

Astrocytes Produce Interferon That Enhances the Expression of H-2 Antigens on a Subpopulation of Brain Cells

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Abstract. Using primary culture methods, we show that purified astrocytes from embryonic mouse or rat central nervous system (CNS) can be induced to produce interferon (IFN) activity when pretreated with a standard IFN-superinducing regimen of polyribonucleotide, cycloheximide, and actinomycin D, whereas IFN activity was not inducible in neuronal cultures derived from mouse CNS. Astrocyte IFN displays inductive, kinetic, physicochemical, and antigenic properties similar to those of IFN- α/β , but is dissimilar to lymphocyte IFN (IFN- γ). Treatment of pure astrocytic cultures or astrocytes cultured with neurons with astrocyte IFN or IFN- α/β induced a dramatic increase

in the expression of H-2 antigens on a subpopulation of astrocytes. Neither neurons nor oligodendroglia expressed detectable levels of H-2 antigens when exposed to astrocyte IFN, IFN- α/β , or to IFN- β . Injection of astrocyte IFN or IFN- α/β directly into brains of newborn mice indicated that H-2 antigens were also induced *in vivo*. None of the IFNs (astrocyte, α/β , or β) tested induced Ia antigens on CNS cells *in vitro* or *in vivo*. Since H-2 antigens have a critical role in immune responses, astrocyte IFN may initiate and participate in immune reactions that contribute to immunoprotective and immunopathological responses in the CNS.

THE central nervous system (CNS)¹ has classically been thought to be an immunologically privileged site in that: (a) it lacks lymphatic drainage (32); (b) the CNS cells express low levels of antigens encoded by the major histocompatibility complex (MHC) which function to both initiate and effect immune responses (8, 20); and (c) it is protected by the blood-brain barrier which is impermeable to many substances, including the known classes of interferons (IFN α , β , and γ) (5, 22, 30, 35).

The brain has been shown to produce high levels of IFN when injected with viruses (1, 35) or polyribonucleotides (6), but neither the CNS cell types that produce high levels of IFN nor the function of brain IFN has been determined. IFNs are a heterogeneous group of proteins that are largely defined by their ability to protect cells against viral infection (17, 28). Three classes of IFN have been discovered which are the primary IFN products of three cell types: leucocytes, IFN- α , fibroblasts, IFN- β , and lymphocytes, IFN- γ (2). Recent work has shown that IFN- γ , a T cell lymphokine, induces a dramatic increase in the expression of MHC antigens on normal and leukemic human myeloid cells (21), on neural cell lines (23), and on brain cells *in vitro* and *in vivo* (41, 42). All major

types of brain cells, including neurons, astrocytes, oligodendrocytes, and microglia, showed enhanced MHC expression in response to IFN- γ (41, 42).

The MHC in the mouse, termed H-2, codes for a variety of cell surface glycoproteins involved in the immune response. Two classes of molecules can be distinguished: class I transplantation antigens (H-2 K, D, and L) are present on most cells of the body, but these antigens are undetectable on normal brain cells (8). H-2 antigens mediate immune reactions of cytotoxic T cells (44). Class II, the immune response associated (Ia) antigens, are found predominantly on B cells, macrophages, and dendritic cells, and function to present foreign antigens to regulatory T lymphocytes. Approximately 1–2% of CNS cells have been reported to be Ia⁺, but the nature and origin of these cells has not been established (14, 36). IFN- γ modulation of MHC antigens on neurons and/or glia might enable normally sequestered immunoincompetent cells of the brain to participate in T cell-mediated responses, and may play an important role for brain diseases that have an immunological component. However, this cooperation of brain and immune cells appears to involve passage of T cells across the blood-brain barrier, so that they enter the brain and release lymphokines as IFN- γ , which normally cannot pass the blood-brain barrier (5, 22, 30, 35).

In this paper, we show that cells (astrocytes) within the CNS produce IFN that is biologically and antigenically similar to IFN- α/β . Astrocyte IFN is a potent inducer of H-2 antigen

1. *Abbreviations used in this paper:* CPE, cytopathic effect; CNS, central nervous system; GC, galactocerebroside; GFAP, glial fibrillary acidic protein; Ia, immune response associated; IFN, interferon; MHC, major histocompatibility complex; poly rI-C, polyribonucleosinic-polyribocytidylic acid; TT, tetanus toxin; VSV, vesicular stomatitis virus.

expression on a subpopulation of astrocytes. These results suggest that astrocyte IFN has an important role in defending the brain against viruses and in the generation of immune responses in the CNS.

Materials and Methods

Cell Cultures

Astrocyte Cultures. Cerebral cortices were dissected from embryonic CBA/J mice (E-19), freed of meninges, and dissociated by gentle repetitive pipetting. The cell suspensions were plated on collagen-polylysine coated tissue culture dishes. Loosely adherent oligodendrocytes were preferentially removed by gently pipetting a stream of medium across the surface of the cells. Cultures were grown to confluence in N5 medium (18) that contained 5% horse serum, then treated with 10^{-5} M cytosine arabinoside for 48 h to kill rapidly dividing cells.

Neuronal Cultures. Neuronal cultures were obtained by dissociation of 15-d CBA/J mouse embryo brains. The tissue was disrupted into a cell suspension by gentle trituration and the cells were grown on collagen-polylysine coated tissue culture dishes in N5 medium that contained 5% neurotrophic factor from horse serum as described (18).

Immunohistochemical Procedures

Cell cultures were immunohistochemically labeled as described by Keane et al. (19). For immunolabeling of the cell surface (tetanus toxin [TT], A2B5, galactocerebroside [GC], H-2, and Ia), cultures were treated with antibody to cell surface antigens, washed in N5 medium, and exposed to fluorochrome-coupled second antibody. Cells were fixed in 10% buffered formalin. For labeling of intracellular antigens (neurofilaments, glial fibrillary acidic protein [GFAP]), cells were fixed in 10% buffered formalin, permeabilized with 95% ethanol at -20°C , and exposed to antibodies to intracellular antigens. The cultures were washed in N5 medium and treated with fluorochrome-coupled second antibodies. Cells were then mounted in 50% glycerol/phosphate-buffered saline (PBS) and visualized in a Nikon diaphot epifluorescence microscope equipped with the appropriate filters.

Antibodies and Toxins

Expression of H-2K^k and Ia antigens on CBA/J (H-2K^k, D^b) mouse neurons, astrocytes, and oligodendrocytes was detected by double immunohistochemical labeling of cells using anti-H-2K^k monoclonal antibodies from cell line 11-4-1 (American Type Culture Collection, Rockville, MD) and anti-Ia monoclonal antibodies from cell line 14-4-4S (American Type Culture Collection). Specificity of binding was evaluated in control labelings using monoclonal antibodies (34-1-2S) of the same class (IgG 2a), but specific for a different haplotype (H-2K^d, D^d). Rabbit anti-neurofilament (43) was a gift of Dr. S.-H. Yen (Albert Einstein University), rabbit anti-GFAP (10) was a gift of Dr. L. Eng (California Institute of Technology), and rabbit-anti-GC (31) was a gift of Dr. K. Fields (Albert Einstein University). All rabbit antisera were used at a 1:150 dilution. Mouse monoclonal antibodies to the neuronal marker, A2B5 (9) (a gift of Dr. M. Nirenberg, NIH), and mouse monoclonal antibodies of H-2 and Ia antigens were used at a 1:200 dilution. Rabbit antibodies were visualized with either tetramethylrhodamine- or fluorescein isothiocyanate-conjugated goat antibodies to rabbit immunoglobulins (IgA, IgG, IgM, heavy and light chains, Cappel Laboratories, Cochranville, PA). Mouse antibodies were visualized with tetramethylrhodamine- or fluorescein isothiocyanate-conjugated goat immunoglobulins to mouse immunoglobulins (Cappel Laboratories). Antibodies and staining procedures for GC, GFAP, and TT receptors were according to previously described procedures (19).

