The effect of irradiation on expression of HLA class I antigens in human brain tumors in culture

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The immunosuppressive effects of irradiation are well known; however, under certain circumstances irradiation also augments the local immune response by as yet undefined mechanisms. Because of the importance of HLA class I antigen in immune regulation and the fact that killing of tumor cells by cytotoxic T cells is HLA antigen-restricted, the authors studied HLA class I antigen expression in eight glioblastomas multiforme, four meningiomas, and four medulloblastomas. Twenty fragments of each tumor specimen were placed in short-term cultures immediately after resection. For each tumor, control Sample 1 was not irradiated, Sample 2 was irradiated on Day 1, and two groups of the remaining pieces of each tumor (specimens 3 to 10) were irradiated on two consecutive days. Escalating radiation doses were given, starting at 200 cGy/day for Sample 2 up to 1000 cGy/day for Sample 10. The total dose range was 200 to 2000 cGy. Corresponding nonirradiated tumor fragments served as controls. Four hours after irradiation, each sample was processed and stained for HLA class I antigen using the immunoperoxidase technique. The tumor cells were intensely stained in non-irradiated glioblastomas and meningiomas, whereas no staining was observed in medulloblastomas. In four of the eight glioblastomas and in all four meningiomas, irradiation augmented HLA class I antigen expression compared to controls. This effect was dose-dependent and was maximum in the 1200 cGy-treated specimens. No change was observed in the other four glioblastomas or in the medulloblastomas. The data suggest that irradiation does not decrease and may even induce HLA class I antigen expression in some brain tumors. This may be one of the mechanisms by which immunotherapy operates after irradiation. Further studies are required to elucidate optimum radiation doses and fractionation as well as optimum timing of immunotherapy.

KEY WORDS • HLA class I antigen • radiation therapy • brain neoplasm • immunotherapy

The major histocompatibility complex (MHC) plays an important role in regulating some functions of the immune system.\textsuperscript{2} Cytotoxic T cells are restricted by HLA class I antigens and kill tumor cells only in the presence of MHC class I antigens,\textsuperscript{8} whereas T helper cells are restricted by class II antigens.\textsuperscript{3} It has been demonstrated in experimental tumors that a reduction in class I antigens enables the tumor to evade the immune system and develop metastasis.\textsuperscript{3} A decrease in HLA class I antigens has been reported in several tumors resected from humans; in some, this finding was correlated to the aggressiveness of the tumor.\textsuperscript{2,5,10}

The brain was originally thought to be immunologically privileged because of the existence of the blood-brain barrier, the absence of lymphatic drainage, and the weak expression of MHC antigens in normal brain tissue.\textsuperscript{11} Recently, however, tumor-infiltrating lymphocytes (mainly T cells) have been described in some brain tumors,\textsuperscript{14} and several investigators have reported that brain tumors express HLA class I antigens more markedly than normal brain tissue.\textsuperscript{16}

Radiotherapy is the primary treatment for brain tumors following surgery. Due to the limitations of this modality, interest has been renewed in the use of immunotherapy. It was the purpose of the present work to study the influence of irradiation on the expression of HLA class I antigens in human brain tumors in short-term culture.

Materials and Methods

Tissue Specimens

Surgical material was removed at operation and immediately processed. The specimens consisted of eight glioblastomas multiforme, four meningiomas, four
Human brain and tumor irradiation and HLA class I antigens

![Graph](image)

**Fig. 1.** Bar graphs illustrating the effect of irradiation on HLA class I expression in the three primary tumor cell cultures. The percentage of cells stained positively is on the vertical axis. MEN = meningioma; G.M. = glioblastoma multiforme; MED. = medulloblastoma.

medulloblastomas, and two normal brains. After removal, each tumor was cut into 20 pieces (each 30 to 40 μm in diameter) and placed in tissue culture flasks containing RPMI medium with glutamine (without fetal calf serum). One fragment of each tumor was taken immediately for histological confirmation. Tissues were held in short-term culture in a humidified chamber until used. The tumor-containing flasks were irradiated, and the nonirradiated tumor fragments in culture served as controls. The same procedure was performed for the normal brain tissue.

