Coexpression of Neuronal, Glial, and Major Histocompatibility Complex Class II Antigens on Retinoblastoma Cells

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ABSTRACT

This study identifies the presence of major histocompatibility complex class II antigens on retinoblastoma cells. In addition, the modulation of HLA-DR by interferon-γ as well as the preferential expression of this major histocompatibility complex molecule over HLA-DQ is described. Double labeling experiments revealed that HLA-DR antigen is shared concomitantly with cells of glial and neuronal character. Investigations such as these underscore the possibility that expression of major histocompatibility complex class II antigens may function as immunological components in the host or play a role in the cellular differentiation of these tumor cells.

INTRODUCTION

MHC class II antigens (HLA-DR, HLA-DQ, and HLA-DP) are integral membrane glycoproteins which are encoded by genes of the major histocompatibility complex. Unlike class I antigens (HLA-A, B, and C) which are expressed on most nucleated cells, class II antigen expression is limited to selected cell types, such as B-lymphocytes, macrophages, and activated T-lymphocytes. Over the past few years, it has become apparent that class II antigens can also be induced on cells which normally do not express these antigens. These molecules have been demonstrated on thyroid epithelial cells, kidney tubular cells, and retinal pigment epithelial cells in immunologically mediated diseases and on selected tumor cells in malignant conditions (1-6). Furthermore, recent experiments have shown that the biosynthesis and cell surface expression of these antigens are under the regulatory control of the lymphokine, IFN-γ (3, 7-9).

The precise function of the MHC class II antigens on each of these diverse cell types is not yet completely understood. However, it is clear that these antigens play an essential immunological role and it has been speculated that they may be involved in cellular differentiation. Immunologically, the expression of the class II antigens enables a variety of cells to present antigen to the T-lymphocyte which then allows for the initiation and perpetuation of immune responses (10, 11). Alternatively, some evidence suggests that these antigens have a more general role in cellular differentiation, i.e., expressed in early differentiation and lost upon maturation (12-18).

The retinoblastoma is the most common malignant ocular tumor of childhood. Although retinoblastoma tissue has been evaluated using a variety of approaches the pathogenesis is poorly understood and the cell(s) of origin is still controversial (19, 20). Many investigators believe that retinoblastomas originate from neuroectodermal tissue of the retina and consist of multipotential embryonic cells that have the potential to differentiate into neuronal and glial-like elements (21-23). Tso et al. (21) provided histopathological and electron microscopic data supporting photoreceptor differentiation. In a later study he suggested that retinoblastomas may also have a glial origin (22). Investigations on the well characterized retinoblastoma cell lines, Y-79 and WERI-Rb1, have provided additional evidence that this tumor may have both neuronal and glial properties originating from a primitive multipotential progenitor (24-28).

The present study was initiated to determine if retinoblastoma cells in situ and in vitro contain MHC class II antigens. Moreover, we wanted to see if the expression of these determinants is selectively restricted to either of the two primitive tumor cell types (i.e., neuronal versus glial) or is shared concomitantly with both. We report herein that in each case evaluated, retinoblastoma cells contain markers for class II antigens and the expression of the HLA-DR antigen is specifically modulated by IFN-γ. Double-labeling experiments demonstrated that both the neuronal-staining cells and the glial-staining cells contained these antigens.

MATERIALS AND METHODS

Patients

Patient 1 was a 4-year and 9-month-old boy with a 6-month history of leukocoria in the right eye (23). Family history was negative for blindness and other ocular problems as well as retinoblastoma or other neoplasms. Vision was 20/400 in the right eye and 20/30 in the left eye. The retina showed a large mass occupying one-half of the vitreous cavity. The subretinal and retinal mass showed dilated surface vessels. The retinal tumor showed large areas of necrosis and scattered calcification. The neoplastic cells were mostly poorly differentiated with scattered Flexner-Wintersteiner rosettes arranged in one or more layers surrounding a central lumen. The lumens showed staining with alcian blue, colloidal iron, and periodic acid-Schiff. The tumor did not invade the laminar cribrosa of the optic nerve. The choroid, sclera, and emissary channels were free of tumor.