Superinduction of IFN

Superinduction was done by preincubating astrocytes or neurons with polyribonucleosinic-polyribocytidylic acid (poly rI-rC) (50 $\mu\text{g}/\text{ml}$) and cycloheximide (10 $\mu\text{g}/\text{ml}$) for 6 h and with actinomycin D (1 $\mu\text{g}/\text{ml}$) for the final 2 h of the induction period (15, 29, 34). Astrocytic cultures were washed thoroughly with N5 medium that contained 5% horse serum, and then incubated with N5 medium for 24 h. Neuronal cultures were washed thoroughly with N5 medium that contained 5% neurotrophic fraction from horse serum (18), and then incubated in N5 medium for 24 h. Neuronal cultures were also treated with other IFN-inducing regimens using either phytohemagglutinin (5–500 $\mu\text{g}/\text{ml}$) (40) or B-lipopolysaccharide (5–500 $\mu\text{g}/\text{ml}$) (26) for 6 h. No measurable IFN activity was obtained from neurons, even after the superinducing regimen.

Cytopathic Effect (CPE) Assay

IFN activity was measured by the CPE assay that is based on dye (crystal violet) incorporation into fixed cells (3, 11). Mouse L929 cells were plated in 96-well Costar microtiter dishes (Costar, Cambridge, MA) at a density of 1×10^4 cells/ml and grown to confluence for 24 h. L929 cells were then incubated with medium from superinduced astrocytes or neurons for 24 h, and then infected with vesicular stomatitis virus (VSV, Indiana strain) that normally lyses these cells. After 24 h of infection, L929 cells were washed with N5 medium, fixed, and stained with 0.5% crystal violet, washed with distilled water, and air-dried. Crystal violet stain was eluted by addition of ethylene glycol monoethyl ether to each well and absorbance (OD_{590}) determined in a Titertek Multiscan Photometer. IFN activity was determined by cell survival, calculated as percent dye uptake (3, 11). For each experiment, log dilutions of standard mouse IFN- α/β (Lee BioMolecular, Inc.) were co-assayed with superinduced astrocyte or neuronal conditioned medium samples to determine the titer of IFN.

Neutralization Assays

Mouse astrocyte IFN (500 U/ml) was incubated with rabbit antiserum against mouse IFN- α/β (Lee BioMolecular, Inc.) or IFN- γ (a gift from Dr. S. Baron, University of Texas, Galveston) with neutralizing specific activity varying from 200 to 5,000 neutralizing U/ml for 3 h at 37°C and the antibody-IFN mixture tested for antiviral activity in the CPE assay (3, 11).

Induction of H-2 Antigens in the Brain

1-d-old CBA/J mice were injected intracerebrally with 50 U of IFN- α/β , IFN- β , astrocyte IFN, or control supernatants from uninduced astrocyte cultures showing no IFN activity. After various periods of time, cell suspensions and fresh-frozen cryostat sections (10 μm) of brains were prepared and assayed for the expression of H-2 and Ia antigens by indirect immunofluorescence procedures.

Results

Purified astrocytic and neuronal cultures used in these studies are shown in Figs. 1 and 2, respectively. The cell types present were assessed immunohistochemically with cell-type specific antibody markers. The majority of cells (>99%) in cortical astrocytic cultures displayed a flattened, sheet-like morphology (Fig. 1a), and stained with antibodies to GFAP (Fig. 1b),

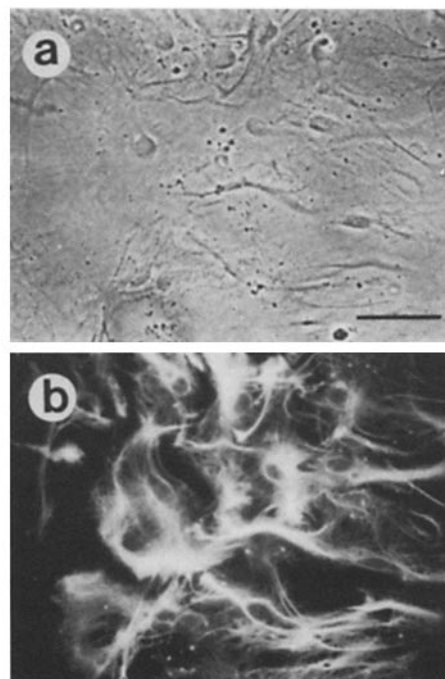


Figure 1. (a and b) Astrocytes from mouse cerebral cortex viewed with (a) phase contrast and (b) GFAP staining. Bar, 20 μm .