**Irradiation Technique**

Tumor irradiation was performed using a photon beam and a roentgen orthovoltage machine 200 kV HVL (half value layer) of 1 mm copper. Tumor Samples 3 to 10 were irradiated for 2 consecutive days with different doses and Sample 2 received radiation only on Day 1. Thus the radiation dose ranged from 200 cGy/day for Sample 2 to 1000 cGy/day for Sample 10, for a total dose ranging from 200 to 2000 cGy. Sample 1 was not irradiated and served as a control. Four hours after irradiation, the tumor was fixed in formalin and processed.

**Immunoperoxidase Technique**

The technique has been described elsewhere. In brief, formalin-embedded blocks of tumor and tissue from two normal brains were studied. Sections were cut 3 μm from the tumors and adjacent normal tissue. The sections were placed on glass slides covered with poly-L-lysine, dried at 45°C overnight, and subjected to routine immunocytochemical techniques.

Sections were dewaxed and rinsed in absolute alcohol; endogenous peroxidase activity was blocked by 0.5% hydrogen peroxide in methanol. The slides were then rinsed in distilled water and Tris-buffered saline, pH 7.6, before incubation in 20% (vol/vol) normal swine serum in Tris-buffered saline for 30 minutes at room temperature. After the blocking steps, the tissue sections were incubated with the primary antibody, rabbit anti-human β2-microglobulin in a volume of 1:1000, for 60 minutes at room temperature in a humidified chamber. Unbound material was washed from the slide, and the tissue sections were then incubated with biotin-conjugated secondary antibody. Unbound material was washed from the slide and the tissue sections were incubated with streptavidin-peroxidase conjugate. Following another wash, the enzyme substrate and chromogen were added to the slide; after incubation for 10 to 15 minutes in a humidified chamber, an insoluble highly chromogenic product was deposited at the site of the antigen. We used chromogen 3-amino-9-ethylcarbazole. The slides were counterstained for 30 to 60 seconds with hematoxylin and mounted in glycerol gelatin.

Each section was examined in its entirety; the degree of staining in the appropriate cells was estimated by comparison with the intensity of the staining of normal controls and classified as follows: (−) for negative staining; (+) for slightly positive staining; (+ +) for moderate staining; and (+ + +) for significant staining. In addition, the percentage of cells stained was scored for each irradiation fraction.

**Soluble β2-Microglobulin Determination**

Supernatants of the irradiated as well as the nonirradiated control cultures were aspirated from the culture flasks 4 hours after irradiation. Determination of β2-microglobulin content was performed by an enzyme-linked immunosorbent assay technique, which has been described in detail elsewhere.

**Tissue Typing**

Blood samples were obtained from the patients and from tumor homogenates of the same patients. The blood was defibrinated, and the peripheral blood mononuclear cells were separated using the Ficoll-Hypaque method. All cell donors were typed as HLA-A, -B, -C, and -DR using standard methods.

**Results**

**HLA Antigen Expression**

The majority of neurons and glial cells from the normal brain specimens were weakly (+) stained for HLA class I antigen; only occasional cells were stained for HLA class II antigen. Among the brain tumor specimens, the tumor cells were intensely stained for HLA class I in glioblastomas multiforme and meningiomas; however, no staining was observed in medulloblastomas.

**Effect of Irradiation on HLA Class I Antigen Expression**

**Normal Brain.** No significant increase in β2-microglobulin staining was observed in neurons and glial cells of normal brain after irradiation.