Patient 2 was a 2-year and 6-month-old girl with bilateral retinoblastoma. The tumor was advanced in the right eye and the right eye was enucleated. The left eye contained two small tumors which were treated with photocoagulation and cobalt. There was no family history of retinoblastoma or other eye disorders. The retinal tumor filled most of the vitreal cavity and was mostly composed of diffuse infiltration and neoplastic cells with hyperchromatic nuclei and scant cytoplasm. There were numerous areas of necrosis and focal calcification within the tumor mass. There were occasional Flexner-Wintersteiner rosettes. The choroid and optic nerve were free of tumor.

Patient 3 was an 18-month-old black female with retinoblastoma in the right eye. The anterior chamber was normal but occluded by a large intraocular tumor. The retina was detached and replaced by tumor. Microscopic examination revealed that the vitreous cavity was almost totally replaced by a large intraocular tumor that displayed areas of calcification and necrosis. The tumor was moderately differentiated with scattered Flexner-Wintersteiner rosettes. The optic nerve showed tumor cells at the level of the laminar cribrosa.

Retinoblastoma Tissue

Cell Cultures. The Y-79 human retinoblastoma cell line used was originally characterized by Reid et al. (24). This cell line was maintained...
in suspension culture. Cytospins of the Y-79 cells were prepared and evaluated in the immunoperoxidase and immunofluorescent assays.

Tissue. Retinoblastoma tissue was processed in two ways. First the freshly collected tumor tissue was gently teased and placed in short term culture. Cytospins of both the teased and cultured cells were prepared and evaluated in the immunoperoxidase and immunofluorescent assays. Second, the tissue was frozen in ornithine carbamyl transferase and sectioned for immunohistochemical evaluation.

Antibodies

The antibodies which were used in this study are summarized in Table 1. All of the antibodies were used in the immunofluorescent and immunoperoxidase assays. In addition, the antibodies directed against HLA-DR, HLA-DQ, S-antigen, IRBP, and GFAP were directly biotinylated. This procedure enhanced the staining for HLA-DR, HLA-DQ, and S-antigen and was necessary for staining with the IRBP and GFAP. Two sources of the L243 anti-HLA-DR antibody were used. The antibody obtained from Becton Dickinson was biotinylated. The antibody kindly provided by Dr. Barton Haynes (Duke University) was not subject to this procedure. The specificity of this antibody was previously described (29). The monoclonal anti-S-antigen antibody was a generous gift from Dr. Paul Stein (Lahey Clinic, MA) and the heteroantiserum against S-antigen was donated by Dr. Igal Gery (National Eye Institute). Anti-IRBP, a heteroantiserum prepared in rabbits, was kindly provided by Dr. Barbara Wiggert (National Eye Institute).

Immunofluorescent Procedure and Reagents

Fresh frozen eyes were obtained from three patients with retinoblastoma and two normal individuals. Cryostat sections (6–8 μ thick) and cytospin preparations of retinoblastoma cells were air dried and fixed in cold acetone for 10 min. Slides were then rehydrated in PBS for 5 min and preincubated with normal goat serum (1:20) (Cappel Laboratories, Cochranville, PA) for 30 min at 35°C in a humidified chamber. Primary antibodies were prepared for 1 h in a humidified chamber. Slides were washed three times in PBS for 10 min followed by incubation with fluorescein or rhodamine conjugated goat antimouse IgG (for monoclonal antibodies) or goat anti-rabbit IgG (for heterosera prepared in rabbits) for 30 min. Slides were washed three times in PBS, mounted, and viewed in a fluorescence microscope.

Immunofluorescent Double-Labeling Technique

Retinoblastoma (Y-79) cells were grown as previously described (27). The cells in suspension culture were always tested 24 h after they were subcultured. The Y-79 cells were stained for the presence of the class II antigens (HLA-DR and HLA-DQ), neuronal markers (S-antigen and IRBP) and the glial marker (GFAP) by double-antibody immunofluorescence.