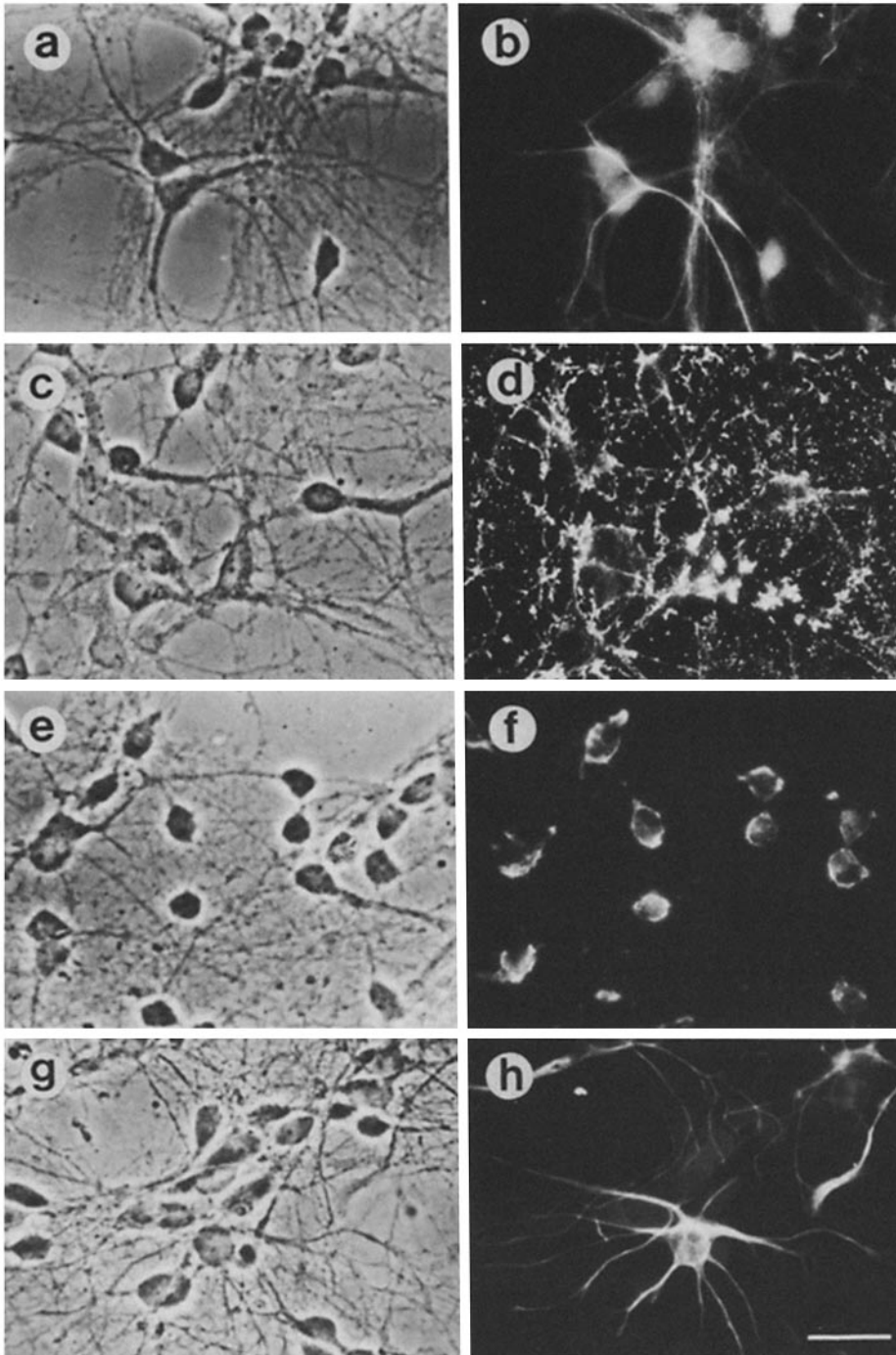


Figure 2. Phase-contrast micrographs (*a*, *c*, *e*, and *g*) of neuronal cultures that were stained with antibodies against neurofilament (*b*), TT (*d*), A2B5 antigen (*f*), or GFAP (*h*). Bar, 20 μm .

a specific marker for astrocytes (4). The remaining cells (<1%) were either fibrous astrocytes, identified by binding of antibodies to GFAP, TT (27) and monoclonal antibodies to A2B5 (9), or oligodendrocytes as evidenced by binding of anti-GC, a marker for oligodendroglia (31).

Neuronal cultures were obtained by dissociation of 15-d CBA/J mouse embryo brains (Fig. 2). The neuronal nature of the majority of cells (95%) was confirmed by their ability to fire action potentials (18) and to bind antibodies to NF (Fig. 2, *a* and *b*), TT (Fig. 2, *c* and *d*), and A2B5 (Fig. 2, *e* and *f*). The remaining cells (5%) in the neuronal cultures were astrocytes as evidenced by staining with anti-GFAP antibodies (Fig. 2, *g* and *h*). Astrocytes cultured in the pres-

ence of neurons assume a process-bearing morphology, much like that of neurons (Fig. 2, *g* and *h*), whereas astrocytes cultured alone have a flattened, sheet-like appearance (Fig. 1, *a* and *b*) (13).

Astrocytic and neuronal cultures were superinduced by a 6-h exposure to poly rI-rC (50 $\mu\text{g}/\text{ml}$) and cycloheximide (10 $\mu\text{g}/\text{ml}$) (Fig. 3*a*). For the final 2 h of the induction period, actinomycin D (1 $\mu\text{g}/\text{ml}$) was added. Cultures were extensively washed and then incubated from 1 to 24 h in a N5 medium that contained 5% horse serum. IFN antiviral activity in the collected medium was assayed by its ability to protect cells against VSV infection in the CPE assay. Superinduction of astrocyte IFN yielded from 500 to > 10,000 U/

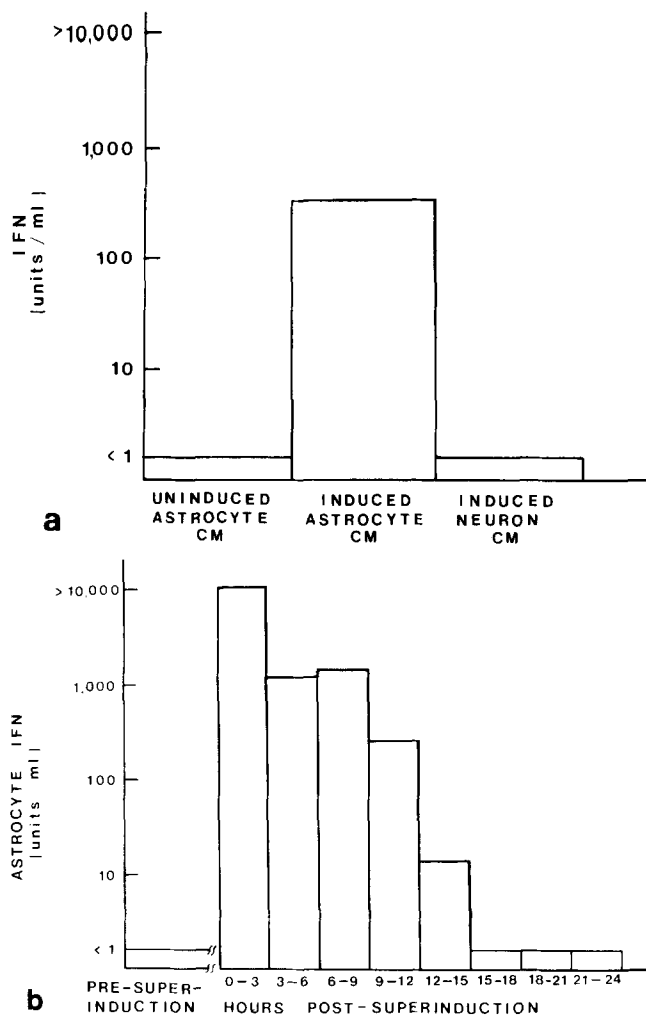


Figure 3. (a) Interferon antiviral activity (log scale) superinduced in mouse astrocytic or neuronal conditioned medium (CM). (b) Kinetics of IFN superinduction by poly rI-rC, cycloheximide, and actinomycin D in astrocytes. IFN produced by astrocytes was collected at 3-h intervals after the superinduction followed by extensive washing of cells with N5 medium between collection periods. Astrocyte IFN activity in each sample was measured by the CPE assay as described in Materials and Methods.

ml IFN activity (Fig. 3a). No measurable IFN activity was detected in either uninduced astrocyte cultures or purified neuronal cultures treated with the same superinduction regimen or with other IFN-inducing protocols using phytohemagglutinin (a T cell mitogen) or B-lipopolysaccharide (a B cell mitogen) (Fig. 3a). Thus, it appears that IFN activity is produced by astrocytes, but not by neurons.