**Brain Tumors.** The effect of irradiation on HLA class I antigen expression was dose-dependent (Fig. 1). Glio-
blastomas demonstrated a gradual increase in staining that reached a maximum at 1000 cGy, compared to controls (Fig. 1B). At 1200 cGy, a gradual decrease was noted in four glioblastomas. No change in class I expression was observed in the other four glioblastomas, and at 2000 cGy, the class I expression was still higher than in the control specimens. The same pattern was observed in meningiomas, although to a greater magnitude (Fig. 1A); however, no change was observed in medulloblastoma (Fig. 1C). The effect of irradiation was seen on Day 1 and persisted 24 hours later when stained again.

To study further the effect of irradiation on HLA class I antigen expression, supernatants from the irradiated tumor cultures as well as from the nonirradiated tumors were examined for the presence of soluble \( \beta_2 \)-microglobulin. In the irradiated tumors, the supernatants had significantly higher levels of \( \beta_2 \)-microglobulin compared to the nonirradiated tumor supernatants (Table 1). The highest levels were observed after irradiation with 1200 rad. In medulloblastoma, \( \beta_2 \)-microglobulin was not detected before or after irradiation.

Tissue typing of peripheral blood and the tumor was performed in all glioblastoma multiforme specimens. There was no difference in tissue typing between the peripheral blood and the tumor.

**Discussion**

The presence of tumor-infiltrating lymphocytes in brain tumors,\(^{15}\) together with the enhanced expression of MHC class I antigens, enable the tumor cell to react in a cell-mediated immune reaction. Because of the poor results with surgery and irradiation, immunotherapy of brain tumors\(^{11}\) may be an attractive approach.

The immunosuppressive effect of ionizing irradiation is well known.\(^{2}\) Irradiation suppresses primary antibody formation and cell-mediated immunity, and has been shown to decrease MHC class II expression in uveal melanoma.\(^{3}\) However, under certain conditions, irradiation can enhance the immune response and increase the efficacy of immunotherapy.\(^{15}\)

In the present study, HLA class I antigen expression was observed in gliomas and meningiomas. This is in agreement with the study by Saito, et al.,\(^{16}\) which found that irradiation did not decrease HLA class I antigen expression but, in many cases, enhanced it. This effect was dose-dependent and was observed when irradiation reached 1400 cGy in two equal fractions 1 day apart. Moreover, the significantly higher level of soluble \( \beta_2 \)-microglobulin in the supernatants from the irradiated cultures compared to control cultures is further support of our findings and cannot be attributed to measurement error. The only study of human tumors was reported by Jager, et al.,\(^{7}\) who described a decreased expression of HLA class II antigen after irradiation of uveal melanoma. In the 9L gliosarcoma brain tumor model in rats, Wen, et al.,\(^{17}\) reported that a single irradiation dose of 1200 cGy did not change the MHC class I or class II antigen expression in the tumors. We applied two doses up to 2000 cGy in 2 days, but these dose ranges are biologically equivalent to the doses used for patient irradiation.

The fact that HLA class I antigen expression was enhanced and not reduced may explain one of the mechanisms by which immunotherapy operates. Radiotherapy induces augmentation of the local immune reaction, possibly via modification of the antigenicity of the tumor cells and influence on the infiltrating lymphocytes.\(^{4}\) Indeed, in our study as well as in the report of Wen, et al.,\(^{17}\) no effect of irradiation on normal brain tissue was found. In addition, Imanaka, et al.,\(^{8}\) reported that the strongest immune reaction was observed after exposure to 2000 cGy in the MM46 tumor transplanted in C34/HE mice. In the same tumor model, Ogawa, et al.,\(^{13}\) reported that active, specific immunotherapy given after irradiation caused marked tumor regression and increased survival compared to immunotherapy without irradiation. Our findings may add another explanation for the augmentation of the immune response after irradiation. The enhanced expression of HLA class I antigen, which is a restriction molecule for cytotoxic T cells, may be the basis of this phenomenon. Further studies with longer observation periods are needed to confirm that our findings are not transient and to elucidate the effects of timing and irradiation dose in immunotherapy of brain tumors.

**References**

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