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Immunology

Weak HLA and β_2-microglobulin expression of neuronal cell lines can be modulated by interferon

(Ia molecules/immunoblot/major histocompatibility complex/monoclonal antibodies/neuroblastoma)

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ABSTRACT **Previous work showed that each of four human neuronal cell lines expresses ≤0.5% of the HLA-A,B,C and β_2-microglobulin seen in glial, lymphoid, and other cell types, and there is a corresponding weak expression in neuroblastoma tumor and adult brain. Here, we probe the genetic basis of this weak expression. For each of three neuroblastoma cell lines, we show that HLA-A,B,C and β_2-microglobulin can be induced by interferon and that the induction occurs within every cell of the population. Class II (Ia) molecules are not detected. Microscopic assay and radioimmunoassay of intact cells suggest that the induced antigen appears at the cell surface as well as within each cell. Immunoblot analysis confirms that the induced proteins have the structure of class I molecules. Thus, the normal weak HLA and β_2-microglobulin expression of these cell lines appears to reflect a regulatory control rather than a primary genetic lesion. According to current theory, lack of HLA-A,B,C should protect transformed, infected, or damaged neurons—but also neurons in neural transplants—from T-cell-mediated immunosurveillance. The possibility that neuronal HLA-A,B,C expression may be under regulatory control is of importance in this context.**

The HLA-A,B,C molecules are highly polymorphic widely distributed products of the major histocompatibility complex (MHC), whose known physiological role is to mediate the interaction between cytotoxic T lymphocytes and antigen-bearing target cells (1). Previous work shows that HLA-A,B,C is expressed weakly, at best, in human brain and cell lines and tumors of neuronal origin (2–4, 28). According to current theory, the implication of this weak expression is that damaged, infected, transformed, or transplanted neurons may be protected from T-cell-mediated surveillance, even if they bear tumor-specific, viral, or other foreign antigens (1, 5). However, the genetic basis of the weak neuronal HLA-A,B,C expression is not known. The aim of this study was to determine whether the observed weak expression reflects a primary genetic lesion or, rather, regulatory control. Here, we present evidence that, for each of three neuronal cell lines, HLA-A,B,C expression is under regulatory control and can be induced by γ interferon (IFN-γ).

The cell lines examined here are derived from neuroblastoma, a major tumor of early childhood that arises from the sympathetic neuroblast (6). The lines express ≤0.1–0.5% of the HLA-A,B,C and β_2-microglobulin (β_2-m) seen on lymphoid cells and many other cell types (4). Our probes included monoclonal antibodies to β_2-m, the invariant chain of HLA-A,B,C, and other class I molecules, and to an invariant determinant on native HLA-A,B,C molecules, w6/32 (Table 1).

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MATERIALS AND METHODS

Cell Lines. Neuroblastoma cell lines IMR-5, NMB, and CHP-126 were grown in standard medium (RPMI 1640/5% calf serum/2% fetal calf serum/glutamine/penicillin/streptomycin for IMR-5 and NMB; Dulbecco's modified Eagle's medium/10% fetal calf serum/glutamine/penicillin/streptomycin for CHP-126) (4). At time 0, half of the medium in each flask was replaced with fresh medium containing *Escherichia coli*-derived human IFN-γ, the gift of Genentech, at 2000 units/ml, or fresh medium alone. After 48 hr, the cells were harvested with a rubber policeman. Cells to be used for binding assays were then fixed with 0.1% glutaraldehyde for 5 min at room temperature (4). Cells to be used for immunoblot analysis were extracted with Nonidet P-40 (NP-40) (4).

Antibodies. Antibodies used in this study are listed in Table 1.

Assays. Radioimmunoassay (RIA) was performed against fixed cell pellets (250,000 cells per well) in microtiter plates, using ^{125}I-labeled rabbit antibody to mouse Fab fragment as the detecting reagent (4). For microscopic assay, fixed cells were embedded in agarose, and 50-μm Vibratome sections were stained by the unlabeled antibody peroxidase-antiperoxidase (PAP) technique (4). For immunoblot assay, NP-40 extracts were electrophoresed on sodium dodecyl sulfate/7% or 13% polyacrylamide slab gels and transferred electrophoretically to nitrocellulose. Strips cut from the nitrocellulose were assayed individually with monoclonal antibody, followed by ^{125}I-labeled rabbit antibody to mouse Fab fragment, and then reassembled for autoradiography (13, 29).