After three washes in PBS, Y-79 cells (5 x 10⁵/0.5 ml) were then incubated with the first biotinylated antibody. After an incubation at room temperature for 30 min, cells were washed twice in PBS, incubated with avidin-rhodamine or avidin-fluorescent reagent for 30 min and then washed again. A second antibody was then added to the cells. After an incubation at room temperature for 30 min, cells were washed two times in PBS, incubated with a secondary fluorescein or rhodamine labeled anti-mouse or anti-rabbit immunoglobulin which was directed against the second antibody. Following a final incubation at room temperature for 30 min, the cells were washed twice in PBS, mounted with 10% PBS and 90% glycerol, coverslipped and sealed.

Cells were examined with a Nikon microscope under epi-illumination. When rhodamine filters were used, the stained cells appeared red. When the same fields were examined with fluorescein filters, the stained cells appeared green. By double-exposure photography of the same field taken with different filter systems, the cells reacting with both rhodamine-labeled antibodies and fluorescein-labeled antibodies now appeared yellow-orange and were easily distinguished from other cells.

Immunoperoxidase Procedure and Reagents

Immunohistochemistry was performed using the avidin-biotin-peroxidase complex method (30). Briefly, tissue sections of cytospin preparations of retinoblastoma cells were fixed in acetone for 5 min, transferred to Tris-buffered saline (pH 7.6), and then immersed in 10% normal horse serum for 5 min. Monoclonal antibodies were used at a

![Fig. 1. Immunoperoxidase analysis of retinoblastoma tissue. A, retinoblastoma tissue stained with an irrelevant monoclonal antibody (anti-T-cell); B, retinoblastoma tissue reacted with anti-HLA-DR antibody. Arrows, the specific black staining observed in a large percentage of the tumor cells; C, the same tumor tissue incubated with anti-HLA-DQ antibody. Occasional positive cells can be identified by the arrow. A, B, and C were photographed at 400x magnification.](cancerres.aacrjournals.org)
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concentration of 1–2 μg/ml. After incubation in a moist chamber at room temperature for 1 h, the slides were washed in Tris-buffered saline, then the secondary antibody, biotin-conjugated horse anti-mouse IgG (Vector Laboratories, Burlingame, CA), was layered on the slides. The slides were again incubated in a moist chamber at room temperature for another hour. After washing in Tris-buffered saline, avidin-biotin-peroxidase complexes at a 1:100 dilution were applied for 45 min. The slides were washed again in Tris-buffered saline, and developed in diaminobenzide-8% nickel sulfate-3% hydrogen peroxide solution. The slides were then counterstained with methyl green (1% in methanol), dehydrated, cleared, and mounted as in routine processing.

Complement-Mediated Cytotoxicity Assay

HLA-DR antigen-bearing cells were also detected by the complement-mediated cytotoxicity assay which is the standard reference technique of the international HLA workshop (3). Two monoclonal anti-HLA-DR antibodies were used (i.e., anti-HLA-DR antibody (OKla+) obtained from Ortho Pharmaceutical Corporation (Raritan, NJ) and anti-HLA-DR (L243). Antibody dose-response curves were established for both anti-HLA-DR antibodies. For example, monocytes (obtained from normal individuals) or the Y-79 retinoblastoma cells (3 x 10⁷/0.2 ml) were incubated with varying dilutions of the anti-HLA-DR antibody for 30 min at 4°C in a total volume of 50 μl/well (3). Supernatant fluids were decanted and the cells were incubated (50 μl/well) with rabbit complement (1:4) (low-tox-H-rabbit complement; Accurate Chemical Co., Wesbury, NY) at 37°C for 30 min. Supernatant fluids were decanted and the trypan blue dye exclusion method was used to determine cell death (31). Each test was performed in triplicate and in a masked fashion.

The ability of IFN-γ to modulate HLA-DR antigen expression was evaluated when we compared percentage of cytotoxicity of retinoblastoma cells (or monocytes) before and after IFN-γ treatment. A single antibody concentration was chosen for the experiments with IFN-γ. The cytotoxicity assay was performed as described above.