Fig. 3b shows the kinetics of astrocyte IFN induction. After the termination of the 6-h induction period, IFN produced by astrocytes was collected at 3-h intervals (up to 24 h) followed by extensive washing with fresh media between collection points. The aliquots of astrocyte-conditioned media collected at each 3-h interval were then assayed for antiviral activity by the CPE assay. Induction of astrocyte IFN activity was rapid with the highest levels produced during the first 3 h after the 6-h induction period. IFN activity gradually declined so that no measurable astrocyte IFN activity remained 15 h after the induction period. The kinetics of astrocyte IFN induction are similar to those reported for IFN- α and IFN- β ,

but not for IFN- γ which is induced much more slowly (29, 34).

Table I shows physical and biological properties displayed by astrocyte IFN. Astrocyte IFN is sensitive to trypsin digestion, which suggests that it is a protein. Astrocyte IFN activity is stable at both pH 2.0 for at least 24 h and at 56°C for 3 h, characteristic properties of IFN- α and IFN- β , but not IFN- γ . Since the antiviral protection conferred by IFN- α , β , and γ is species- and cell-specific, we tested host cell specificity of mouse astrocyte IFN on several different fibroblast cell lines often used in the CPE assay (Table I). Antiviral activity was greatest on homologous mouse cell lines (~75% of VSV-infected L929 cells were protected from viral infection), whereas little or no protection was conferred on human foreskin fibroblasts or rat kidney fibroblasts. Additionally, induced IFN from neonatal Sprague-Dawley rat cortical astrocytes protected ~70% of VSV-infected rat kidney fibroblasts, but afforded little or no protection for VSV-infected human foreskin fibroblasts or mouse L929 fibroblasts (data not shown). These results show that the antiviral protection conferred by astrocyte IFN is species-specific. The response to inducers, kinetics of induction, and physical properties of astrocyte IFN are similar to those reported for IFN- α and IFN- β , but not for IFN- γ (29, 34).

To test whether astrocyte IFN is antigenically similar to other IFN classes, medium conditioned by superinduced astrocytes was incubated with dilutions of rabbit antiserum against mouse IFN- α/β or IFN- γ (Table II). After a 3-h incubation of antibody and astrocyte IFN mixtures, the astrocyte IFN antiviral activity was measured by the CPE assay. Antiserum against mouse IFN- α/β effectively neutralized astrocyte IFN antiviral activity, while antiserum against mouse IFN- γ did not. Thus, astrocyte IFN is antigenically similar to IFN- α/β and dissimilar to IFN- γ .

Table I. Physical and Biological Properties of Astrocyte IFN

Treatment	Titer
	U/ml \pm SD
Induced IFN (control)	6,408 \pm 2,800
+ Trypsin (0.25%, 3 h, 37°C)	1
pH 2.0 (24 h)	5,564 \pm 1,956
+ Heat (56°C, 3 h)	5,877 \pm 2,711
Cross-species activity	% Dye uptake
Mouse L929 fibroblasts	76.5 \pm 12.5
Human foreskin fibroblasts	3.1 \pm 3.5
Rat kidney fibroblasts	2.7 \pm 2.6

Mouse astrocyte IFN was superinduced and collected as described in Materials and Methods. Three (0.5 ml) aliquots were taken. One sample was treated with trypsin (0.25%) for 3 h and the reaction stopped with 0.25% soybean trypsin inhibitor. The second sample was acidified (pH 2.0) with 1.0 N HCl for 24 h and then neutralized to pH 7.0 with 1.0 N NaOH. The third sample was heated to 56°C for 3 h and then cooled to 37°C. IFN activity in all three samples was then assayed for antiviral protection on mouse L929 fibroblasts in the CPE assay. Samples containing 0.25% trypsin and 0.25% soybean trypsin inhibitor did not affect viability (crystal violet uptake) of uninfected mouse L929 fibroblasts. Cross-species activity of mouse astrocyte IFN was tested on mouse L929 cells, human foreskin fibroblasts, and rat kidney fibroblasts. L929 cells, human foreskin fibroblasts (1×10^4 cells), and rat kidney fibroblasts (2.5×10^4 cells) were seeded in 96-well Costar microtiter dishes and grown to confluence. Antiviral activity conferred by mouse astrocyte IFN on each cell type was measured by the CPE assay. Each mean (\pm SD) is based on six values ($n = 6$).

Table II. Neutralization of Astrocyte IFN Activity with IFN- α/β or IFN- γ Antiserum

IFN- α/β antiserum neutralizing U/ml	Astrocyte IFN U/ml	Percent neutralization astrocyte IFN
0	545 \pm 90	0
200	330 \pm 60	34
500	185 \pm 80	63
2,000	0 \pm 0	100
3,000	0 \pm 0	100
5,000	0 \pm 0	100

IFN- γ antiserum neutralizing U/ml	Astrocyte IFN U/ml	Percent neutralization astrocyte IFN
0	565 \pm 95	0
200	550 \pm 110	0
500	610 \pm 125	0
2,000	590 \pm 80	0
3,000	555 \pm 105	0
5,000	625 \pm 130	0

Mouse astrocyte IFN (500 U/ml) was incubated with rabbit antiserum against mouse IFN- α/β or IFN- γ (neutralizing specific activity varying from 200 to 5,000 neutralizing U/ml) for 3 h at 37°C and tested for antiviral activity in CPE assay as described in Materials and Methods. Each mean (\pm SD) was based on eight samples ($n = 8$).

Table III. Induction of MHC Antigens on 48 h Cultured CBA/J Brain Cells

	H-2K ^k	H-2K ^d	Ia
Astrocyte IFN	23 \pm 3	0	1 \pm 1
IFN- α/β	26 \pm 2	0	1 \pm 1
IFN- β	1 \pm 1	0	1 \pm 1
Uninduced	1 \pm 1	0	1 \pm 1

Brains of 1-d-old CBA/J (H-2K^k, D^k) mice were removed, freed of meninges, and disaggregated by gentle repetitive pipetting. Approximately 1.5×10^7 cells were recovered from each brain with viability of 85%. Cells were cultured in N5 medium that contained 5% neurotrophic factor from horse serum (18). After 48 h, cells were treated with 10 U of IFN (α/β , β , or astrocyte) or left untreated (uninduced). After 48 h of IFN treatment, the cells were stained with monoclonal antibodies against H-2K^k, H-2K^d, or Ia antigens. The percentage of cells expressing MHC antigens was determined by counting $\sim 1,000$ cells, and each mean (\pm SD) was based on four separate determinations.

Inducible Expression of H-2 Antigens on Brain Cells In Vitro

Since brain cells and many other cell types respond to IFN- γ by enhancing levels of H-2, and in some cases Ia antigens (41, 42), we examined brain cells to determine whether MHC antigen expression could be induced by astrocyte IFN, IFN- α/β , or IFN- β (Table III). Single cell suspensions were prepared from 1-d-old CBA/J mice and then treated with different concentrations of IFN (1–500 U/ml). After 48 h of exposure to IFN, the expression of MHC antigens was determined by indirect immunofluorescence staining using monoclonal antibodies against H-2 and Ia antigens. Treatment with astrocyte IFN or IFN- α/β caused expression of H-2 antigens on $\sim 25\%$ of the brain cell population, whereas treatment of cells with IFN- β showed H-2 levels similar to uninduced brain cell controls (Table III). Detection of the observed fluorescence was specific for H-2 antigens in that monoclonal antibodies (13-1-2S), specific for a different H-2 haplotype (H-

2K^d, D^d), did not bind to brain cells of CBA/J (H-2K^k, D^k) mice. None of the IFN treatments stimulated the expression of Ia antigens.