RESULTS

Fig. 1 shows RIAs of three neuroblastoma cell lines that had been grown for 48 hr in standard medium, with or without the addition of IFN-γ. There is a clear increase in both β_2-m and w6/32 activity of each IFN-treated cell line, as compared to the untreated control (Fig. 1 *A–F*). This assay uses whole, fixed cells, suggesting that the measured increase is due to cell surface antigens. In the same assays, no increase in a second class of MHC products, the class II or Ia molecules, was revealed (Fig. 1 *G–I*). Thus, the IFN-γ has not induced expression of all MHC products, or all proteins. The B-cell line Daudi, which lacks the normal gene for β_2-m (14, 15), was included as a specificity control in these studies. As expected (15), even after IFN treatment, neither β_2-m activity nor w6/32 activity (which depends upon the presence of β_2-m) was increased in the Daudi cells (Fig. 1 *J* and *K*).

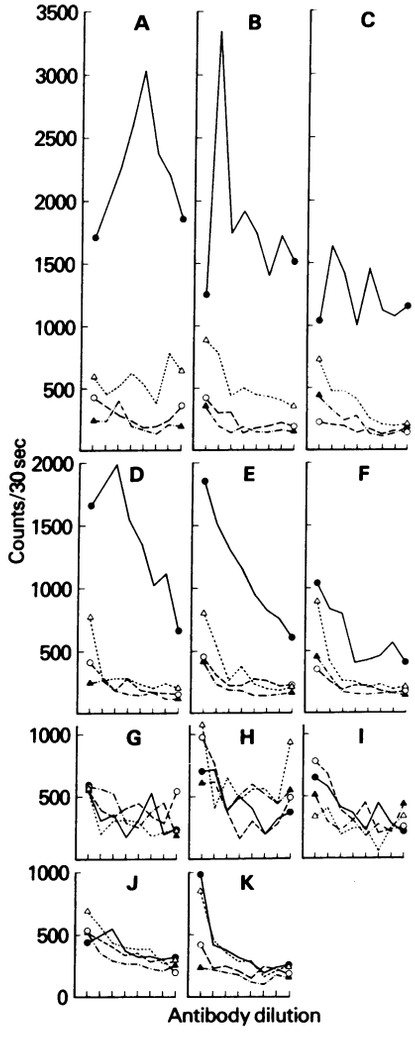
To examine the distribution of the antigenic activity within the induced populations, a microscopic assay was used. Cells were prepared as for RIA, but embedded in agarose blocks, which were then cut into 50-μm sections for assay by

Abbreviations: MHC, major histocompatibility complex; IFN, interferon; β_2-m, β_2-microglobulin; NP-40, Nonidet P-40; PAP, peroxidase-antiperoxidase.

Table 1. Antibody panel

Antibody (source)	Isotype and description	Specificity	Refs.
L368	γ1 mouse monoclonal, used as culture supernatant	β_2-m, the invariant chain of all HLA-A,B,C molecules, and some structural analogs	4, 7
BBM.1 (American Type Culture Collection)	γ2b mouse monoclonal, used as culture supernatant	As for L368	8
Anti-β_2-m serum (Dako-Immunoglobulins)	Conventional polyclonal rabbit antiserum	As for L368	4
W6/32 (Sera-Lab)	γ2a mouse monoclonal, used as serum/ascites	Invariant determinant on native two-chain HLA-A,B,C molecules; does not cross-react with known analogs	7, 9, 10
"anti-HLA" (Cappel Laboratories 0201-2027)	γ1 mouse monoclonal, used as purified immunoglobulin	Invariant determinant on HLA chain; cross-reactivity with known analogs not defined	29
L203	γ1 mouse monoclonal, used as culture supernatant	Invariant determinant on human class II (Ia) molecules	11
C4 (gift of J. Lessard)	γ1 mouse monoclonal, used as purified immunoglobulin	Interspecies determinant on actin	12

the unlabeled antibody PAP technique. These assays revealed that β_2-m and w6/32 activity was increased in every cell in the population and was increased within each cell as well as at the periphery (Fig. 2). Thus, the increase in antigenic activity cannot be wholly ascribed to changes in cell size or shape, to surface antigenic modulation, or to adsorption of antigens that had been secreted by a small subpopulation of cells or were present in the medium. Although the results shown were obtained with *E. coli*-derived recombinant IFN-γ, similar results were also obtained with a natural IFN-γ (Interferon Sciences, New Brunswick, NJ). Studies of IFN-mediated MHC induction in other cell types have shown that synthesis of MHC proteins and mRNA are both increased in IFN-treated populations (16, 17). Here, we complement the previous analyses by showing that MHC expression is increased within each cell. Again, no evidence for class II activity was seen in either IFN-treated or control neuroblastoma cells.



