Heterotransplantation of retinoblastoma into the athymic “nude” mouse. 


Fresh surgical specimens of retinoblastoma were successfully transplanted into the anterior chamber of the “nude” mouse (a homozygous mutant, nu/nu, with a severe defect in cellular immunity), filling the eyes but failing to grow subcutaneously. Retinoblastoma cells from an established cell line, Y-79, spread from the intraocular injection site to invade the orbit, optic nerve, and brain and formed large tumors when implanted subcutaneously. Tumor cells injected into the anterior chamber of immunologically normal litter-mates (heterozygotes, nu/+ ) survived for varying periods in the anterior chamber but showed little growth.

The “nude” mouse is a hairless mutant (homozygous for the mutation nude, nu/nu), which is born without a thymus and possesses a severe defect in cellular immunity. Recently, a variety of human malignant tumors have been transplanted subcutaneously and intraperitoneally into the nude mouse. Some tumor cell lines, maintained in vitro prior to injection, have shown infiltrative and metastatic growth when so transplanted. Fresh tumor specimens, on the other hand, typically grow slowly, if at all, when similarly implanted.

The experimental study of retinoblastoma, the most common intraocular tumor of childhood, has been limited by its low incidence and by the consequent scarcity of surgical specimens. Although short-term propagation of retinoblastoma is readily achieved, there are only two well-characterized, established cell lines of retinoblastoma in vitro. The results of heterologous transplantation into the anterior chamber of the eye has led to consideration of this site as an area of relative “immunologic privilege.” Recent studies have suggested that the mechanisms involved and the extent of “immunologic privilege” are not yet fully defined. Our attempts at propagating numerous explants of retinoblastoma in the anterior chamber of rabbit, hamster, and monkey eyes and the cheek pouch of hamsters, over a 10 year period, have been unsuccessful. This is in marked contrast to the results with ocular melanomas and a variety of other tumors.

In the present experiments, portions of fresh surgical specimens of retinoblastoma as well as cells from the established Y-79 cell line were transplanted into the anterior chamber of nude mice (nu/nu) and their heterozygote (nu/+ ) litter-mates, which have been shown to be immunologically normal, and into the subcutaneous site in nu/nu mice.

Methods. Portions of 13 fresh retinoblastomas were obtained at the time of enucleation. Part of each specimen was placed in tissue culture with the use of methods previously described. A single cell suspension of the fresh tumor was easily obtained by agitation in the tissue culture medium; trypsinization was unnecessary. Swiss background nu/nu and nu/+ mice were obtained from the animal facility at Memorial Sloan-Kettering Cancer Center and were kept in a conventional environment in cages with filter tops. Fresh tumor cells or the cell line Y-79, in a single-cell suspension in RPMI 1640 with 1 percent penicillin and streptomycin, were implanted into the anterior chamber and subcutaneously into the nude mice and into the anterior chamber of their nu/+ litter-mates. The tumor cells were injected into the anterior chamber under sterile conditions, with a 30-gauge needle, under a dissecting microscope. The maximum volume of the anterior chamber injection was approximately 0.0001 ml., containing approximately 6,000 cells. As the needle was withdrawn, there was some slight reflux of fluid, so that the final number of cells remaining in the anterior chamber was less. The volume injected subcutaneously was approximately 0.20 ml. containing from 10⁶ to 10⁷ cells.

Four nu/nu mice were treated with 300 mg./kg. of cyclophosphamide 4 days prior to subcutaneous implantation of fresh retinoblastoma, lowering their white cell counts from 10,000 to 400/mm³.

Results. Growth of retinoblastoma in the various sites is shown in Table I.

Fresh retinoblastoma heterotransplanted into nu/nu mice. Twelve of the 13 fresh retinoblastoma transplants filled the anterior chamber of the nu/nu mouse. Spread into the vitreous and retina was typically seen. The fresh retinoblastomas failed to acquire a demonstrable stroma or vascular supply and remained as loosely clumped tumor cells floating inside the eye. Although infiltration of the iris and retina was seen, the tumor was avascular. No extraocular extension was seen. The histologic appearance of the tumor which grew within the eye remained similar to that in the original tumor. In two cases in which Flexner-Wintersteiner rosettes had been seen on original histopathologic study, similar structures were seen in the tumor which spread intraocularly in the nu/nu mice (Fig. 1). Tumor harvested from the eyes of nu/nu mice could be passed to the eyes of other nu/nu mice. Several tumors are now in the fifth and sixth passage.