Identification of Cell Types That Respond to Astrocyte IFN

To determine which CNS cell types respond to astrocyte IFN, we double-labeled cells using anti-H-2 or Ia monoclonal antibodies together with cell-type-specific antibodies that identify the major CNS cells.

In neuronal cultures (4 wk old), $>95\%$ of the cells are neurons and the remaining 5% of the cells are process-bearing astrocytes (Fig. 2). When neuronal cultures were treated with 1, 10, 50, 100, 500, or 1,000 U of astrocyte IFN, IFN- α/β , or IFN- β , no detectable levels of H-2 or Ia antigens were found on neurons (Fig. 3). However, when we double-labeled the population (5%) of process-bearing astrocytes in these cultures, $\sim 45\%$ of the astrocytes showed enhanced expression of H-2 antigens in response to treatment with either astrocyte IFN or IFN- α/β (Table IV, Fig. 4, *a-c*). This selective response of a subpopulation of astrocytes to IFN- α/β or astrocyte IFN is in contrast to the action of IFN- γ which has been reported to enhance the expression of H-2 antigens on all CNS cell types (41, 42).

Since the antiviral activity of astrocyte IFN and IFN- α/β in the CPE assay was neutralized by anti-IFN- α/β , but not by anti-IFN- γ antibodies, we tested whether the MHC-inducing activity showed a similar neutralization with these antisera (Fig. 4, *d-i*). H-2 inducing activity of astrocyte IFN was effectively neutralized by anti-IFN- α/β (Fig. 4, *d-f*), but not by anti-IFN- γ (Fig. 4, *g-i*) antibodies. Anti-IFN- α/β also neutralized the H-2 inducing activity of IFN- α/β (not shown). No detectable Ia staining was observed on cells treated with any of the IFN classes (Table IV). Thus it appears that astrocyte IFN and IFN- α/β enhance the expression of H-2 antigens on a subpopulation of astrocytes within the CNS.

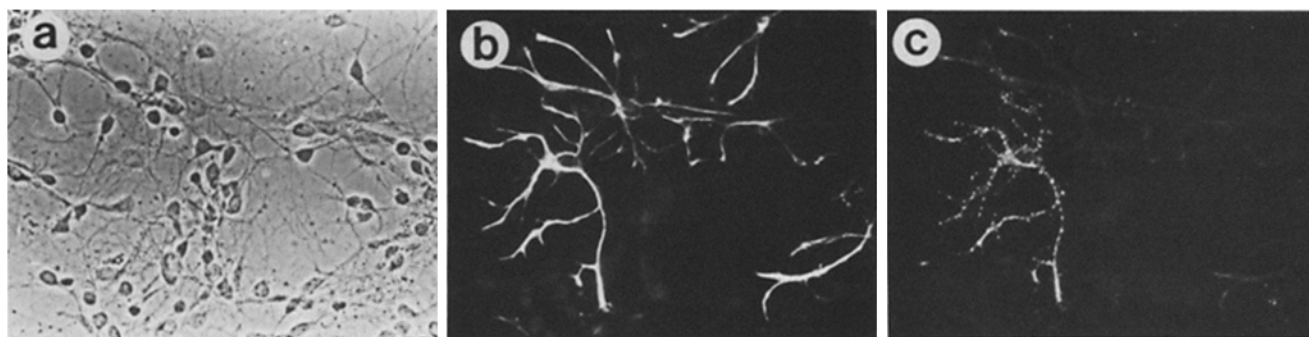
Failure of H-2 process-bearing astrocytes to express MHC antigens could result from a suppressive trophic response from the neuronal population. To test this possibility, we cultured pure populations of astrocytes from cerebral cortices of embryonic mice, induced them with astrocyte IFN, IFN- α/β , and IFN- β , and then determined the expression of H-2 and Ia antigens by immunohistochemical staining (Fig. 5, Table IV). Astrocytes cultured for 1–3 wk did not respond to IFN induction with enhanced MHC antigen expression. Cells in

Table IV. Percentage of Astrocytes Expressing MHC Antigens

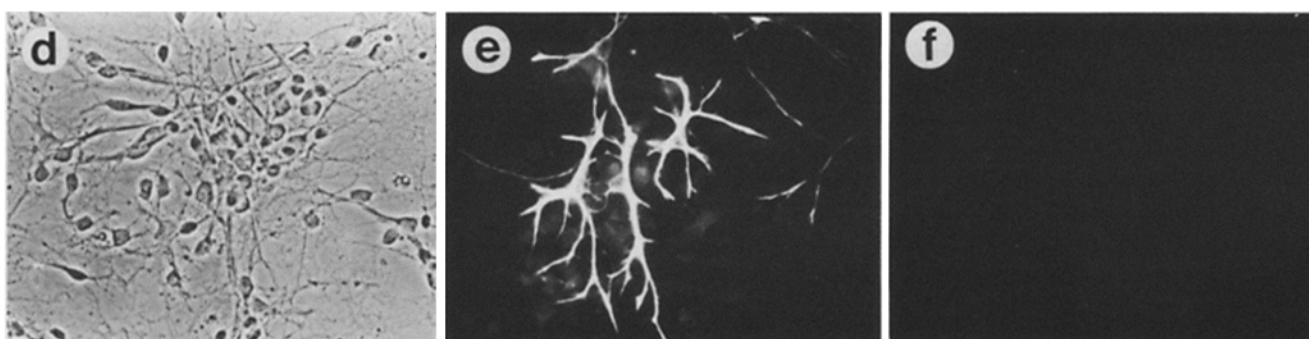
Treatment	Astrocyte Morphology			
	Process-bearing		Sheet-like	
	H-2	Ia	H-2	Ia
Astrocyte IFN	41 \pm 3	0	43 \pm 2	0
IFN- α/β	45 \pm 4	0	48 \pm 4	0
IFN- β	0	0	0	0

Astrocytes from cerebral cortices (sheet-like) and astrocytes in neuronal cultures (process-bearing) were prepared as described in Materials and Methods. Cells were grown for 5 wk and then treated with 10 U of IFN (astrocyte, α/β , or β) for 48 h. Cells were then double-labeled with rabbit anti-GFAP and monoclonal antibodies to H-2 or Ia antigens. The percentage of GFAP⁺ cells that express MHC antigens was determined by counting ~ 200 cells. Values are expressed as the mean (\pm SD) of four separate experiments.