To examine the structure of the induced proteins, an immunoblot assay was used (Fig. 3). Here, the IFN-treated cells showed a strong increase in expression of a polypeptide that reacted with each of three antibodies to β_2-m and migrated with the β_2-m from lymphoid cells (Fig. 3 *C* and *D*). Similarly, the IFN-treated cells showed a strong increase in expression of a polypeptide that reacted with a monoclonal antibody to a nonpolymorphic determinant on HLA chains and migrated with the HLA chains from the lymphoid control. This polypeptide was shown to be distinct from actin, a major cellular component of molecular weight similar to that of HLA chains (Fig. 3 *A* and *B*). The actin activity did not appear to be increased in the IFN-treated cells. These results confirm that both component chains of HLA-A,B,C or multiply cross-reactive structural analogs have been specifically induced in the IFN-treated cells. The anti-HLA antibody also defined a specific band in the IFN-treated neuroblastoma cells that was of lower molecular weight than the lymphoid HLA chain. Whether this represents a precursor, a breakdown product, or a novel class I product is not yet known.

The antibody specificities used here include those used previously to detect "HLA" induction in melanoma and other IFN-treated cells (16). The increased activity of W6/32 against whole fixed cells in RIA and at the periphery in the microscopic assays suggests that native two-chain HLA-

FIG. 1. RIA. Neuroblastoma cell lines IMR-5, NMB, and CHP-126 were grown in standard medium. At time 0, half of the medium in each flask was replaced with fresh medium plus IFN-γ to a final concentration of 1000 units/ml, or fresh medium alone. After 48 hr, the cells were harvested and fixed. Antibodies used were as follows: *A–C* and *J*, L368; *D–F* and *K*, W6/32; *G–I*, L203. Antibody reactivities are summarized in Table 1. The negative control for L368 and L203, which were used as culture supernatants, was P3/X63 spent medium; the control for W6/32, which was used as serum/ascites fluid, was normal mouse ascites fluid. Antibodies were tested at 2-fold serial dilutions. Initiation dilution for culture supernatants (*A–C*, *G–J*) was 2-fold; initial dilution for serum/ascites fluid (*D–F*, *K*) was 10-fold of supplier's initial preparation. ●, Antibody tested against IFN-treated cells; ○, negative control with IFN-treated cells; △, antibody against untreated cells; ▲, negative control with untreated cells. Cells used as targets were as follows: *A*, *D*, and *G*, IMR-5; *B*, *E*, and *H*, NMB; *C*, *F*, and *I*, CHP-126; *J* and *K*, Daudi.

