparation used

Table 1. *Specific Suppression of IFN-γ–inducible Proteins by Gangliosides*

		IFN-γ (units/ml)			
		0	1	10	100
MHC class I	–*	286 (28)	502 (42)	872 (40)	1241 (72)
	+	84 (10)	117 (13)	507 (40)	1085 (75)
MHC class II	–	4 (1)	5 (1)	36 (4)	89 (7)
	+	5 (1)	3 (1)	12 (2)	29 (3)
ICAM-1	–	115 (12)	141 (14)	173 (16)	232 (21)
	+	89 (10)	101 (10)	124 (12)	152 (15)
NCAM	–	48 (6)	46 (6)	44 (5)	48 (5)
	+	47 (6)	42 (7)	40 (5)	40 (4)
Thy-1	–	130 (21)	156 (23)	163 (24)	148 (22)
	+	150 (23)	146 (21)	154 (22)	150 (21)

Values represent the MFI of each sample. Values in parentheses represent the SE of the mean.
* (+) Presence or (–) absence of 25 μg/ml gangliosides.

Table 2. *Densitometric Quantification of mRNA Levels*

	−IFN-γ		+IFN-γ	
Gangliosides	−	+	−	+
MHC class I	6.86	0.20	20.17	6.63
MHC class II (I-A_α)	0.13	0.44	21.28	1.69
MHC class II (I-E_β)	0.30	0.35	3.02	0.05

8-d astrocyte cultures were treated with either medium alone (−IFN-γ) or medium with 10 U/ml IFN-γ (+IFN-γ) for 2 d in the presence or absence of 50 μg/ml bovine brain gangliosides. RNA was extracted and Northern blots were probed for MHC class I, MHC class II, and β-actin mRNA, and autoradiograms were scanned by a densitometer. Values represent the density of bands relative to β-actin mRNA.

in the studies described above contained a mixture of tetraose gangliosides. To determine (*a*) whether the suppressive activity on astrocytes involved a cooperative effect of the mixed gangliosides; (*b*) whether individual ganglioside species are able to suppress MHC class I and II molecules; and (*c*) whether the number of sialic acid groups per ganglioside molecule is important for suppression, the relative suppressive activity of equimolar amounts of G_{M1}, G_{D1a}, G_{T1b}, and G_{Q1b} was tested. As well, asialo-G_{M1} was used to determine the absolute requirement for sialic acid. As shown in Table 3, individual sialylated gangliosides suppressed the expression MHC molecules on astrocytes. In contrast, asialo-G_{M1} had no suppressive activity. Suppressive activity depended on the number of sialic acid groups such that $G_{Q1b} = G_{T1b} > G_{D1a} > G_{M1}$. Ganglioside components ceramide and sialic acid (NANA) were totally nonsuppressive (Table 3).

The Effect of Gangliosides on the Expression of Transcription Factor Binding Activities in Astrocytes. Because the ability of gangliosides to suppress steady state levels of MHC class I and II protein and mRNA may be related to direct effects on transcription, levels of transcription factors important for these genes were analyzed. The transcription of MHC class I genes in a variety of cells, including astrocytes (31) is controlled primarily by two juxtaposed enhancers, designated the MHC-CRE and the ICS, located in the upstream promoter region of these genes (31, 40, 46). These enhancers function by binding specific nuclear protein transcription factors, including NF-κB (47, 48). Treatment of astrocytes with gangliosides suppressed both constitutive and IFN-γ–inducible expression of both NF-κB–like and ICS binding activities (Table 4). IFN-γ (10 U/ml) induced both ICS-binding protein (ICS-BP) (by 10-fold) and NF-κB–like activity (by fourfold) in astrocytes not treated with gangliosides. Induced levels were suppressed by 3.6-fold for ICS-BP and by 60-fold for NF-κB (Table 4) by gangliosides. This suppression is likely to be important in the suppression of MHC class I and II mRNA in astrocytes (Table 2) and indicates that ganglio-

Table 3. *Ganglioside Sialylation and Suppressive Activity*

	−	G_{M1}	G_{D1a}	G_{T1b}	G_{Q1b}	aG_{M1}	NANA	Ceramide
MHC class I	1.00	0.66	0.27	0.13	0.14	1.17	1.12	1.04
MHC class II	1.00	0.15	0.16	0.11	0.10	1.21	1.06	1.07

Values represent MFI of astrocytes treated with 10 U/ml IFN-γ in the presence of 50 μM ganglioside relative to cultures treated with IFN-γ in the absence of gangliosides.