Astrocyte IFN



Anti-IFN- α/β



Anti-IFN- γ

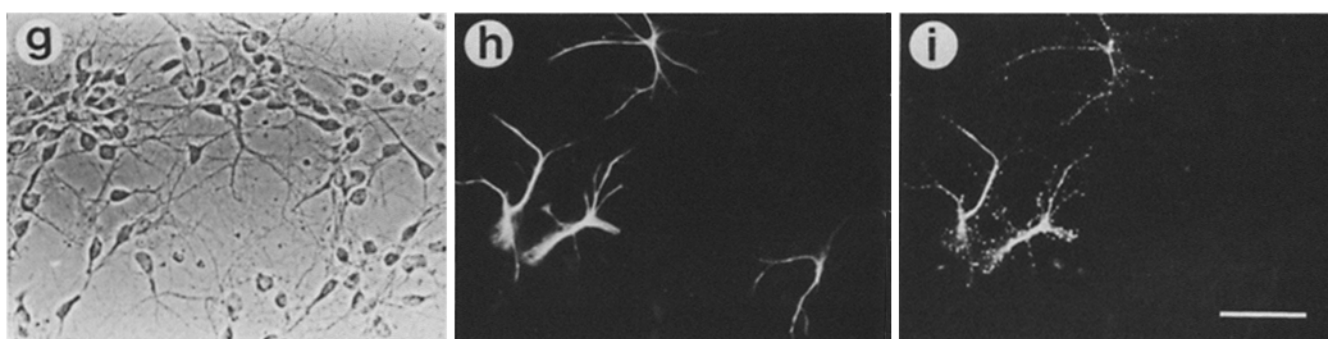
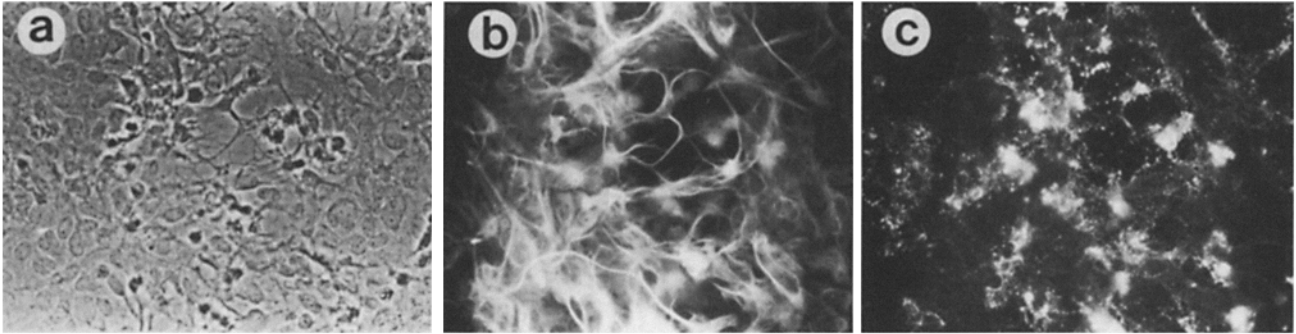


Figure 4. (a-c) Photomicrographs of astrocytes present in neuronal cultures that were treated with 10 U of astrocyte IFN for 48 h. (a) Phase-contrast; (b) GFAP staining; (c) H-2 staining. Approximately 45% of the process-bearing astrocytes in neuronal cultures were H-2⁺. (d-i) Neutralization of H-2 inducing activity of astrocyte IFN with anti-IFN- α/β (d-f) or anti-IFN- γ (g-i) antiserum. 10 U of astrocyte IFN was incubated with 500 neutralizing U/ml of anti-IFN- α/β or anti-IFN- γ antiserum for 3 h at 37°C. The antigen-antibody mixture was then added to cultures for 48 h. Astrocytes were stained with antibodies to GFAP (e and h) or H-2K^b antigens (f and i). Anti-IFN- α/β antiserum effectively neutralized the H-2 inducing activity (d-f) of astrocyte IFN, whereas anti-IFN- γ antiserum did not (g-i). Bar, 20 μ m.

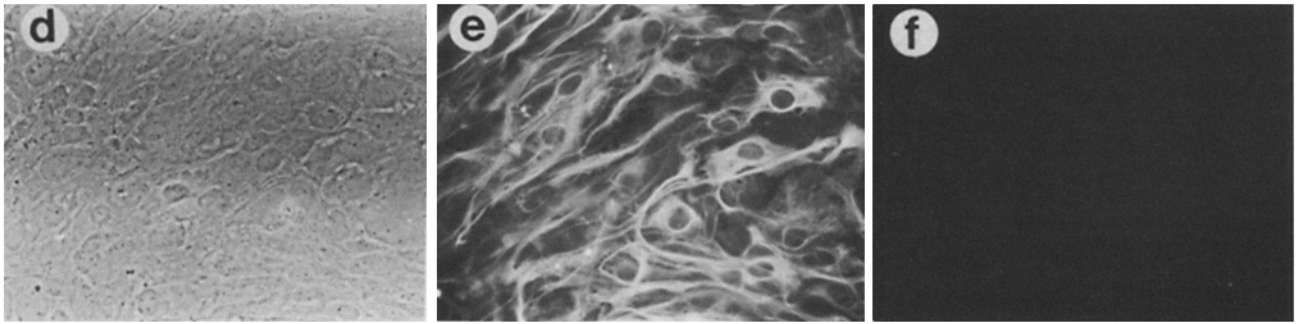
these cultures formed a confluent monolayer that consisted of two populations of astrocytes: those that stained intensely with anti-GFAP antibodies and cells that showed diffuse GFAP staining. By 3-4 wk of culture all of the cells stained

intensely with anti-GFAP antibodies. Induction of older cultures (4-6 wk) with astrocyte IFN or IFN- α/β resulted in ~40% of the astrocytes expressing detectable H-2 staining (Table IV).

Astrocyte IFN



Anti-IFN- α/β



Anti-IFN- γ

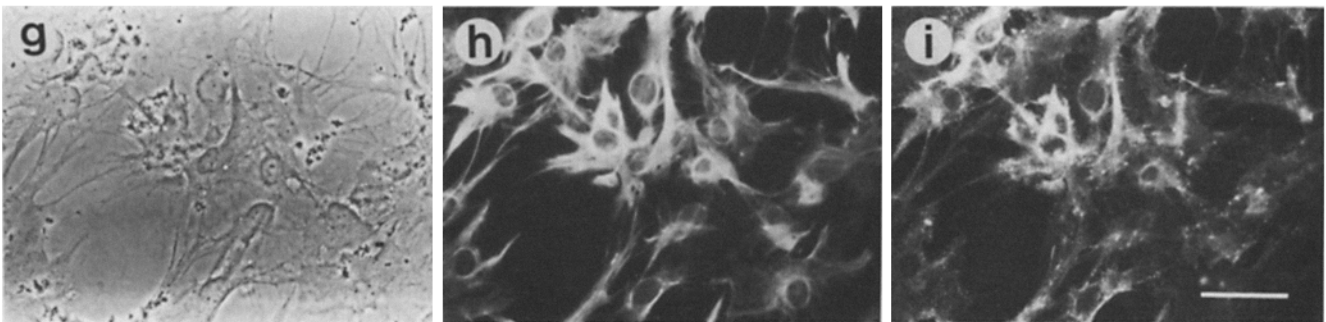


Figure 5. (a-c) Phase-contrast micrograph (a) of 5-wk-old cortical astrocyte cultures treated with 10 U of astrocyte IFN for 48 h and stained with anti-GFAP (b) and anti-H-2K^k (c) antiserum. (d-i) Neutralization of H-2 inducing activity of astrocyte IFN with anti-IFN- α/β (d-f) or anti-IFN- γ (g-i) antiserum. Neutralization assays were done as described in Fig. 4, d-f legend and then cortical astrocyte cultures were stained with antibodies to GFAP (e and h) or H-2K^k (f and i) antigens. Note that while enhancing H-2 antigens (c and i), astrocyte IFN also caused disorganization of GFAP filaments (b and h). Bar, 20 μ m.