Interferon Production and Assay

γ-Interferon was prepared by incubating human peripheral mononuclear cells (2.5 x 10⁶ cells/ml) with concanavalin A (10 μg) for 48 h at 37°C. Supernatant fluids contained 500 units of IFN activity. The antiviral activity was labile at pH 2.0 and was inactivated by anti-IFN-γ and not by anti-IFN-α or anti-IFN-β antisera. A second source of IFN-γ was recombinant IFN-γ obtained from Genetic, Inc., San Francisco, CA. Monoclonal anti-IFN-γ immunoglobulin was kindly provided by Dr. Jan Vilcek, New York University. Anti-IFN-α and anti-

Fig. 2. Immunoperoxidase evaluation of cytocentrifuged retinoblastoma cells. A, tumor cells stained with an irrelevant monoclonal antibody (anti-T-cell); B, tumor cells reacted with anti-HLA-DR antibody. Typical specific black stain is noted in nearly all the malignant cells. A and B were photographed at 400x magnification.

Fig. 3. Evaluation of Y-79 cells by the immunoperoxidase technique. A, retinoblastoma cell line reacted with an irrelevant monoclonal antibody (anti-T-cell); B, this same cell line stained with anti-HLA-DR antibody. Majority of cells demonstrate the positive immunoperoxidase reaction. A and B were photographed at 400x magnification.

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IFN-β were obtained from the Reference Reagent Branch, National Institute of Allergy and Infectious Diseases, NIH.

Antiviral activity was determined by the reduction in vesicular stomatitis virus plaque formation on human amnion (WISH) cells (ATCC; CCL25) grown in microtiter plates (32). The antiviral activity, expressed in IFN units, was calculated as the reciprocal of the highest dilution of the sample that reduced the number of viral plaques by 50%. Human reference of IFN-α (20,000 units; obtained from the Research Reagent Branch, National Institute of Allergy and Infectious Diseases, NIH) contained 10,000 units, when tested in our assay system.

RESULTS

Expression of Class II Antigens on Retinoblastoma

Both immunoperoxidase and immunofluorescence were used to detect class II antigens on retinoblastoma cells. These studies were performed on frozen tissue sections, freshly collected retinoblastoma cells from these cases and finally, on cultured cells of the established retinoblastoma cell line, Y-79. Each cell source will be described separately.

Frozen Tissue. The expression of HLA-DR antigen on frozen eye tissue sections from a patient with retinoblastoma is illustrated in Fig. 1B. Both anti-HLA-DR antibodies were used in the immunoperoxidase technique to show that HLA-DR antigen was present on almost all of retinoblastoma cells in situ. Fig. 1B represents the typical positive pattern of staining observed when anti-HLA-DR antibody is used to identify HLA-DR antigens. In contrast, there was an absence of positive staining when the same cells were treated with mouse ascites fluid or an irrelevant monoclonal antibody (anti-T-cell) (Fig. 1A). Using this same assay system HLA-DQ, another class II antigen gene product, was also evaluated and compared to HLA-DR antigen expression. When the monoclonal anti-HLA-DQ antibody (Leu 10) was reacted with the retinoblastoma
Fig. 5. Immunofluorescent analysis of retinoblastoma cells. A, retinoblastoma cells viewed under light microscopy; B, tumor cells reacted with anti-HLA-DR antibody tagged with rhodamine (red); C, the tumor cells incubated with anti-S-antigen antibody tagged with FITC (green); D, double-labeling immunofluorescent evaluation of the same field illustrated in A, B, and C. The cells bearing dual markers now appear yellow-orange. This color is a result of double-exposure photography of cells reacting with both the rhodamine labeled antibody and the FITC labeled antibody. A, B, C, and D, were photographed at 400x magnification.

tissue, approximately 5% of the tumor cells expressed this antigen (Fig. 1C). In comparison, greater than 90% of the same cells stained for HLA-DR antigen.