All tumors failed to grow subcutaneously in untreated nu/nu mice, but in three out of four nu/nu mice treated with cyclophosphamide, tumors grew subcutaneously to be as large as the whole animal. These large tumors spread along tissue planes but did not invade or destroy normal tissues.
Fig. 1. Flexner-Wintersteiner rosettes seen on intraocular growth of fresh retinoblastoma in the nude mouse. Similar structures were seen in the original tumor. (*600.)

Table I. Growth of retinoblastoma in nude mice

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<tr>
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<th>nu/nu anterior chamber</th>
<th>nu/nu subcutaneous</th>
<th>nu/+ anterior chamber</th>
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<tbody>
<tr>
<td></td>
<td>Untreated</td>
<td>Cyclophosphamide-treated</td>
<td></td>
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<tr>
<td>Fresh retinoblastoma</td>
<td>41/46</td>
<td>0/26</td>
<td>3/4</td>
</tr>
<tr>
<td>(13 tumors)</td>
<td></td>
<td></td>
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<tr>
<td>Retinoblastoma passed from nu/nu anterior chamber</td>
<td>25/32</td>
<td>0/6</td>
<td>—</td>
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<tr>
<td>Y-79 cell line</td>
<td>8/8</td>
<td>0/4†</td>
<td>—</td>
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<tr>
<td></td>
<td></td>
<td>7/9‡</td>
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*Expressed as number of animals showing tumor growth/number of animals implanted.
†10⁶ tumor cells injected.
‡Microscopic growth only.
§10⁷ tumor cells injected.

Y-79 retinoblastoma cell line heterotransplanted into nu/nu mice. The established Y-79 cell line completely destroyed the eye in all instances and extended into the orbit, optic nerve, subarachnoid space, and the brain (Fig. 2). This tumor characteristically showed a prominent fibrovascular stroma.

The Y-79 cells showed rapid subcutaneous growth at the site of injection in the nu/nu mice when the tumor inoculum contained 10⁷ or more cells (Table I). In four animals in which fewer cells were injected, no subcutaneous growth was seen.

Retinoblastoma heterotransplanted into the anterior chamber of nu/+ immunologically normal mice. Tumor growth was not clinically apparent.
in the eyes of nu/+ mice injected with either fresh tumor or cells from the Y-79 line. Histopathologic examination, however, showed residual, viable tumor cells in the anterior segment, with some infiltration of iris in one instance.

Attempts at tissue culture propagation. Explants and single cell suspensions of all 13 fresh retinoblastoma specimens survived for only 1 or 2 weeks in vitro. Cells harvested from the mouse eyes also survived only a short time in vitro.

Discussion. An experimental model of retinoblastoma using the immune-deficient nude mouse has been established. Two patterns of growth were observed. Tumor from freshly enucleated eyes spread diffusely throughout the ocular cavities when injected into the anterior chamber of nude mice. Cells from the established Y-79 retinoblastoma cell line, which retain the morphologic characteristics of retinoblastoma, when similarly injected, showed a much more invasive growth pattern. The basis for the observed difference in growth pattern between fresh and cultured tumor is not well understood but may reflect the observation that tumor cells in cell culture become less differentiated and more anaplastic than the cells of the original tumor.

The growth of fresh surgical specimens of retinoblastoma in the eye of the nude mouse was consistent; the only retinoblastoma tumor cells which did not grow intraocularly were derived from a tumor that had received 3,500 rads of radiation several months before enucleation. Relatively large amounts of fresh tumor could be grown in subcutaneous sites in the nude mouse but only when their immunologic defenses were further impaired by treatment with cyclophosphamide.

The nude mouse provides an in vitro system for maintaining retinoblastoma for relatively long periods through serial transplantation. This system will make available relatively large amounts of tumor, suitable for biochemical assays such as those for lactic acid dehydrogenase (which some investigators have found to be elevated in the aqueous humor of eyes with retinoblastoma) or catecholamines or their breakdown products. Moreover, this system provides a promising in vivo model for studying tumor response to chemotherapy.

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