While effectively enhancing the expression of H-2 antigens, IFN- α/β and astrocyte IFN caused alterations in the cytoskeletal arrangement of GFAP filaments (Fig. 5, b and h). The MHC inducing activity was neutralized by anti-IFN- α/β

(Fig. 5f), but not by IFN- γ antibodies (Fig. 5i). None of the IFNs induced the expression of Ia antigens on astrocytes (Table IV).

To determine whether the other major glial cell population,

the oligodendroglia, showed enhancement of H-2 or Ia antigens in response to astrocyte IFN, we treated oligodendrocytes with 10 U of astrocyte IFN for 48 h and then double-labeled the cells with rabbit antibodies to the oligodendrocyte-specific marker, galactocerebroside and with monoclonal antibodies to H-2 or Ia antigens (Fig. 6, *a-c*). None of the oligodendrocytes examined showed detectable levels of H-2 or Ia antigens after exposure to astrocyte IFN (Fig. 6*c*).

Inducible expression of H-2 Antigens in the Brain

We attempted to test whether the H-2 induction observed *in vitro* had relevance to a physiological response *in vivo*. Astrocyte IFN, IFN- α/β , IFN- β , or supernatants from uninduced astrocyte cultures that exhibit no IFN activity were injected into cerebra of 1-d-old CBA/J mice. After different periods of treatment, brains were dissected, the meninges removed, and the tissue was dispersed into single cell suspensions. The cells were then stained for H-2 and Ia expression. Fig. 7 shows the time courses of the percentage of brain cells that express H-2 antigens. Approximately 20% of the brain cells that received IFN- α/β or astrocyte IFN were found to express H-

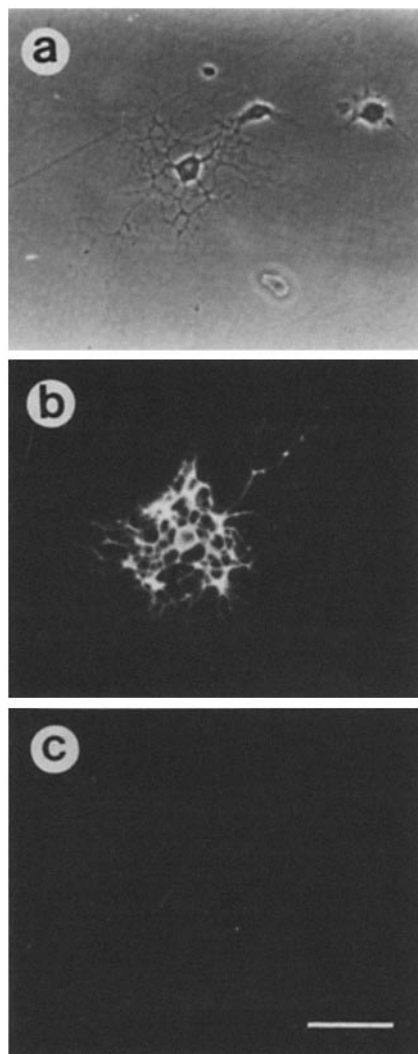


Figure 6. (*a-c*) Photomicrograph of an oligodendrocyte from embryonic mouse brain treated with 10 U of astrocyte IFN. (*a*) Phase-contrast; (*b*) GC staining; (*c*) H-2 staining. Bar, 20 μ m.

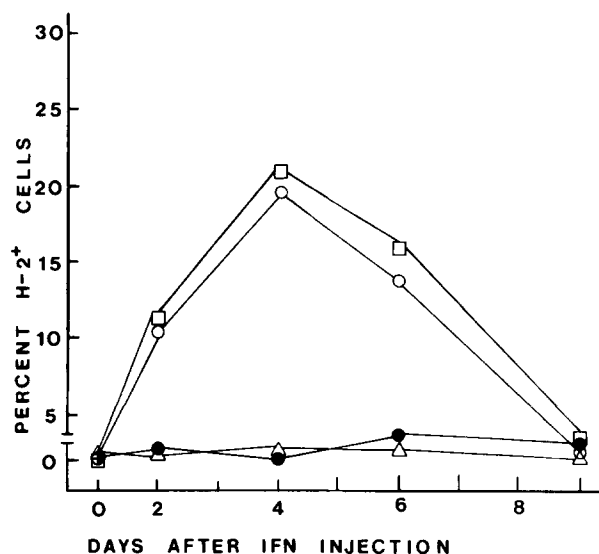


Figure 7. Expression of H-2 antigens on brain cells *in vivo*. Brains of 1-d-old CBA/J mice were injected with 50 μ l (1 U/ μ l) of IFN- α/β (\square), IFN- β (Δ), or astrocyte IFN (\circ), or control supernatant that lacked IFN activity (\bullet). After different times, single cell suspensions were prepared and stained with monoclonal antibodies against H-2K^k (14-4-4S). The percentage of H-2⁺ cells was determined by counting \sim 1,000 cells per time point.

2 antigens by 2 d after treatment. The percentage of H-2⁺ cells decreased so that by 7 d after IFN injection into brains, few, if any, positive cells were present. Cells from brains injected with IFN- β or control supernatants did not express detectable levels of H-2 antigens.

To determine whether cells in different brain regions expressed H-2 or Ia antigens in response to IFN, we made fresh-frozen cryostat sections from brains injected with IFN- α/β or astrocyte IFN and from brains injected with supernatants from uninduced astrocyte cultures that show no IFN activity (Fig. 8). H-2⁺ staining was detected on groups of cells scattered throughout the frontal lobes and on cells lining the ventricles in brains injected with astrocyte IFN or IFN- α/β . No staining was observed in cerebellum or brain stem regions or in control sections of brains which were injected with supernatants from uninduced astrocyte cultures. Thus, it appears that H-2 antigens can be induced on CNS cells *in vivo* by astrocyte IFN.