Freshly Collected Cells. We next separated cells from cases 1 and 2 and prepared cytospins for use in the immunoperoxidase test. The results obtained from one of these cases is illustrated in Fig. 2. Two sources of monoclonal anti-HLA-DR antibody were used to show that HLA-DR antigen was present on these freshly collected cells. A positive reaction is shown by the dark staining (Fig. 2B). When the same freshly collected cells were treated with mouse ascites fluid or an irrelevant monoclonal antibody (anti-T-cell), the positive dark black stain was absent (Fig. 2A). In both cases, greater than 90% of the cells stained for HLA-DR antigen. Freshly collected retinoblastoma cells from one of the three cases described above were further evaluated. The tumor cells were maintained in vitro for up to 6 months. During this time an immunoperoxidase assay was performed and the presence of HLA-DR antigen was examined at 7, 30, 60, and 180 days. In each instance, HLA-DR antigen was detected on the cells (data not shown). These data indicate that short term incubation (i.e., several months) of retinoblastoma cells does not result in a detectable loss of the antigen.

Cell Line Y-79. Based on these studies, we next wanted to determine if class II antigens were present on the Y-79 retinoblastoma cell line, which has been maintained in vitro for several years. The expression of HLA-DR antigen on Y-79 cells is illustrated in Fig. 3. Again, two sources of monoclonal anti-HLA-DR antibody were used in the immunoperoxidase test to demonstrate the presence of these molecules. It was noted again that greater than 90% of these cells stained for HLA-DR antigen (Fig. 3B). In contrast, when these cells were treated with mouse ascites or an irrelevant monoclonal antibody (anti-T-cell), there was an absence of immunoperoxidase staining (Fig. 3A). In order to further support the specificity of the two
monoclonal anti-HLA-DR antibodies, it should be noted that
WI38 cells, a continuous fibroblast cell line and WISH cells, a
human amnion cell line, failed to react with these antibodies.

Using both immunoperoxidase and immunofluorescence,
HLA-DQ was also evaluated and compared to HLA-DR anti-
gen expression. The results obtained with the Y-79 cells were
similar to those generated with the frozen tissue (i.e., approxi-
mately 5% of the cells contained HLA-DQ while 90% of this
population stained for HLA-DR antigen).

These studies clearly illustrate the presence of class II anti-
gens on retinoblastoma cells in situ, on freshly collected cells
and on cells maintained in vitro for several months to several
years.

Lack of Class II Antigen Expression in the Normal Retina

Retinoblastoma consists of a heterogeneous population of
tumors which may be derived from multipotential, primi-
tive cells which are capable of differentiation along several
different lines (27). The mature cells at the end of this differ-
entiation sequence are thought to be mainly neuronal (e.g.,
photoreceptors) and/or glial cells. We therefore examined fro-
zened eye sections to determine if class II antigens were detected
on photoreceptor cells or other neuronal elements of the normal
retina. Using both immunoperoxidase and immunofluorescent
assays, class II antigens were not detected in ocular tissue from
normal adults. These studies are consistent with previous ob-
servations in which tumor cells were recorded to contain class
II antigens while their normal mature counterparts failed to
express these molecules (33, 34).

Coexpression of Markers for Neuronal Cells, Glial Cells, and
MHC Class II Antigens

Direct Immunofluorescence. The Y-79 cell culture maintained
in suspension was analyzed further by immunofluorescence.
The same results were generated when the Y-79 cells were
incubated with anti-HLA-DR antibodies, i.e., greater than 90%
of the cells stained for the class II antigen, HLA-DR. Two
distinct staining patterns were observed when either mono-
clonal anti-HLA-DR antibody was used. More than 80% of the
cells displayed a punctate pattern of staining (Fig. 4B). This
pattern of staining was usually observed in the larger cells in
the heterogeneous population. In contrast, approximately 10%
of the cells stained with a diffuse pattern (Fig. 4B). This diffuse
pattern of staining was detected in the smaller cell type.

Using either a hetero anti-S-antigen antibody or a mono-
clonal anti-S-antigen antibody, neuronal, photoreceptor-like
cells were identified in approximately 10% of the retinoblas-
toma population (Fig. 5C). Likewise, when anti-IRBP antibody
was used to characterize photoreceptor-like cells, 10% of the
retinoblastoma population stained for IRBP. Anti-GFAP, a
marker for glial cells, also indicated that approximately 10% of
these cells contained glial-like elements (Fig. 4C). It is note-
worthy that when the morphology of the neuronal or glial
staining cells was examined, only the smaller cell type consist-
ently contained these markers.