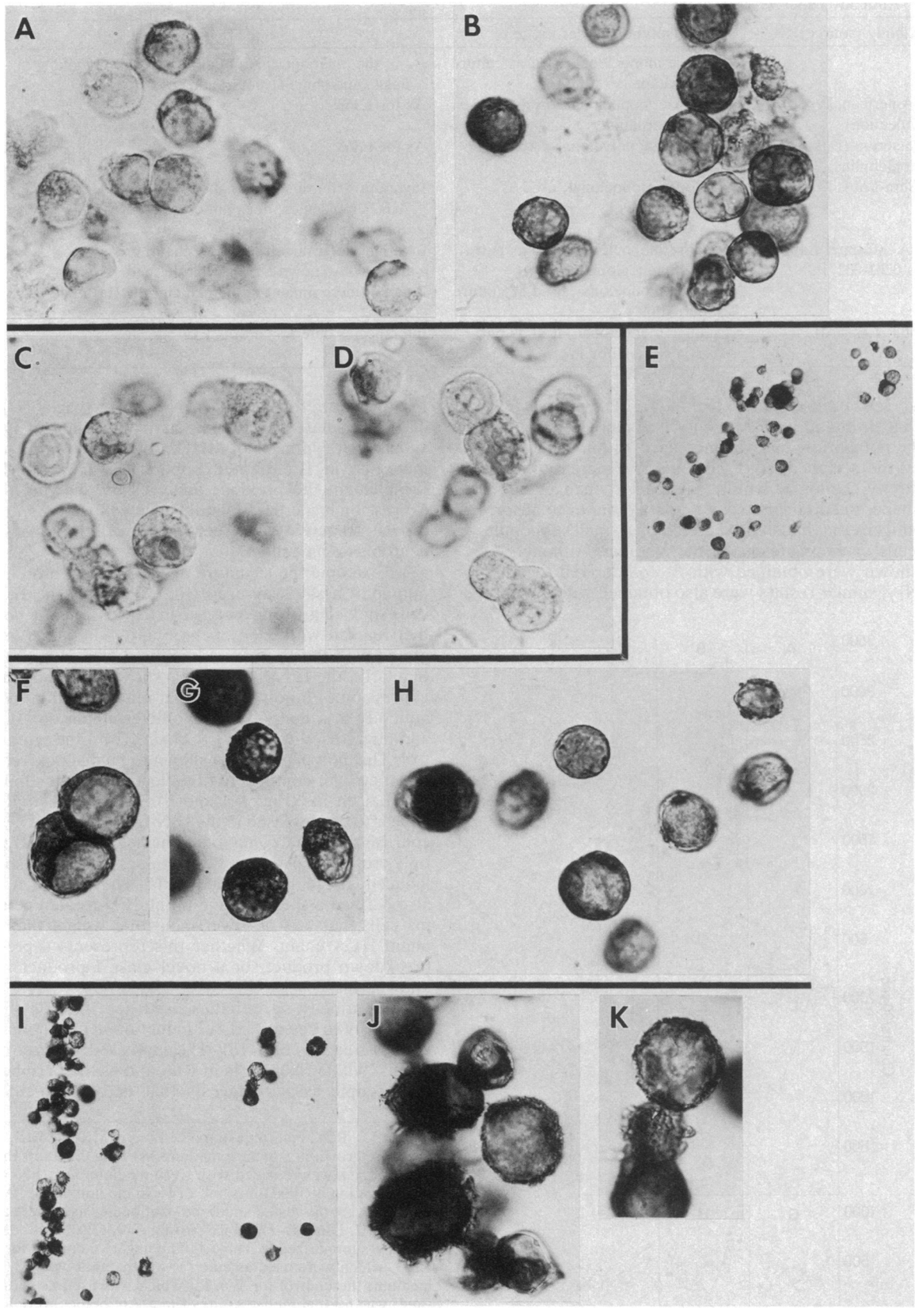


FIG. 2. Microscopic assay. IFN-treated and control cells were prepared as for Fig. 1. The fixed cells were embedded in agarose and assayed as 50-μm Vibratome sections by the PAP technique (4). Description of the antibodies is in Table 1. (*A*) Untreated IMR-5 cells, assayed with L368; (*B*) a human B-cell line, Raji, assayed with L368, used as a positive control; (*C* and *D*) IFN-treated IMR-5 cells assayed with P3/X63 spent medium; (*E–H*) IFN-treated IMR-5 cells, assayed with L368; (*I–K*) a second IFN-treated cell line, NMB, assayed with L368. Similar results were obtained with W6/32 and with the third neuroblastoma cell line, CHP-126. [Cells were photographed using D.I.C. (Nomarski) optics. Original magnifications of *E* and *I* were ×200 and those of other panels were ×1000. Photographs are printed at 67% of original.]