Discussion

Our results demonstrate that astrocytes, but not neurons, can produce IFN that is both biologically and antigenically similar to IFN- α/β . Treatment of CNS cells with astrocyte IFN enhances the expression of H-2 antigens on a subpopulation of astrocytes. This finding may have particular relevance to the generation of CNS immune responses. For example, cytotoxic T cells are actively involved in certain brain lesions and in the pathology of certain viral infections of the brain. Since there is little or no expression of MHC antigens on normal CNS cells (8), it might be expected that virally infected brain cells could escape recognition and destruction by cytotoxic T cells, which can recognize viral antigens only in association with cellular MHC antigens (44). Since CNS cells show enhanced expression of MHC antigens in response to IFN, and the peripheral IFN classes (α , β , and γ) do not

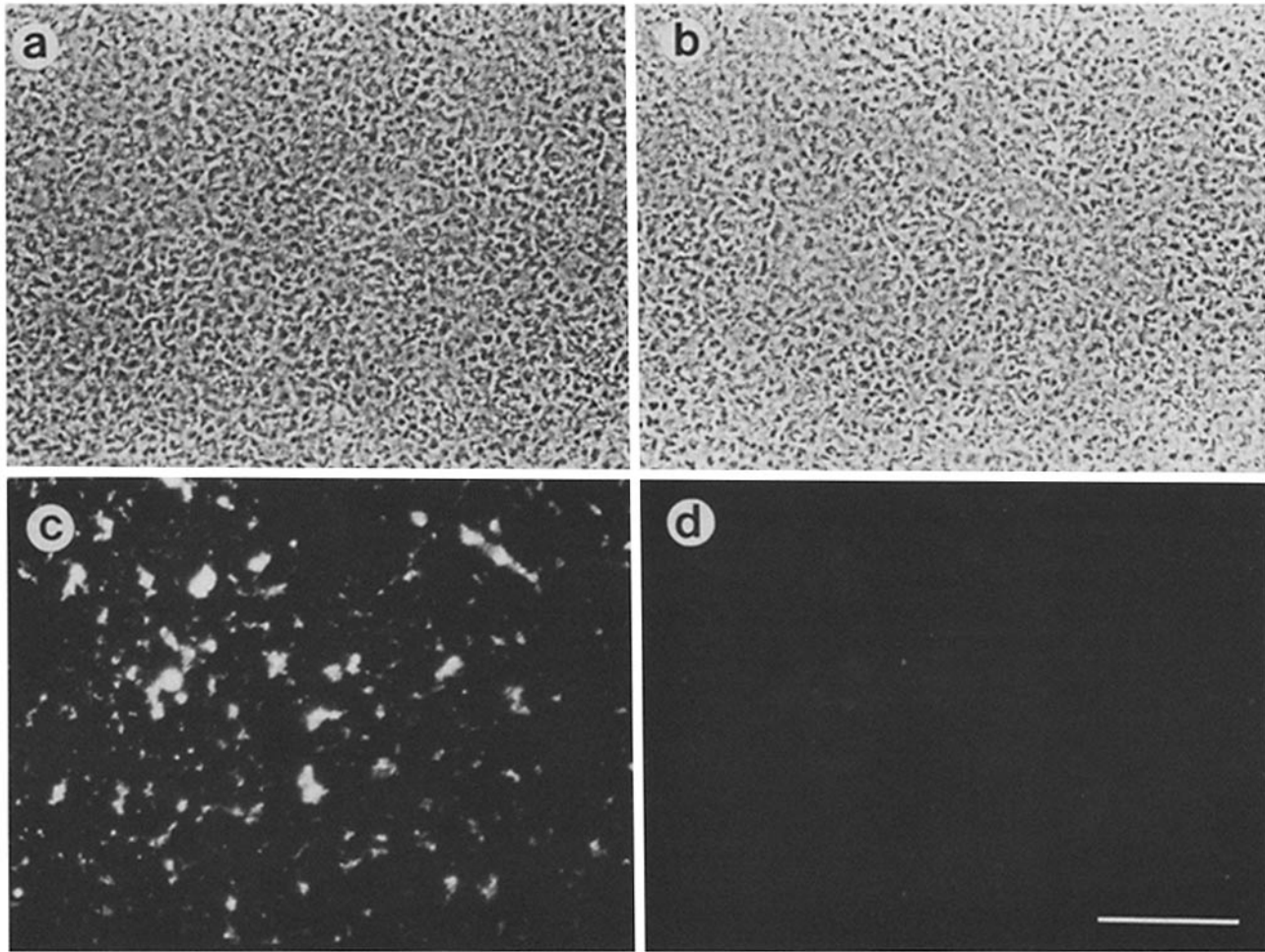


Figure 8. Cryostat sections of CBA/J frontal lobes injected with 50 U of astrocyte IFN (*a* and *c*) or with control supernatants that lacked IFN activity (*b* and *d*). (*a* and *b*) Phase-contrast micrographs and (*c* and *d*) H-2K^k staining. Bar, 20 μ m.

appear to cross the blood-brain barrier (5, 22, 30, 35), IFN produced by astrocytes may play an important role in CNS immune responses by enhancing the expression of MHC antigens on previously immunologically incompetent brain cells, which then become competent to participate in T cell-mediated responses. This mechanism may contribute to CNS degenerative diseases such as multiple sclerosis, where T cells infiltrate the CNS (7, 38).

The brain has been shown to produce high levels of IFN when injected with viruses (1, 35) or poly rI-rC (6). Within the brain there also appears to be compartmentation of IFN. Luby and co-workers (25) found that patients dying from St. Louis encephalitis had IFN in frontal lobes, cerebellum, basal ganglia, but not brain stem. Marked differences in IFN content between certain areas of the brains infected with Western equine encephalitis have also been reported (24). It is possible that astrocytes in different locations of the brain produce IFN, thus accounting for the compartmentation of IFN observed in CNS infections and diseases. We are currently investigating whether astrocytes from different CNS locations can be induced to produce IFN activity.

Although astrocyte IFN and IFN- α/β share many biological, physicochemical, and antigenic properties, further neutralization assays with antisera against subclasses of IFN- α/β are required to determine whether these are identical IFNs

or different subclasses of IFN- α/β . All types of IFN genes have been cloned. There is a single IFN- β gene (16) and IFN- γ gene (12), whereas from Southern blot analysis on chromosomal DNA the number of IFN- α genes was estimated to be at least 9–11 (33, 45). Since there are multiple subclasses of IFN- α/β , it is possible that astrocytes exclusively produced one IFN subtype that is not expressed by other cells of the body.

Tamm and co-workers (39) have reported IFN- β disorganizes the microtubule and 10-nm filament network in a subpopulation of macrophages. Microtubules from the peripheral cytoplasm coiled around the nucleus without evidence of depolymerization after IFN- β treatment. We have found that IFN- α/β and astrocyte IFN treatment of astrocytes results in the disorganization of intermediate GFAP filaments, and we have obtained data that prolonged astrocyte IFN treatment of neurons leads to disorganization of neurofilaments in neurons (data not shown). Whether these intermediate cytoskeletal alterations affect cellular functions is unclear, but these observations suggest a possible explanation for the production of neurofibrillary tangles and neuritic plaques in Alzheimer's and Parkinson's diseases (37).

CNS cells have been categorized by morphological and antigenic criteria (27). Two broad classes of astrocytes, protoplasmic and fibrous, differ in their antigenic phenotype,

developmental history, morphology, and location in the CNS. We have demonstrated that cells within each of these broad astrocyte categories can be further subdivided based on their ability to express H-2 antigens in response to IFN treatment. Whether these H-2⁺ astrocytes are functionally different from H-2⁻ astrocytes remains to be determined, but clearly the CNS probably contains many more astrocyte subtypes than previously realized.

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