Double-Labeling Immunofluorescence. In order to determine
if either glial or neuronal cells expressed class II antigens, we
performed double-labeling experiments in which the first mono-
clonal antibody was tagged with fluorescein isothiocyanate
(green) and second monoclonal antibody was tagged with rho-
damine (red). Cells were first viewed under phase microscopy.
This field was then examined with a fluorescein filter (green)
and then with a rhodamine (red) filter. By double exposure
photography of this field, the cells reacting with both fluores-
sein and rhodamine-conjugated antibodies demonstrated a yel-
low-orange color. As can be seen in Fig. 4D, cells that contained
the glial marker, GFAP, also displayed HLA-DR antigen.
Likewise, cells displaying neuronal properties (S-antigen and
IRBP) also coexpressed HLA-DR antigen (Fig. 5D). Once again
the smaller cell type was noted to be the cell which coexpressed
both markers. Finally, using both the neuronal markers (S-
antigen or IRBP) and the glial marker in a double-labeling
experiment, the same small cell type was shown to coexpress a
combination of neuronal (i.e., photoreceptor) and glial proper-
ties (data not shown). Although the presence of both neuronal
and glial markers has been noted in the same retinoblastoma
cell line (27, 28), this finding significantly extends present
information because it demonstrates dual staining (i.e., neu-
ronal and glial) in the same tumor cell.

Modulation of Class II Antigen Expression by γ-Interferon

Since the lymphokine, γ-interferon, has been identified as
the regulatory protein that enhances the synthesis and expres-
sion of class II antigens, we next incubated Y-79 cells with
medium alone or with medium containing recombinant human
IFN-γ (60 units). After incubation periods of 24 and 48 h the
retinoblastoma cells were reacted with anti-HLA-DR antibody
(OKl4+) (1:4 dilution) and complement and the percentage of
cytotoxicity was determined. Percentage of cytotoxicity is re-
corded as the number of dead cells determined by trypan blue
dye uptake (31). As is seen in Fig. 6, IFN-γ treatment results
in an increase in percentage of cytotoxicity. This same enhance-
ment is observed when monocytes were used as a control. This
enhancement by IFN-γ occurred in 24 h and is maintained for
48 h. Following this time (72 h), the percentage of cytotoxicity
returned to values detected prior to IFN-γ treatment. These
studies demonstrate that IFN-γ can modulate the expression
of class II antigens on retinoblastoma cells.

DISCUSSION

This study describes for the first time the presence of MHC
class II antigens on retinoblastoma cells. Regardless of the
assay used to detect these determinants, HLA-DR antigen was
present on the majority of retinoblastoma cells evaluated. This
antigen distribution was observed on frozen intact tissue, freshly
collected dispersed cells, and on the established cell line, Y-79.
In addition, HLA-DR-positive cells coexpressed the glial
marker, GFAP (35) or either putative photoreceptor cell
marker, S-antigen or IRBP (36–39). Taken together these data
support and extend previous observations that, under selected
conditions such as malignant transformation, cells of glial as
well as neuronal character can express class II determinants.

The retinoblastoma, a tumor, consisting of a population of
highly undifferentiated tumor cells, has been scrutinized in
terms of cellular composition. Both glial and neuronal cell
types have been described. Astrocytes containing GFAP were
initially reported in retinoblastoma tissue by Lane et al. (26).
Subsequently, Kyritsis and Jiang examined the retinoblastoma
cell lines, Y-79 and WERI-Rb1, and documented the presence
of cells staining for GFAP as well as neuronal-specific-enolase
in this tumor (27, 28). We also identified both cell types in the
Y-79 cell line. In fact, using double-labeling techniques, we
observed the dual presence of both glial and neuronal markers
in the same retinoblastoma cell. Our use of antibodies to S-
antigen and IRBP are of some interest, since it not only indi-
cates a neuronal but also a photoreceptor-like character of the
cells. Similarly, Kyritsis et al. (40) have demonstrated signifi-
cant IRBP induction in the Y-79 cell line. These data add
antigen expression have been compared (52, 53). Data gathered from examination of hematopoietic progenitor cells and selected tumor cells indicate that there is a differential as well as preferential expression between these molecules (52–54). In nearly all cases, the HLA-DR determinant is observed more often, appears more dense, and seems to precede the expression of the HLA-DQ determinant. A similar discordant expression is seen on retinoblastoma cells: that is, 90% of tumor cells contain HLA-DR antigen and only 5% of these cells weakly express HLA-DQ antigen. Although the significance of HLA-DQ antigen expression remains obscure, it is possible that this variable distribution may serve a regulatory function during cellular development.