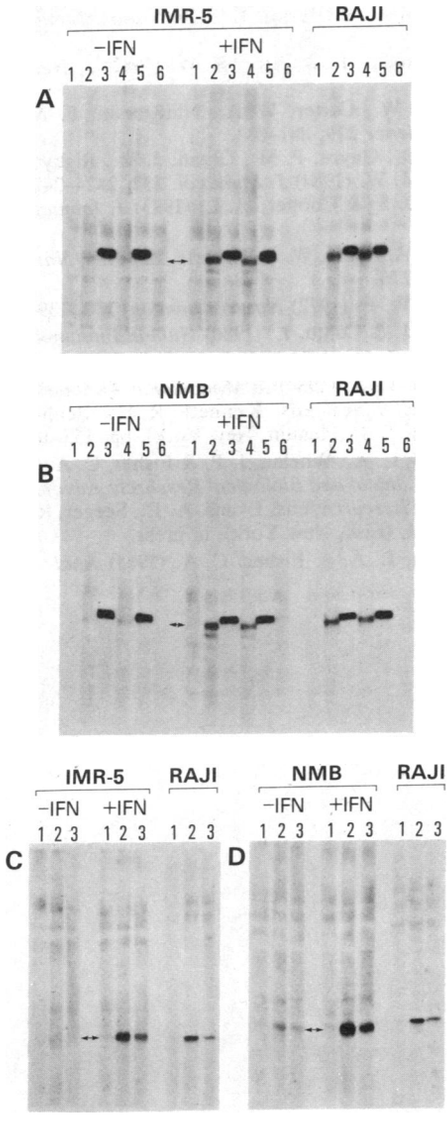


FIG. 3. Immunoblot assay. NP-40 extracts from IFN-treated and control cells were electrophoresed on sodium dodecyl sulfate/7% (*A*, *B*) or 13% (*C*, *D*) polyacrylamide slab gels and transferred to nitrocellulose. Strips cut from the nitrocellulose were assayed individually with monoclonal antibody. Antibodies used in *A* and *B*: lanes 2 and 4, "anti-HLA" (Table 1); lanes 3 and 5, C4 (anti-actin); lanes 1 and 6, negative controls. Antibodies used in *C* and *D*: lane 1, P3/X63 spent medium; lane 2, L368; lane 3, BBM.1. Similar results were obtained with a rabbit anti-β_2-m serum. Extracts from the B-cell line Raji were included as positive controls on each gel. The results shown in this figure were also obtained with CHP-126.

A,B,C molecules are being expressed at the cell surface (10). Direct analysis of polymorphic specificities is needed to confirm this; further studies will also reveal whether other β_2-m-associated proteins are induced as well.

We have observed increased HLA and β_2-m expression in neuroblastoma cell lines treated with IFN-γ from both natural and recombinant DNA sources, at doses from 10 to 10^4 units/ml, and at times from 1 to 5 days after IFN addition. In nine experiments, the mean viability of both IFN-treated and control cells was 91% at 48 hr after IFN addition; in six experiments, the cell yields from IFN-treated cultures were 110% of those from controls. The control cells showed at best weak HLA-A,B,C and β_2-m activity (Figs. 1–3) at all times during culture, including 10 days after plating. Thus, the IFN-induced changes in HLA and β_2-m expression cannot be ascribed to general changes in viability or metabolic rate.

In the microscopic assays shown in Fig. 2, the neuroblastoma cells had been harvested before fixation, and so assumed a rounded form. In complementary experiments with cells that had been fixed as they grew on coverslips, the increased antigenic expression was observed in all morphological forms and throughout cell bodies and processes. The IFN treatment did not appear to shift the populations towards more differentiated neuronal forms (unpublished observations).

DISCUSSION

IFN-γ has been shown to induce HLA-A,B,C expression in many different cell types, including cell lines derived from melanoma, which, like neuroblastoma, is a tumor of the neural crest (16). The parameters of dose and timing are consistent with those reported here. Different forms of interferon have been shown to affect excitability (18), or inhibit viral growth (19), in neuronal cultures. Our finding of continued cell growth 48 hr after IFN addition is consistent with previous results with neuronal cultures and melanoma; we do not know whether cytostatic or cytotoxic effects would be seen at later time periods or with other forms of IFN (16, 18–20). Although our findings are consistent with previous work, the demonstration that MHC products can be modulated in cells of neuronal origin is of particular interest because of the low initial neuronal HLA-A,B,C expression, as compared to other cell types that have been examined (4).

The demonstration that weak HLA and β_2-m expression in neuroblastoma cell lines reflects a regulatory control, rather than a primary genetic lesion, is of interest *per se* in the context of understanding potential mechanisms of MHC regulation. Taken together with recent work from other laboratories, our findings support a developing picture of plasticity in MHC expression in a variety of cell types (21–23).

Both neuroblastoma tumor and normal brain resemble the neuroblastoma cell lines in their paucity of class I expression (28). If class I modulation occurs, or can be induced, in neural tissues *in vivo*, this would be of interest in several contexts. An *in vivo* modulation of neuronal HLA-A,B,C might enable neurons, which are normally sequestered from immunocompetent cells by a blood–tissue barrier, to participate in T-cell-mediated responses at appropriate times, for example, during inflammation. This model is consistent with recent evidence for modulation of class II expression on astrocytes after specific contact with T lymphocytes (23). From a practical viewpoint, tumors, such as neuroblastoma, with weak but inducible HLA-A,B,C expression might be particularly appropriate targets for therapies that can modulate MHC levels. In a nonimmunological context, a temporal change in expression of these cell surface proteins might mediate other kinds of cellular interaction in neural tissue (24–27). Although currently speculative, these possibilities are amenable to direct testing; the microscopic analysis and antibody panel used here will help to evaluate them in complex tissue.

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