Table 4. *Gel Shift Assay of Specific ICS and MHC-CRE Region I (NF-κB-like) Binding Activities: Effect of IFN-γ and Bovine Brain Gangliosides*

	NF-κB-like		ICS-BP	
	−IFN-γ	+IFN-γ	−IFN-γ	+IFN-γ
Gangliosides (μg/ml)				
0	93,307	398,044	15,756	152,289
50	12,368	6,671	7,252	42,678
Fold suppression	7.5	60.0	2.2	3.6

8 d primary astrocyte cultures were incubated in medium alone (−IFN-γ) or medium containing 10 U/ml IFN-γ (+IFN-) in the presence or absence of 50 μg/ml bovine brain gangliosides for 2 d. Nuclei were prepared and proteins were extracted and analyzed by gel mobility shift assay. Specific competible bands in autoradiograms were quantified by densitometry and are listed in the table. All densities are relative to a background of 1,100 for the probe alone. Fold suppression represents the level of binding activities in astrocytes without gangliosides divided by the level of binding activities in astrocytes with gangliosides.

sides may directly affect transcription factors and transcriptional activity of these genes.

Discussion

To identify possible intrinsic or extrinsic factors present in the CNS environment that suppress the expression of both MHC class I and II molecules within the CNS in vivo, the effect of brain-derived gangliosides on cultured astrocytes was analyzed. Brain gangliosides were chosen for study because of previous reports on the immunosuppressive capacity of gangliosides (27, 28, 49). The present study identifies, for the first time, a defined factor, enriched within the CNS, that can selectively suppress the expression of both MHC class I and II molecules on CNS cells. This suppression may partially account for the immunosuppressive activity of gangliosides, particularly within the CNS.

The mechanism of ganglioside action on astrocytes with respect to MHC class I and II gene suppression is unknown. The induction of MHC class I and II molecules by IFN-γ has been shown to involve either protein kinase C (PKC) or Ca^{2+}/calmodulin (CaM) activation, depending on cell type (17, 50–53). This may be relevant in the present study because gangliosides have been shown to specifically bind to and suppress the activity of both PKC and CaM (54, 55). Such ganglioside interactions are implicated in the present study because the suppressive activity of individual gangliosides on PKC and CaM closely correlates with MHC-suppressive activity as presently shown (Table 3). Also, possible ganglioside-mediated modulation of CaM or PKC may relate to mechanisms that specifically downregulate MHC gene expression, subsequent to signal transduction, such as increasing intracellular cAMP (38, 56) and/or alteration of transcription factors (42, 57). This latter possibility is consistent with observations that (*a*) gangliosides can suppress constitutive expression of both MHC class I and ICAM-1 molecules; and (*b*) posttreatment of astrocytes with gangliosides can also suppress MHC class I and II genes subsequent to induction by IFN-γ (Massa, P. T., unpublished observations).

Because gangliosides appeared to ultimately regulate MHC molecules at the transcriptional level, the ability of gangliosides to specifically downmodulate the binding activity of transcription factors important for the expression of both MHC class I and II genes was analyzed. An especially profound suppressive effect on NF-κB–like binding activity and an effect on the binding activity to the ICS enhancer of MHC class I gene promoters was observed. This suppression may account for the tissue-specific lack of these transcription factors both in the brain (47) and in cultivated neurons, as recently described (46). As for MHC class I expression, the suppressive effect of gangliosides on NF-κB–like activity may play a role in MHC class II I-A_α chain, MHC class II invariant chain, and ICAM-1 gene expression (58–62). Further analysis of other transcription factors of MHC class II genes is in progress, in particular, those that act at highly conserved X, Y, and W box enhancers (59, 63, 64).

Of all CNS cell types, the suppression of MHC class I and II molecules appears to be most complete in neurons. Neurons do not constitutively express MHC class I or II molecules (1, 46) nor can these molecules be induced by cytokines on these cells, either in vivo or in vitro (12, 46). With respect to the present study, this suppression is consistent with the ability of these cells to synthesize high levels of complex polysialogangliosides compared with other CNS cell types or to cells of other tissues (25, 65–68). It is further proposed that the transfer of tetraose polysialogangliosides from neurons to astrocytes (65, 66) may be an important mechanism for the suppression of MHC class I and II molecules on astrocytes as well as on other CNS cells in vivo. This hypothesis is consistent with the sharp decrease in the levels of complex tetraose gangliosides in cultured astrocytes (65, 66), the concomitant increase in expression of constitutive and inducible MHC class I and II molecules on astrocytes with time in culture (6, 10, 31, 69), and the suppression of constitutive and inducible expression of both MHC class I and II molecules by exogenous application of CNS-enriched gangliosides to astrocytes in vitro as presented in this study.

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