The biological significance of MHC class II antigen expression on tumors is unknown. However, there are at least two possible consequences of this expression on malignant cells. The first involves an immunological function while the second speculates a role in cellular differentiation.

The expression of MHC class II antigens on cells of the immune system is critical to immune regulation. Recently, the appearance of these determinants has been reported on cell types not considered to have an immune role (11). Such cells include epithelial and endothelial cells which have been exposed to immunological insult (55–58), cells of the nervous system which have been involved in a degenerative disorder (42, 43), and cells which have undergone malignant transformation (5, 33, 59). The fact that many different cell types not belonging to the immune system express HLA-DR antigens constitutionally or can be induced to do so raised the possibility that these cells could function as antigen-presenting cells. Whether or not retinoblastoma cells which express HLA-DR antigen can function in this capacity will be a fertile area for future investigation. A number of reports have demonstrated that IFN-γ, a potent immunoregulating protein, can induce or augment the expression of class II antigens on a variety of different cell types (9, 49, 60). Although in this report we demonstrate that retinoblastoma cells constitutively express HLA-DR antigens, it was of interest to examine whether or not IFN-γ could enhance the expression of this antigen. Using the complement-mediated cytotoxicity assay, we observed an increase in the percentage of cytotoxicity after retinoblastoma cells were treated with IFN-γ. This specific modulation of HLA-DR molecules is consistent with other published data describing the induction or enhancement of these antigens on tumor cells by IFN-γ (8, 34). Since IFN-γ is a product of activated T-lymphocytes, it is possible that the expression of MHC class II antigen in retinoblastoma is a reflection of the host's immune response to the tumor.

Most intriguingly, much evidence suggests that the MHC class II antigens could function in another, nonimmune context. The appearance of these antigens during defined stages of erythroid and myeloid development as well as on certain tumor cells arrested at selected phases of their cell cycle, support the concept that class II antigens may have a role in cellular differentiation (10, 14–16, 18). Houghton et al. showed that while class II antigens can be seen on a proportion of both cultured and noncultured specimens of melanoma, no HLA-DR antigen is detected on normal melanocytes (34). The authors conclude that one explanation is that class II molecules are expressed on an early, as of yet, unidentified cell in the normal melanocyte lineage and that the HLA-DR-positive melanoma arises from this progenitor. Accordingly, HLA-DR antigen would be classified as a differentiation antigen in the melanocyte pathway. The retinoblastoma represents cells at one or more stages of early neuronal and glial development. The
fact that retinoblastoma cells contain HLA-DR antigens and that their normal mature counterparts, e.g., photoreceptors and glial cells, lack this determinant add support to the concept that HLA-DR antigen is also a differentiation antigen in the neuronal and glial pathway. Moreover, γ-interferon may actively mediate this process (60).

In conclusion, these studies identify the presence of the MHC class II antigens on retinoblastoma cells. We describe the modulation of HLA-DR antigen by γ-interferon as well as the preferential expression of this determinant over HLA-DQ antigen. In addition, double-labeling experiments revealed that HLA-DR antigen is shared concomitantly with cells that demonstrate glial and neuronal properties. These findings confirm previous reports demonstrating, that under selected conditions, glial cells can be induced to express HLA-DR and offer new evidence that cells of neuronal character can also be activated to express this determinant.

A role for the MHC class II antigens, especially HLA-DR, in the immunological process appears secure. However, the importance of this determinant in cellular differentiation is less well defined. Regardless of the precise role this molecule exercises in either dynamic event, analysis of MHC expression and modulation may offer a fruitful approach to a better understanding of the nature and molecule basis of this tumor.

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